Journal of
Science &
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in the Tropics Volume 7 Number 2 Dec 2011

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# JOURNAL OF SCIENCE AND TECHNOLOGY IN THE TROPICS

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2. Beveridge W.I.B. (1961) *The Art of Scientific Investigation*. Mercury Book, London.
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*Journal of Science and Technology in the Tropics* (2011) **7**: 67

# EDITORIAL

## Mahathir Science Award

The Academy of Sciences Malaysia (ASM) at its 53rd meeting in February 2004 approved the establishment of the Mahathir Science Award to encourage scientists to address problems of the tropics using science and technology. This award was in appreciation of Y.A.Bhg. Tun Dr. Mahathir and his contribution in the establishment of ASM and his support to S&T in the country during his tenure as Prime Minister. The award was established and launched in conjunction with ASM's tenth Anniversary celebrations on the 17th August 2004. The award is to be bestowed on any scientist, institution or organization worldwide in recognition of contributions to solving problems in the tropics through science and technology. It was the hope of ASM Council that this award would set a new benchmark for scientific research in the country.

There are four categories to the award, viz. tropical medicine, tropical agriculture, tropical architecture and engineering, and tropical natural resources. One award is conferred each year covering any of the four categories. The prize is RM100,000.00, a gold medal and a certificate. The scientific criteria for the award are scientific breakthrough, impact of that breakthrough and solving problems of the tropics. A stringent vetting process by an evaluation committee is held comprising Fellows of the ASM, an international panel of experts and invited Nobel Prize winners. Submissions close in March each year.

The recipients of the award to-date are: **2005** – Professor John Sheppard Mackenzie of Australia "for his contribution in solving the problems related to Japanese encephalitis virus" in the field of tropical medicine; **2006** – Faculty of Medicine, University of Malaya "for outstanding contribution to the understanding and treatment of the Nipah virus" in the field of tropical medicine; **2007** – Professor Joseph S.M. Peiris of Sri Lanka "for his discovery of the aetiological agent causing SARS leading to the understanding of pathogenesis and epidemiology of the disease in 2003" in the field of tropical medicine; **2008** – Professor Gurdev Singh Khush from India "for his perseverance, leadership, commitment and revolutionary work in systematically directing and developing several rice varieties that have overwhelmingly contributed towards reducing global hunger" in the field of tropical agriculture; **2009** – Forest Research Institute Malaysia "for technology and development of the rubberwood furniture industry in Malaysia and globally" in the field of tropical natural resources; **2010** – no winner; and **2011** – Professor Yuan Long-Ping from China in recognition "of his courage in independent thinking in rice breeding resulting in the innovative development of the hybrid rice, that has revolutionized rice production and sustainability" in the field of tropical agriculture.

It is thus hoped that the ASM Award for Scientific Excellence in Honour of Tun Dr. Mahathir will help spur Malaysian scientists in particular to achieve excellence in R&D and in so doing help develop the scientific research culture in the country.

**Salleh Mohd. Nor and Ong Eng Long**
Co-Chairman, JOSTT

*Journal of Science and Technology in the Tropics* (2011) **7**: 115-119

# Effects of water treatment processes on basic water quality index (WQI) parameters, overall WQI and class of water

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**Abstract** This study, carried out in May and November 2007 at the Semenyih River Water Treatment plant, Precinct 19, Putrajaya, Peninsular Malaysia, examined the effects of five water treatment processes on the basic parameters of the water quality index (WQI), the overall WQI and the Class of Water. The basic WQI parameters include pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), dissolved oxygen (DO) and ammoniacal-nitrogen ($NH_3$-N). The water treatment processes under study were coagulation, flocculation, sedimentation, filtration and disinfection. In this study, TSS and BOD are among the studied WQI parameters that underwent significant change during the treatment processes, resulting in more than 95% TSS and 25-67% BOD reduction. Results of this investigation show that the five studied treatment processes seem to affect most of the individual WQI parameters and the overall WQI but not necessarily the Class of Water.

**Keywords** water treatment – WQI parameters – Class of Water

## INTRODUCTION

Water is the most important inorganic liquid that exists naturally on earth. Oceans contain over 97% of the earth's water. However, salt water cannot be consumed directly by humans or used for many industrial processes. This means only less than 3% of water (fresh water) is readily available for use. Freshwater sources include rivers and lakes (surface water), groundwater and ice as well as glaciers [1].

In most countries, the major sources of water include surface water, groundwater and precipitation. In Malaysia, over 99% of drinking water comes from surface supplies, mainly rivers which are facing increasing levels of pollution, due to rapid industrialization and socio-economic development since the late 1980s [2].

Water Quality Index (WQI) relates a number of water quality parameters in a common scale and combines them in accordance with a chosen method or model of computation into a single number [3]. The main objective of the WQI system is to serve as a preliminary means of assessment of a water body for compliance with the standards adopted for five designated classes of beneficial uses [3].

Chemical oxygen demand (COD) measurements are used in both municipal and industrial wastewater treatment plants to indicate efficiency of the water treatment process [4]. According to Malaysia's Department of Environment (DOE), industrial activity is a major contributor to surface water biochemical oxygen demand (BOD) values in Selangor [5]. Numerous scientific studies suggest that 4 to 5 parts per million (ppm) of dissolved oxygen (DO) as the minimum amount needed to support a large, diverse fish population. Most fish die when dissolved oxygen falls below 3.0 ppm. DO is vital for maintaining aerobic conditions in natural waters that receive pollution matter and also in aerobic treatment processes intended to purify domestic wastewaters [4].

Ammoniacal-nitrogen ($NH_3$-N) is due to the presence of nitrate ($NO_3^-$), nitrite ($NO_2^-$), ammonia ($NH_3$) and organically-bonded nitrogen in water. Sewage and fertilizers are the main source of nitrates in lakes and rivers. The presence of excessive nitrates in water will encourage rapid growth of algae (eutrophication) and this later will increase the value of BOD when the algae start decaying [4].

Total suspended solids (TSS) include all particles suspended in the water which will not pass through a filter. Suspended solids can absorb heat from sunlight which increases the water temperature and decreases levels of dissolved oxygen (DO). Also since less

light penetrates the water, the rate of photosynthesis decreases and this also contributes to lower DO values.

The three major Malaysian river water pollutant indicators are ammoniacal-nitrogen, biochemical oxygen demand and suspended solids [3]. Ammoniacal-nitrogen originates from livestock farming and domestic sewage while suspended solids are due to earthworks and land-clearing activities. Biochemical oxygen demand is mainly due to discharges from agro-based and manufacturing industries. 80% of Malaysian rivers were polluted by $NH_3$-N in 1997, 43% in 1998 and 39% in 2006. Suspended solids polluted 31% of Malaysian rivers in 1997, 34% in 1998 and 40% in 2006 while BOD was responsible for 69% of Malaysian river water pollution in 1997, 21% in 1998 and 21% in 2006 [3]. Improvement in $NH_3$-N and BOD brought about reduction of polluted rivers from 25 in 1997, 16 in 1998 to 7 in 2006 [3].

Surface water from Malaysian rivers, lakes and dams undergo conventional water treatment processes such as coagulation, flocculation, sedimentation, filtration and disinfection before it is distributed to consumers as their domestic water supply or potable water.

This study examined the effects of five conventional water treatment processes on the individual parameters of the DOE water quality index. It also examined how the changes in the individual WQI parameter influenced the overall WQI and eventually the classes of beneficial uses of water.

## MATERIALS AND METHOD

Malaysia's Department of Environment (DOE) formula was used for this investigation and it included the following parameters: dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), ammoniacal-nitrogen ($NH_3$-N) and pH [3].

### Sampling site

This study was conducted at Sungai Semenyih Water Treatment plant in Precinct 19, Putrajaya, Peninsular Malaysia. This plant treats water from Semenyih dam via Semenyih river.

### Sampling frequency

Sampling was done twice, once in May 2007 and the other in November 2007.

### Sampling points

Samples were taken from 6 stages of the water treatment processes. These include raw water, coagulation, flocculation, sedimentation, filtration and disinfection.

### Sample analysis

In-situ parameters for pH, DO and temperature were obtained using the YSI environmental meter (Model 556). Ammoniacal-nitrogen concentrations were determined using Ion Chromatograph and BOD values were obtained using the BOD track apparatus. The DRB 200 digestion reactor and HACH DR 2010 spectrophotometer were utilized for analyzing COD values.

## RESULTS AND DISCUSSION

The various measured water quality parameters as well as calculated WQI and Class of Water values of all the treatment stages for May 2007 and November 2007 are tabulated in Table 1 and Table 2 respectively.

**Table 1**. Average water quality parameters, water quality index (WQI) and Class of Water for six stages of the treatment process at Precinct 19 Putrajaya Water Treatment Plant for May, 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| pH | 6.98 ± 0.05 | 6.13 ± 0.02 | 6.10 ± 0.02 | 5.92 ± 0.08 | 6.07 ± 0.02 | 7.32 ± 0.03 |
| DO (mg/L) | 6.96 ± 1.70 | 7.25 ± 1.10 | 7.01 ± 1.45 | 6.02 ± 1.45 | 6.90 ± 1.10 | 7.24 ± 0.80 |
| BOD (mg/L) | 2.8 ± 0.5 | 2.8 ± 0.7 | 1.1 ± 0.4 | 2.2 ± 0.4 | 2.7 ± 0.5 | 2.1 ± 0.5 |
| COD (mg/L) | 14.32 ± 1.24 | 13.60 ± 0.95 | 9.80 ± 1.04 | 6.00 ± 0.75 | 5.10 ± 0.90 | 4.73 ± 0.54 |
| TSS (mg/L) | 260.0 ± 5.4 | 200.0 ± 2.6 | 6.0 ± 1.2 | 10.0 ± 0.4 | 2.0 ± 0.2 | 0.2 ± 0.1 |
| $NH_3$-N (mg/L) | nd | nd | nd | nd | nd | nd |
| Temperature $^0$C | 25.9 ± 0.2 | 25.1 ± 0.1 | 25.3 ± 0.2 | 27.0 ± 0.1 | 25.9 ± 0.2 | 28.4 ± 0.3 |
| WQI (DOE) | 85.19 | 85.47 | 89.22 | 90.08 | 93.41 | 96.38 |
| Class of Water | II | II | II | II | I | I |

Table 3 and Table 4 show the changes in the status quality of the indexes for the individual major Malaysian river water pollutants ($NH_3$-N, BOD and SS) as these parameters undergo all the treatment stages.

There was no significant change in the pH during the treatment stages (Tables 1, 2). The average pH of raw water of 6.98 in May 2007 and 6.69 in November 2007 was within the recommended range of 5.5-9.0 for raw water quality criteria set by the Ministry of Health, Malaysia [6]. The final disinfection stage had an average pH of 7.32 in May 2007 and 7.18 in November 2007, both of which fell within the range of 6.5-9.0 for drinking water quality standards set by the Ministry of Health, Malaysia. In both studies, the highest average pH was obtained during disinfection stage which indicates the overall water treatment processes seem to improve the pH to alkaline range.

DO for both studies started decreasing during the flocculation stage, reaching the lowest at the sedimentation stage. During this stage, flocculants formed and agglomerated during the coagulation and flocculation stages were allowed to settle to the bottom using gravity force. After sedimentation, DO started increasing again and was the highest (7.24 and 7.50) during the disinfection stage for both studies.

BOD started decreasing during the coagulation stage (Tables 1 and 2). However, there seem to be some differences with BOD readings during flocculation stage between the two studies. The study done in May 2007 shows the lowest average BOD value for in contrast to the highest value for the November 2007. In both studies, it is evident that a significant change in the value of BOD occurred during coagulation-flocculation stage. A wastewater study [7] using 40 to 60 mg alum during coagulation in conventional water treatment processes indicated possibility of 95% BOD, turbidity and SS removal. Coagulation using alum coagulant is a typical process used in drinking water treatment to remove turbidity and natural organic matter [8, 9].

Disinfection processes also shows significant decrease in BOD values. The overall water treatment processes are able to convert the status quality of the individual BOD value (a major Malaysian river water pollutant) which is also one of the six parameters to calculate WQI, from slightly polluted to clean (Tables 3 and 4).

**Table 2**. Average water quality parameters, water quality index (WQI) and Class of Water for six stages of the treatment process at Precinct 19 Putrajaya Water Treatment Plant during November, 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| pH | 6.69 ± 0.02 | 6.68 ± 0.03 | 6.93 ± 0.03 | 6.99 ± 0.03 | 7.02 ± 0.06 | 7.18 ± 0.04 |
| DO (mg/L) | 7.19 ± 1.46 | 7.19 ± 1.20 | 6.50 ± 0.56 | 6.30 ± 0.85 | 7.20 ± 0.64 | 7.50 ± 0.94 |
| BOD (mg/L) | 3.90 ± 1.14 | 3.10 ± 0.94 | 3.70 ± 0.77 | 2.60 ± 1.01 | 2.20 ± 0.34 | 1.30 ± 0.28 |
| COD (mg/L) | 2.30 ± 0.45 | 37.75 ± 2.46 | 7.70 ± 1.15 | 2.40 ± 0.55 | 9.90 ± 1.33 | 4.90 ± 0.65 |
| TSS (mg/L) | 4.30 ± 0.88 | 4.70 ± 0.46 | 4.30 ± 0.22 | 4.00 ± 1.10 | 4.00 ± 0.94 | 4.00 ± 0.44 |
| $NH_3$-N (mg/L) | 0.65 ± 0.05 | 0.51 ± 0.07 | 0.46 ± 0.10 | 0.46 ± 0.08 | 0.43 ± 0.06 | 0.38 ± 0.03 |
| Temperature $^0$C | 25.2 ± 0.3 | 24.9 ± 0.2 | 24.8 ± 0.1 | 25.1 ± 0.1 | 24.1 ± 0.1 | 25.6 ± 0.1 |
| WQI (DOE) | 87.18 | 82.03 | 85.35 | 87.21 | 88.49 | 91.45 |
| Class of Water | II | II | II | II | II | II |

**Table 3**. Classification of major river pollutant indexes during conventional water treatment processes in terms of quality status for May 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| BOD | Slightly polluted | Slightly polluted | Clean | Clean | Slightly polluted | Clean |
| TSS | Polluted | Polluted | Polluted | Clean | Clean | Clean |
| $NH_3$-N | Clean | Clean | Clean | Clean | Clean | Clean |

**Table 4**. Classification of major river pollutant indexes during conventional water treatment processes in terms of quality status for November 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| BOD | Slightly polluted | Slightly polluted | Slightly polluted | Slightly polluted | Clean | Clean |
| TSS | Clean | Clean | Clean | Clean | Clean | Clean |
| $NH_3$-N | Polluted | Polluted | Polluted | Polluted | Polluted | Polluted |

COD values seem to be highest for coagulation where coagulants were added to produce flocculants (Tables 1 and 2). This value decreased further during flocculation and sedimentation. Disinfection shows the lowest average value of COD especially for May 2007 study. Disinfection in our study is the process of adding chlorine to public water supplies to make it safe from microbiological point of view as well as to leave a detectable residual of chlorine inside the distribution network [1].

During the overall water treatment process in May 2007, the value of TSS, a major water pollutant, decreased significantly from 260 mg/L to 0.06 mg/L (Table 1). Significant decrease in TSS occurred during coagulation. Coagulation appeared to be a very effective process to remove TSS if the initial concentration of TSS was high (260 mg/L).

Sedimentation and filtration also contributed significantly towards the decrease in TSS if the initial concentration of TSS in the raw water was high. In the May 2007 study, the combination of coagulation and flocculation processes managed to change the quality status of TSS index from polluted to clean (Table 3). Coagulation, sedimentation and filtration did not seem to work effectively if the initial TSS was very low (4.30 mg/L) as in the November 2007 study with a slight increase during coagulation and remained unchanged during sedimentation and filtration.

The overall treatment process was able to decrease ammoniacal-nitrogen from 0.6 mg/L (raw water) to 0.38 mg/L (disinfection) in the November 2007 study (Table 2). The greatest decrease seemed to occur during the coagulation process. However, it should be noted that both the coagulation and overall treatment processes were unable to remove even 50% of the ammoniacal-nitrogen. This was probably why the Sungai Semenyih plant operators shut down the treatment processes if ammoniacal-nitrogen was detected above the maximum acceptable value (1.5 mg/L) during routine monitoring [3]. The overall treatment processes was unable to improve the quality status of the $NH_3$-N index (Table 4).

In this study, coagulation with alum and disinfection with chlorine are the stages of the overall conventional water treatment processes that seem to have the most impact on the individual WQI parameters especially the major water pollutants like BOD, TSS and $NH_3$-N. Coagulation involves neutralizing negative water pollutants, for example clay and silt by using positive metal coagulants such as aluminium sulphate (alum) to form flocculants. Previous studies on alum coagulation which has been optimized for turbidity and organic matter removal indicated this process is extremely effective in removing *Cryptosporidium parvum* oocysts [9]. Alum has been shown to be a more effective coagulant to remove natural organic matter (NOM) than polyaluminium chloride [8]. A study had also reported that large molecules of NOM such as humic acid could be easily removed by coagulation [10]. Several studies also indicate possible alternative functions of coagulation. This can be achieved by manipulating alum dosage [7] which indicated efficient removal of phosphorus, bacteria and heavy metal with increased alum dosage. Increased alum dosage also improved removal efficiency of certain antibiotics in drinking water [11]. Other alternative coagulation functions can also be achieved by changing the type of coagulant used – for example, almost 100% asbestiform fibre removal was achieved from potable water using iron salts as the coagulant [12]. Similar study using other coagulant such as polyaluminium chloride (PACl) [11] indicated 50% removal efficiency for certain antibiotics.

Chlorine used in the disinfection process is usually added in the form of free chlorine or as hypochlorite [13]. It acts as a potent oxidizing agent in both forms and it often dissipates itself in side reactions so rapidly that little disinfection is achieved unless amounts in excess of the chlorine demand is added [13]. This, in turn creates a serious problem as chlorine also reacts with natural organic matter present in the water to form halogenated trihalomethanes (THMs) and haloacetic acids (HAAs) which are two major disinfection byproducts (DBPs) with harmful long-term effects [14]. However, with the increased use of chlorine for disinfection, there has been a corresponding decrease in the incidences of waterborne diseases such as cholera and typhoid. Disinfection with chlorine has also been shown to be an effective way to control nitrite levels in drinking water [15]. A study indicated 70% removal of certain common pesticides with chlorine disinfection [16].

In summary, the results of this study indicate that conventional water treatment processes especially coagulation and disinfection have the capacity to improve certain basic WQI parameters thus improving the overall WQI values. However, improvement of WQI values does not necessarily bring changes to the Class of Water.

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# From organometallic radicals to complex molecules: structural and mechanistic studies

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**Abstract** The reactivity features of $[CpCr(CO)_3]_2$ (Cp = $\eta^5$-$C_5H_5$) (**2A**) towards (i) homo- and hetero-polyatomic aggregates of the nonmetal elements of Groups 15 and 16, (ii) several classes of organo- P-, S- and N-compounds, and (iii) heterocyclic dithiadiazolyl radicals, are described. The primary products obtained arise from facile cleavage of S–S, P–P and P–S bonds by the 17-electron species $[CpCr(CO)_3]$ (**2**). Further treatment of the product complexes with **2** under thermal activation results in cleavage of C–X (X = N, S), P–S and Cr–E (E = C, N, P, S) bonds, accompanied by C–C and P–P bond formation in some cases, generating new organometallic compounds, belonging to various classes and often incorporating interesting novel structures.

**Keywords** *organotransition-metal chemistry – radical-coupled products*

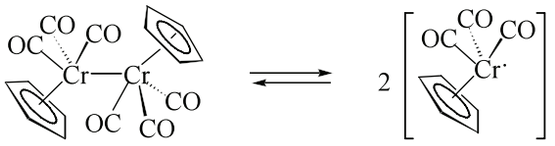
## INTRODUCTION

There is continuing interest in radical species as reactive intermediates in organotransition-metal chemistry [1]. The classical method for generating such radicals is the homolysis of metal-metal bonds under thermal or photochemical activation (eq i).

$$M_2L_{2n} \rightleftharpoons 2ML_n \qquad \text{(i)}$$

$$[Co_2(CN)_{10}]^{6-} \rightleftharpoons 2[Co(CN)_5]^{3-} \qquad \text{(ii)}$$

**1A** **1**



**2A** **2** (iii)

Notable examples are the 17-electron pentacyanocobalt(II)ate species **1** (eq ii) and the cyclopentadienylchromium tricarbonyl monomer $[CpCr(CO)_3]$ (Cp = $\eta^5$-$C_5H_5$) (**2**), readily formed via the facile dissociation of the unusually long Cr–Cr bond (3.281(1) Å) [2] in the dichromium species **2A** (eq iii). This phenomenon has been substantiated by various studies via NMR [3], ESR [4] and electronic spectral [5] and electrochemical [6] techniques.

Reactivity studies of **1A/1** antedated those of **2A/2** by more than two decades. The formation of the first organocobalt complexes was initiated by the facile abstraction of halogen from alkyl halides, as illustrated in eq. (iv) [7]

$$Co(CN)_5^{3-} + RX \longrightarrow R\cdot + XCo(CN)_5^{3-}$$
$$R\cdot + Co(CN)_5^{3-} \longrightarrow RCo(CN)_5^{3-}$$

(R = Me; n-py$CH_2$ (n = 2,3,4)) (iv)

$$CpCr(CO)_3\cdot + RX \longrightarrow R\cdot + CpCr(CO)_3X$$
$$R\cdot + CpCr(CO)_3\cdot \longrightarrow CpCr(CO)_3R$$

(R = Me; $CH_2$ = $CHCH_2$, $PhCH_2$, $CH_2CN$, and others containing α-H's) (v)

Some three decades later, it was established that a similar mechanism operates in the reaction of **2A** with alkyl halides, eq. v [8].)

Early indications of the high reactivity of **2A** came from our observation of a facile ligand substitution with trimethyl phosphite, yielding the derivative complex **3A** (Scheme 1) [9]. The extremely long Cr–Cr bond in **3A**, longer than that in **2A**, renders it highly susceptible to dissociation; the resulting radical species $[CpCr(CO)_2(P(OMe)_3]$ (**3**) is capable of cleaving the O–C bond in the methoxy group, generating a methyl derivative **3a** and a phosphonate complex **3b**.

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## REACTIONS WITH NONMETAL COMPOUNDS

Our subsequent investigations had demonstrated the facile reactivity of **2A** towards nonmetal-nonmetal bonds in the homo- and hetero-polynuclear molecules of the chalcogens (S and Se) and the pnicogens (P, As and Sb) (Chart 1). The cleavage of these bonds yielded organometallic derivatives of nonmetal elements of Groups 15 and 16, shown in Schemes 2 and 3. These results have been reviewed [10].

Clearly, the formation of polynuclear complexes like $[(CpCr(CO)_2]_5P_{10}$ (**5c**), $Cp_4Cr_4(CO)_9P_4X_3$ (X = S, Se) (**7a**) and $[(CpCr(CO)_3]_4(Sb_2S)$ (**8a**) in reactions with $P_4$ [11a,b], $P_4X_3$ (X = S, Se) [11c-e] and polymeric $Sb_2S_3$ [11f], respectively, has involved multiple bond cleavage in the nonmetal polynuclear molecules by **2**, followed by fragment aggregation. In an attempt to probe the generality of such phenomena in the chemistry of **2A**, we have pursued similar investigations with S–S, P–P and S–P bonds in organic substrates shown in Chart 2, including the reactivity of **2A** towards various Cr–E (E = C, N, P, S) bonds in the primary CpCr complexes formed.

## S–S, P–P and P–S BOND CLEAVAGE IN ORGANIC SUBSTRATES

### Reactions with bis(thiophosphinyl)disulfanes (A) and bis(thiophosphoryl)disulfanes (B)

$[CpCr(CO)_3]_2$ (**2A**) reacts readily with the disulfane **A** or **B** yielding $CpCr(CO)_2(S_2PR'_2)$ (R' = Ph, **9a**; R' = O$^i$Pr, **9b**) as the primary products [12-14], via an initial homolytic S–S bond cleavage of the disulfanes by **2,** accompanied by an incumbent coupling reaction (Scheme 4).

Under thermolytic conditions, complex **9a/9b** undergoes degradation via loss of CO ligands and/or

**Scheme 1**

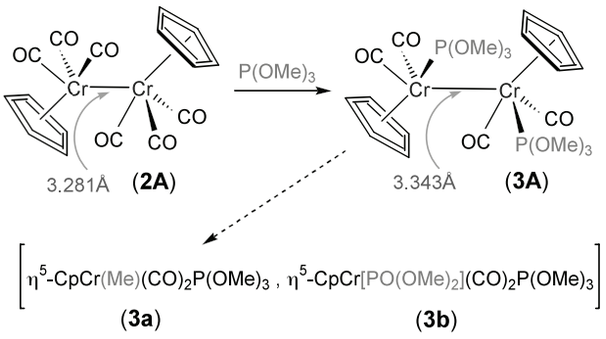
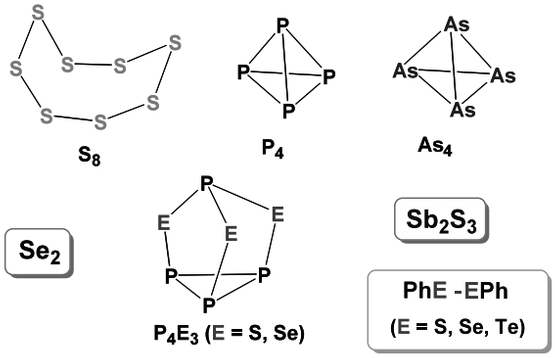
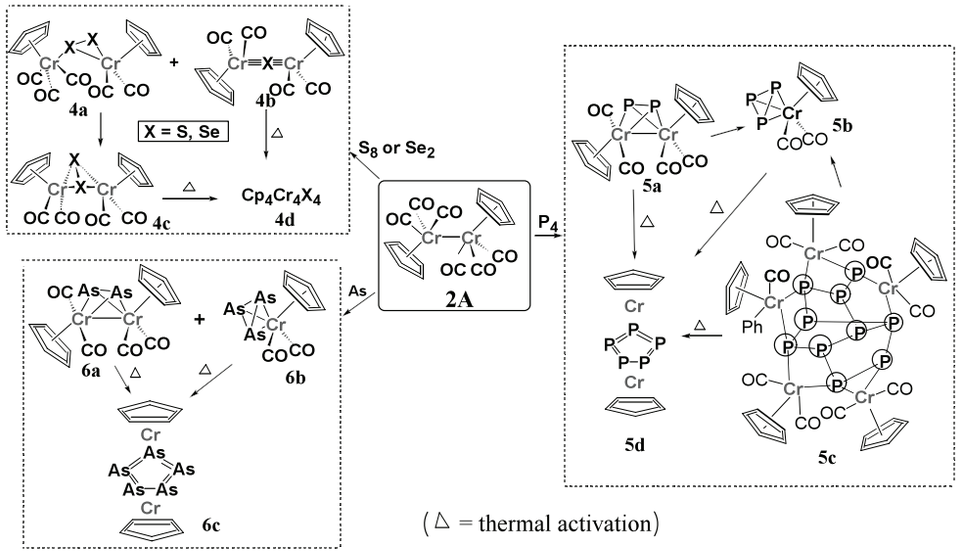



**Chart 1**




**Scheme 2**



**Scheme 3**

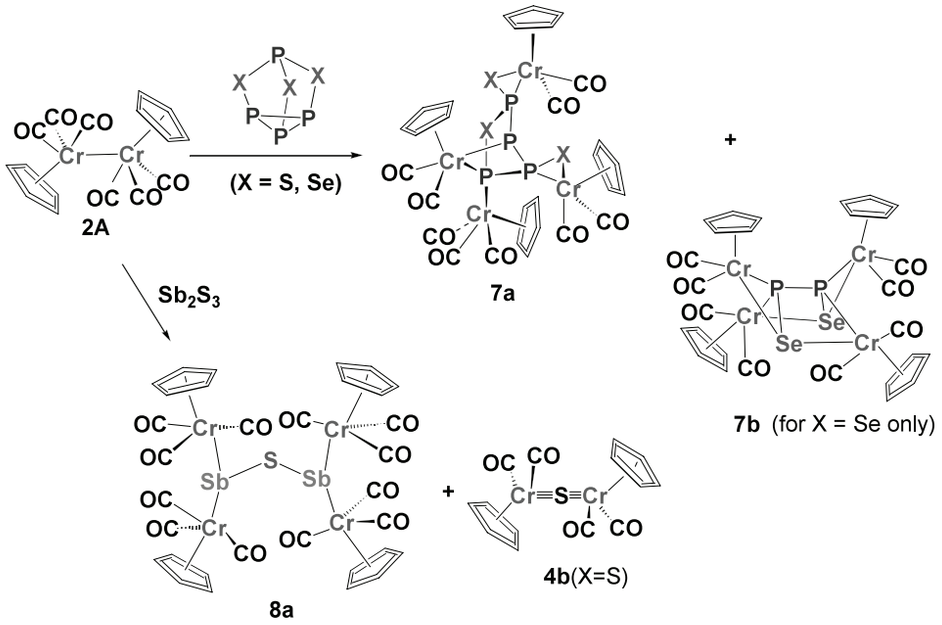
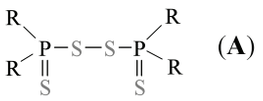
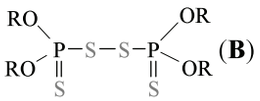


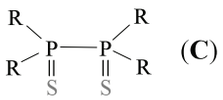

**Chart 2**


(**A**)

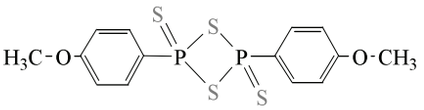
bis(thiophosphinyl)disulfanes


(**B**)

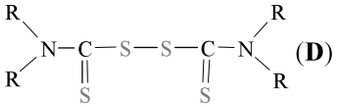
bis(thiophosphoryl)disulfanes


(**C**)

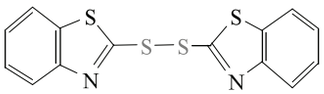
tetraalkyldiphosphine disulfides



Lawesson's Reagent (**LR**)


(**D**)

tetraalkylthiuram disulfides



dibenzothiazolyl disulfide (**E**)

sulfur atoms in the thiophosphinyl/phosphoryl ligands, with concomitant or subsequent intermolecular association to form the thiophosphinito complex $CpCr(CO)_2(SPPh_2)$ (**9c**), $CpCr(S_2PR'_2)_2$ (**9d**), the Cr≡S≡Cr compound $Cp_2Cr_2(CO)_4S$ (**4b**) [10, 15] and the cubane-like complex $Cp_4Cr_4S_4$ (**4d**), the ultimate thermolytic derivative of **4b** [10].

**Reactions with tetraalkyldiphosphine disulfides $R_2P(S)P(S)R_2$ (C)**

As previously found for the cleavage of P–P bonds in $P_4$ by **2A** [10, 11a,b], thermal activation is required for the reaction of **2A** with **C,** which yields the $\eta^2$-thiophosphinito complex $CpCr(CO)_2(SPR_2)$ (R= Me, Et, **10**) (Scheme 5) and desulfurized derivatives, *viz.* the hydrido-phosphido-bridged complexes $Cp_2Cr_2(CO)_4(\mu\text{-H})(\mu\text{-PR}_2)$ (**10a**), the bis(μ-phosphido) doubly metal-metal bonded complex $Cp_2Cr_2(CO)_2(\mu\text{-PR}_2)_2$ (**10b**), and the trinuclear complex $Cp_3Cr_3(CO)_2(S)(\mu\text{-PR}_2)$ (**10c**), together with $Cp_4Cr_4S_4$ (**4d**) as a minor product. These are demonstrated to arise from the thermal interaction of **10** and **2A**

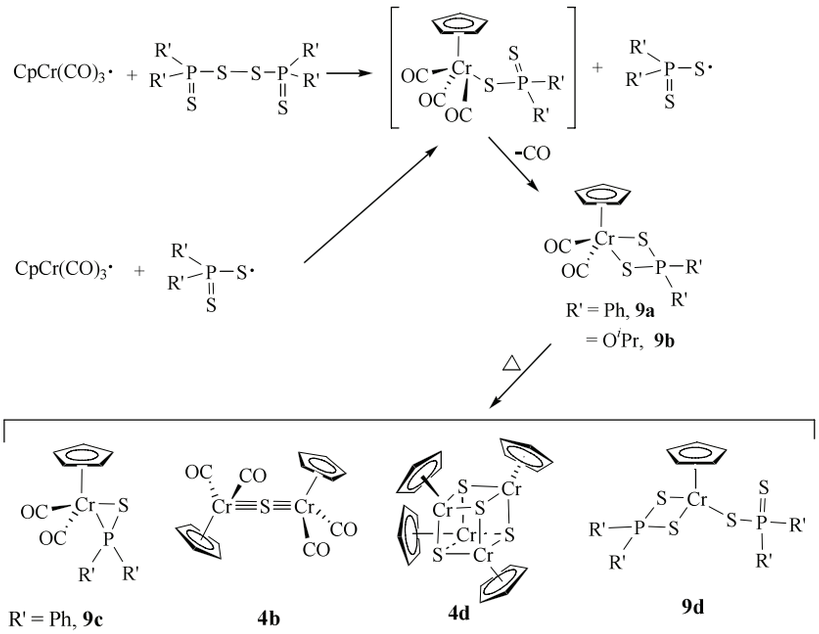
[16]. The structurally characterized complex **10c**, a phosphido-bridged tri-homometal cluster of a Group 6 element, adds to the family of such species of which the butoxide-bridged and nitrene-bridged analogues are known [17].

These results show that desulfurization of a thiophosphinito ligand at a CpCr center as in **10** provides a pathway to $\mu$-phosphido complexes, e. g. **10a – 10c**. These complexes have mainly been prepared from the reaction of metal carbonyls with diphosphanes $R_2PPR_2$ and phenyl phosphines $PPh_2H$ or $PPhH_2$.

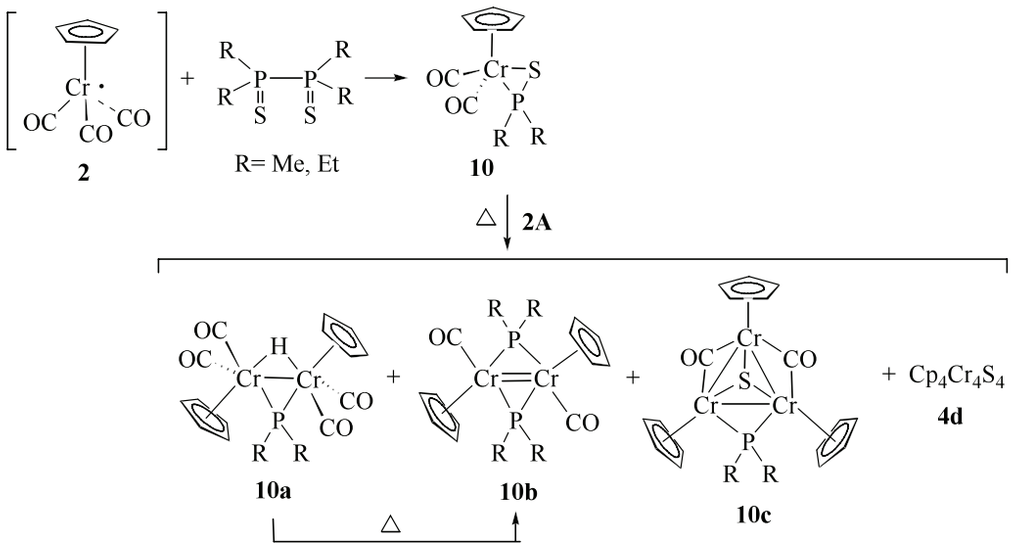
**Reactions with Lawesson's reagent $[CH_3OC_6H_4PS_2]_2$ (LR)**

The four-membered P–S bonded $P_2S_2$ ring with doubly-bonded S substituents on P in the molecule of **LR** presents a class of S- and P-containing substrate, very different from the S–S and P–P bonded systems discussed above. Though an effective thionation

**Scheme 4**




**Scheme 5**



agent towards organic substrates, **LR** has been little studied in transition metal chemistry.

The reaction of **LR** with an equimolar equivalent of **2A** gives products markedly dependent on reaction temperature, as shown in Scheme 6 [18]. Thus the ambient-temperature products are $Cp_2Cr_2(CO)_5(SPAr)$ (**11**), $Cp_2Cr_2(CO)_5(S_2PAr)$ (**12**), $Cp_2Cr_2(S_2P(O)Ar)_2$ (**13**), together with $Cp_2Cr_2(CO)_4S$ (**4b**), of which **11** and **12** are not detected at high temperatures, which result in additional new products, $CpCr(CO)_2(SP(H)Ar)$ (**15**), $[CpCr(CO)_2(SPAr)]_2$ (*cis*-**16**) and its isomer *trans*-**16**.

The molecular structures of **11** and **12** suggest that they both originate from a common intermediate, the radical species **I**, shown in Scheme 7, formed via interaction of **2** and the "monomer" of **LR**, route (i), or direct cleavage of the $P_2S_4$ central unit of **LR** by **2** (route (ii)). Subsequent reactions involving decarbonylation and desulphurization, with or without assistance from **2**, then generates the complexes **11** – **13**, described above.

The temperature-dependent distribution of product species and yields are consistent with bond homolysis 'a', leading to formation of **13** and **4b**, and homolytic dissociations 'b' and 'c', leading to **11**, **15** and **16** (Scheme 8)

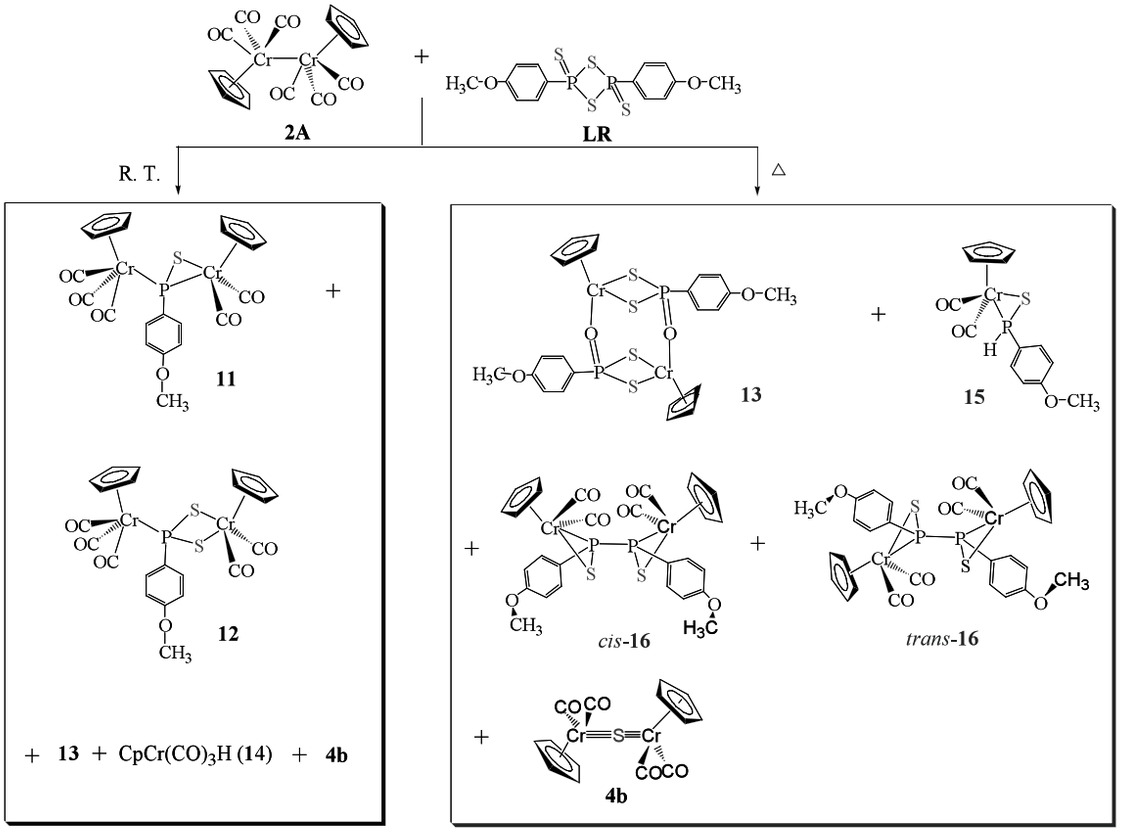
It was demonstrated that complex **16** also arises from Cr–P homolytic bond cleavage in **11**, followed by coupling of the P-centered radical species (**11a**) (Scheme 9).

This proposition is supported by (i) the reversal of the transformation by addition of **2A**, and (ii) the *ambient temperature* facilitation of the process by elemental sulfur or **LR**, which as effective scavengers for **2** drives the reaction towards formation of **16**.

## C–X (X = N, S) BOND CLEAVAGE AND C–C COUPLING

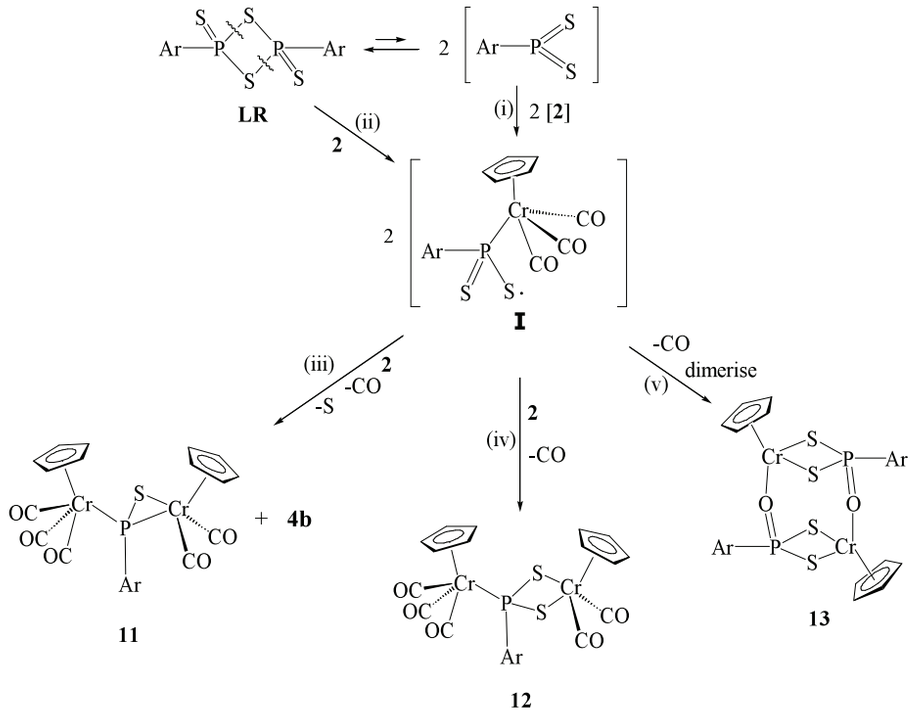
This article so far has shown the effectiveness of **2** in the cleavage of S–S, P–P and P–S bonds. The S- and N- containing organic substrates like thiuram disulfides and benzothiazoles provide situations for a study of the reactivity of **2** towards C–S and C–N bonds in the organic substrate in both its free and coordinated states.

**Scheme 6**



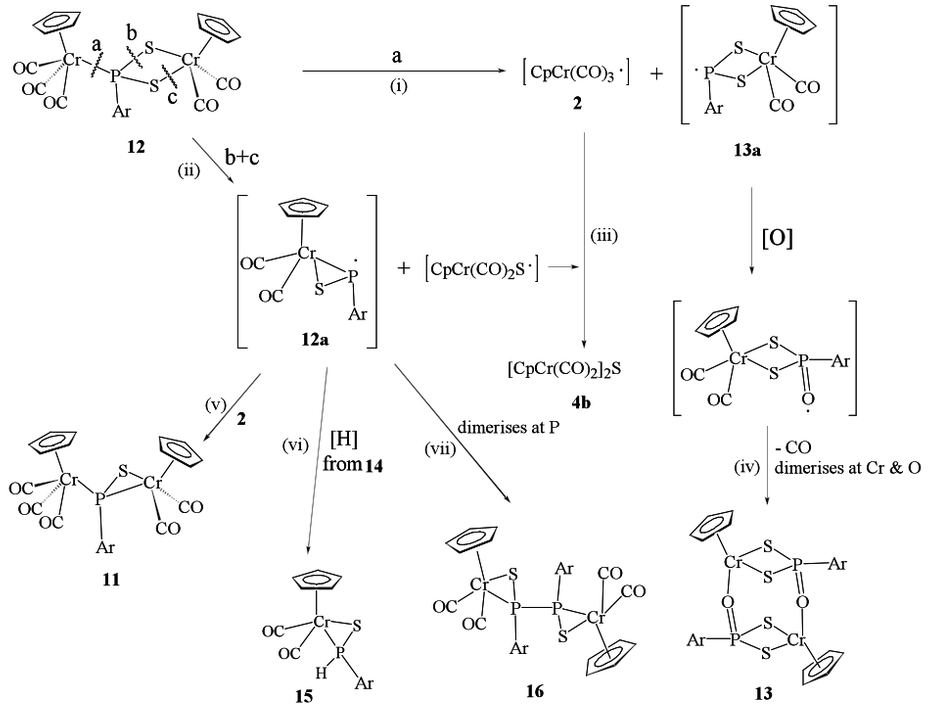
## Scheme 7

[Ar = $C_6H_4OCH_3$, ⌇ = bond cleavage]



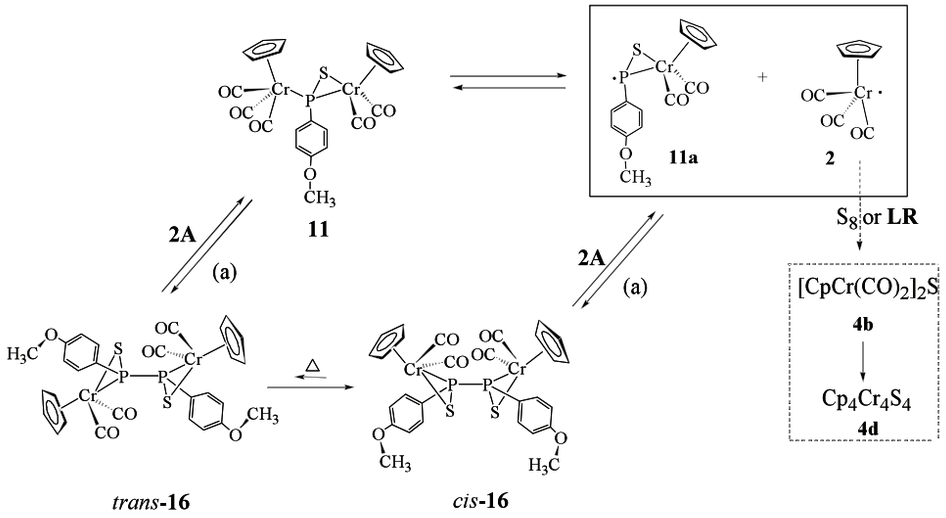

## Scheme 8

[ ⬭ = Cp or Cp*. a, b, c : bond homolysis]



**Scheme 9**

[(a) =△, $S_8$ or **LR**]




**Reaction with tetraalkylthiuram disulfides (D)**

Although the coordination chemistry of dithiocarbamate, $R_2NCS_2^-$, derived from tetraalkylthiuram disulfide (**D**), with both main group and transition metals, is well established, its organometallic chemistry is limited.

The facile reaction of **2** with **D** yields a product mixture, the composition of which is variant with temperature. *At temperatures below ambient*, the usual homolytic reaction of **2** produces the monodentate complex $CpCr(CO)_3(\eta^1\text{-}S_2CNR_2)$ (**17**), which readily decarbonylates at ambient temperature to give $CpCr(CO)_2(\eta^2\text{-}S_2CNR_2)$ (**18**) in high yield (Scheme 10). *At elevated temperatures*, the reaction leads to the isolation of **18** in reduced yield, together with a thiocarbenoid complex $CpCr(CO)_2(\eta^2\text{-}SCNR_2)$ (**19**), a thiocarboxamido dicubane-like cluster $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}SCNR_2)_2$ (**20**), a dithiocarbamate dicubane $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}S_2CNR_2)_2$ (**21**), the coordination compound $Cr(\eta^2\text{-}S_2CNR_2)_3$ (**22**), $Cp_2Cr_2(CO)_4S$ (**4b**) and $Cp_4Cr_4S_4$ (**4d**) [19 a,b]. A similar product composition is obtained from thermolytic degradation of **18** [19b].

In the presence of $[CpCr(CO)_3]_2$ (**2A**), the thermolysis of **18** gives additional products, viz. chromium carbyne complex $CpCr(CO)_2(CNR_2)$ (**23**) and an aminoacyl complex $CpCr(CO)_2(\eta^2\text{-}C,O\text{-}C(O)C(NR_2)CH(NR_2))$ (**24**). (Scheme 11) [19c]. An independent reaction shows that the carbyne complex **23** derives from thermal desulfurization of the thiocarbenoid complex **19** with **2A**.

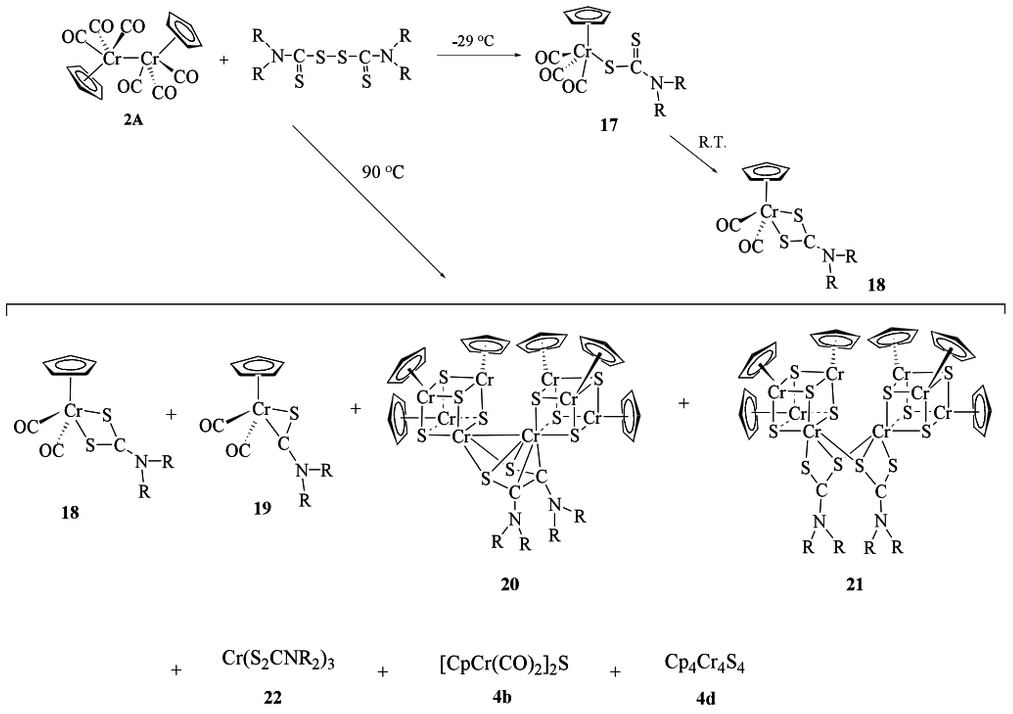
In an unprecedented reaction, complex **2A** "cleaves" the chelate rings in $Cr(S_2CNEt_2)_3$ (**22**) under thermolytic conditions, effecting a transfer of dithiocarbamate ligands to CpCr moieties to give a mixture of complexes **19**, **20**, **23** and **4d** (Scheme 12).

The products profile of these three reactions shows that while the cleavage of one sulfur atom from **18** is a thermally-achievable process, double desulfurization requires assistance from **2**. As in previous cases, the isolation of **4d** in substantial amounts provides evidence for the initial formation of the precursor complex $[CpCr(CO)_2]_2S$ (**4b**), a finding congruent with the observed thiophilicity of **2**. The thermal conversion of **18** to the double cubane-like complexes **20** and **21** containing $(\eta^5\text{-}CpCr)_3CrS_4$, is a new reactivity feature, not observed in the synthesis of the Mo or W analogues of **17** under thermal and photochemical conditions, respectively.

The significant feature of **20** is the presence of a dithiooxamide ligand (DTO = $Et_2NC(S)=C(S)NEt_2$), which links the two cubane-like cores with $\eta^2,\eta^4$ bonding mode, in addition to a weak M–M bond (3.101 Å) between the two 'cubanes'. The formation of the DTO ligand has involved a single C–S bond cleavage of each of two DTC ligands with C–C coupling of the resulting moieties. The cuboidal units in **21** are doubly bridged by two dithiocarbamate ligands, each bonding in a $\eta^1$-S,$\eta^2$-S,S' coordination mode, and do not involve any M–M bonding. In both these double

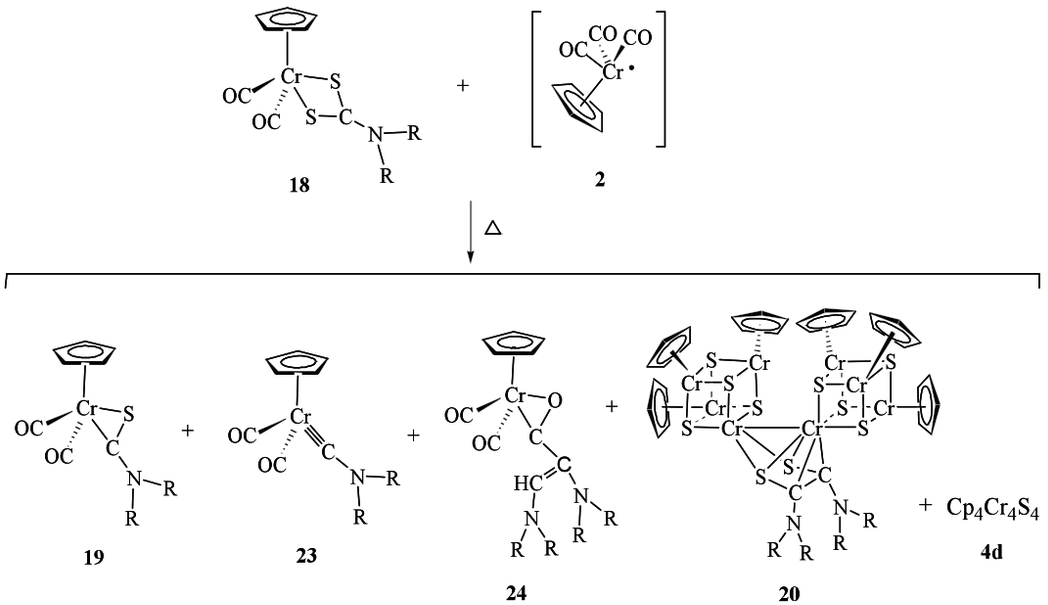
## Scheme 10

[ R= Me, Et or $^{i}$Pr.
the six Cr-Cr bonds in each of the cuboidal cores of **20** and **21**are omitted for clarity]



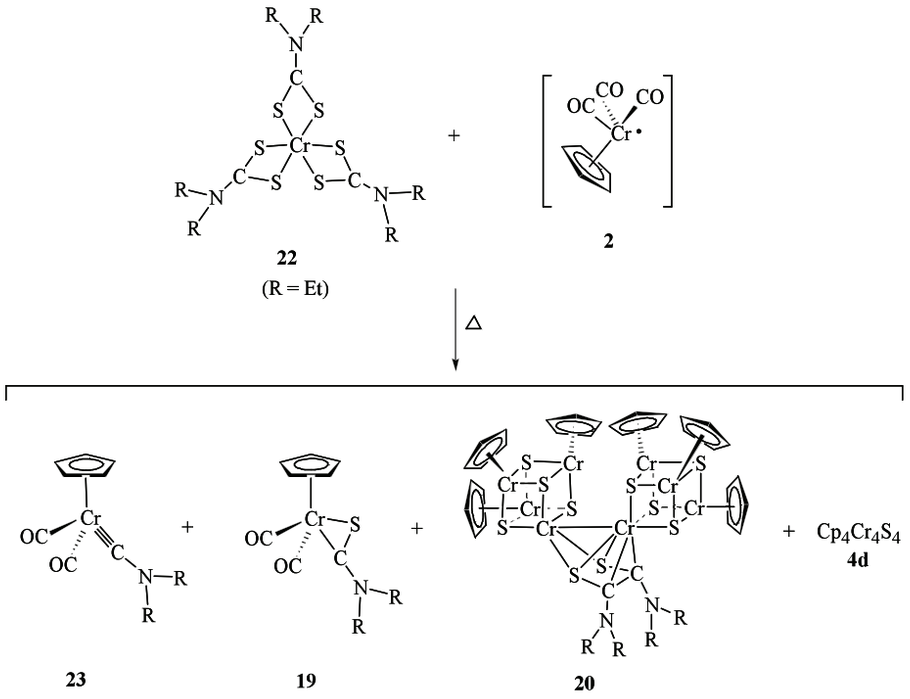

## Scheme 11

[R = Et.
The Cr-Cr bonds in the cuboidal core of **20** are omitted for clarity]



**Scheme 12**

[R = Et
The Cr-Cr bonds in the cuboidal core of **20** are omitted for clarity]




cubane-like molecules, dissociation of a $\eta^5$-Cp ligand has occurred at one Cr corner to accommodate the bridging ligands. Such Cp ligand dissociation seems to be facile in these CpCr systems, though rarely observed in Cp-metal chemistry.

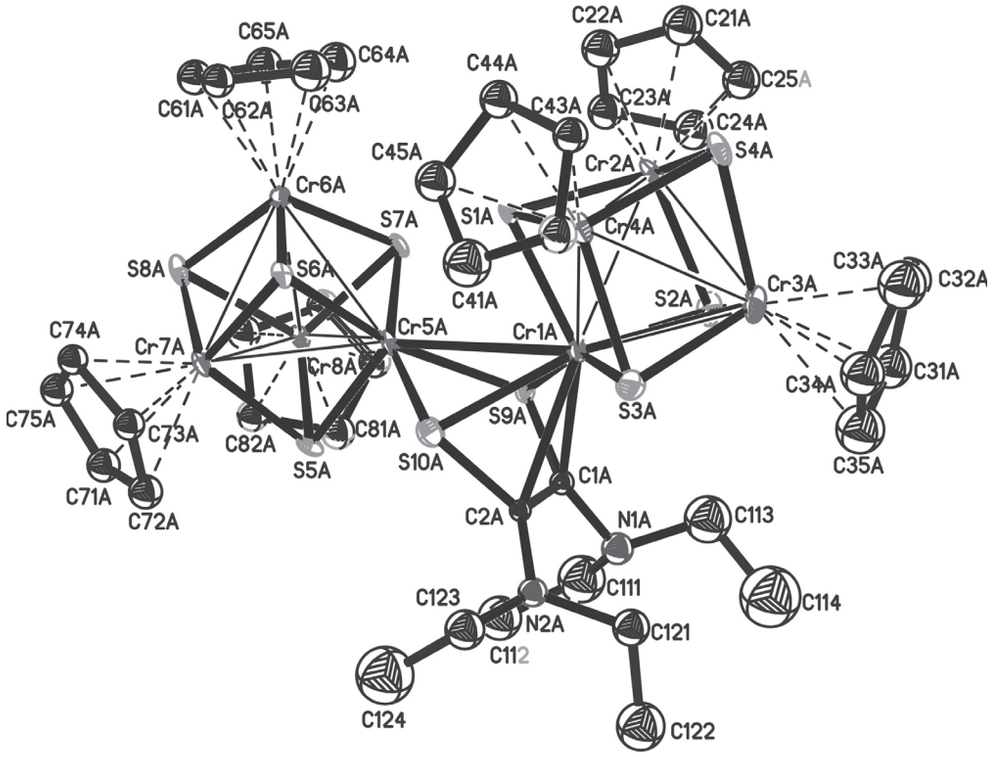
The complex **23** belongs to the rare group of aminocarbyne chromium complexes, the first example of which was isolated by Filippou and coworkers from a multiple-step synthesis from $Cr(CO)_6$ [20]. The intermediate formation of a carbenoid species $R_2NC$**:** is indicated by the presence of alkene and alkenyl acyl moieties in the structural composition of **20** and **24**, respectively, suggestive of carbene dimerisation as found in the formation of the DTO ligand discussed above. The carbyne moiety $R_2NC\equiv$ is evident in the structure of **23.**

The profile of the product composition shows that with respect to sulfur cleavage, the reaction pathways fall into three categories, in which (i) the dithiocarbamate (DTC) ligand remains intact, as in the DTC bridged cubane **21** and the coordination compound $Cr^{III}(DTC)_3$ **22**, (ii) the DTC ligand has undergone mono-sulfur cleavage, as found in the thiocarbenoid complex **19** and the dithiooxamide di-cuboidal compound **20**, and (iii) the DTC ligand has suffered double sulfur cleavage, as found in the Cr-aminocarbyne complex **23**, and the alkenylacyl compound **24,** respectively.

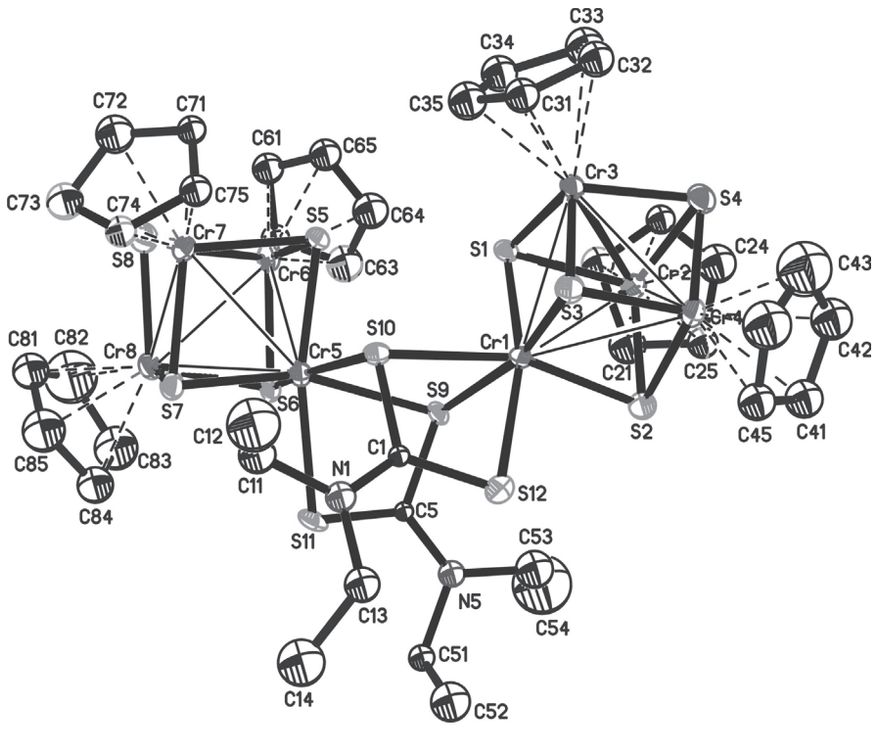
**Reaction with 2,2'-dibenzothiazolyl disulfide (E)**

The attractive features of thiazolyl disulfides for this study are the presence of a homolytically-cleavable S–S bond and a heterocyclic component often encountered in many bioactive molecules [21]. Since mechanisms of ring-opening and closure of heterocycles in biomolecules are of current active interest [21c], our intention was to examine the role of **2/2A** in probable ring-cleavage reactions in a thiazole ligated to CpCr.

The instantaneous reaction of **2A** with one mol equivalent of **E** at ambient temperature gives $CpCr(CO)_2(SCSN(C_6H_4))$ (**25**) in high yield. The further reaction of **25** with **2A** under thermolytic conditions produces $[Cp_2Cr_2(CO)_2(=CNS(C_6H_4))]_2$ (**26**), $Cp_5Cr_6S_4(SN(C_6H_4))(SNC_2(C_6H_4))$ (**27**), $Cp_6Cr_8S_4(OH)(SN(C_6H_4))_2(SNC_2(C_6H_4))_2$ (**28**), 2,2'-bibenzothiazole $(C_6H_4NSC)_2$ (**29**) and $Cp_4Cr_4S_4$ (**4d**) (Scheme 13) [22]. In contrast, in the absence of **2A**, **25** and **27** are thermolysed to non-characterisable compounds, while **26** remains unchanged.

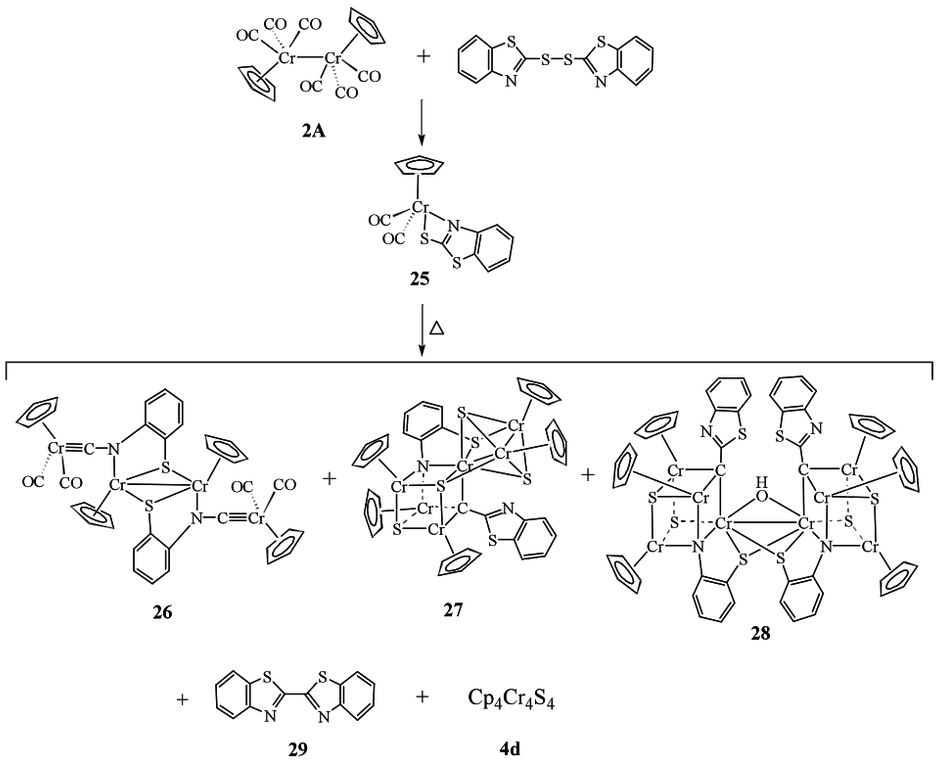



Molecular structure of $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}SCNEt_2)_2$ (**20**)
[Cr1A–Cr5A = 3.101 Å]

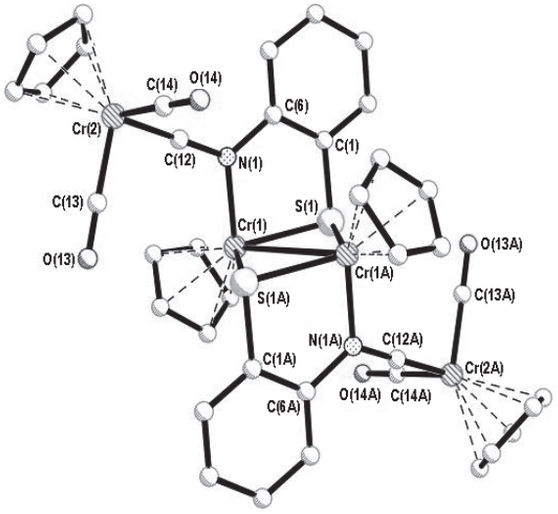



Molecular structure of $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}S_2CNEt_2)_2$ (**21**)
[Cr(1) … Cr(5) = 3.853(7) Å]

**Scheme 13**




The molecular structure of **26** possesses a crystallographic center of symmetry at the midpoint of the Cr(1)–Cr(1A) bond. A salient feature is the chair configuration in the central portion of the molecule with the planar four-membered $Cr_2S_2$ ring forming the "seat", wherein lies the Cr–Cr bond.



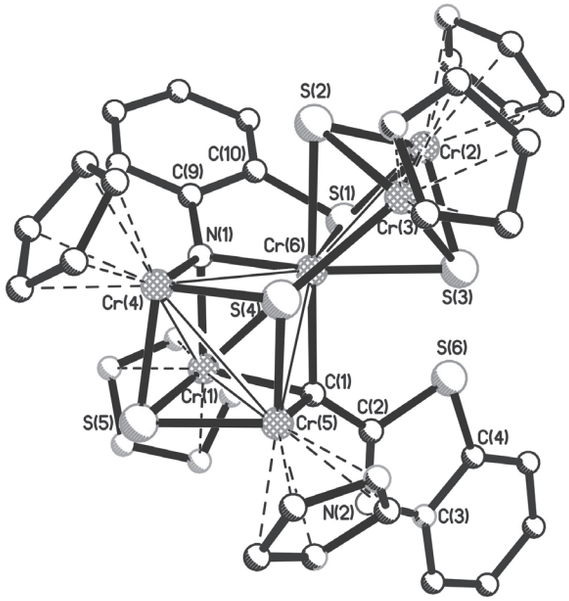

Molecular structure of $[Cp_2Cr_2(CO)_2(=CNS(C_6H_4))]_2$ (**26**) [Cr(1)–Cr(1A) = 3.070(1) Å]

A significant feature in **27** is the $Cr_4S_2CN$ cube, wherein three of the Cr corners are still attached to $\eta^5$-Cp rings, while the fourth corner (Cr(6)) is capped by a dichromium-trisulfur moiety, Cr(2) Cr(3)S(1)S(2)S(3) where S(1) is a component of the benzothiolatonitrido unit, which thus edge-bridges Cr(6) and the N(1) corner of the cube. The $\mu_4$-bonding S(4) is linked to Cr(3), Cr(4), Cr(5) and Cr(6). The carbido C(1) corner of the cube is singly-bonded to C(2), a component atom of a benzothiazole unit.

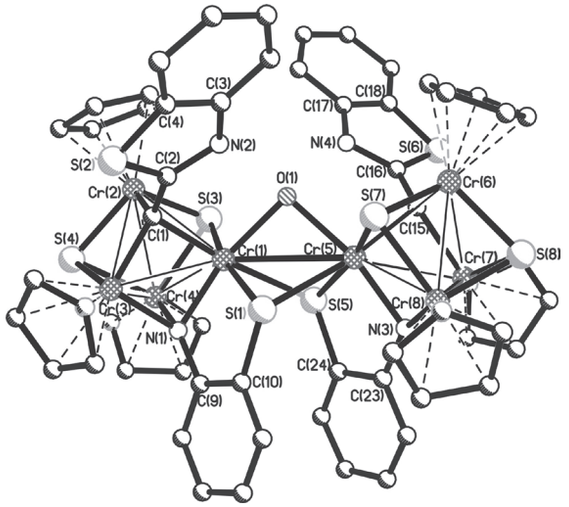
The molecular structure of **28** consists of double cubane moieties ($Cr_4S_2CN$), quadruply bridged by a weak Cr–Cr bond, a hydroxo ligand and the thiolato sulphur atoms of two benzothiolatonitrido units, the N atom of each of which constitutes one of the corners of each cube. The C atom in the cube is linked to a benzothiazole unit. The nature of the components of the cubes and of the bridge of this double 'cubane' has no precedent among the numerous cubane and double-cubane compounds, which have been extensively studied by Holm, Coucouvanis and Sykes [23].

The structural composition of **26**–**29** supports their formation from radical moieties, either discrete or quasi-associated, arising from the sequential

cleavage by **2** of C–S (steps (i) and (iv)), Cr–S (step (ii)), Cr–N (step (iii)) and C–N (step (v)) bonds in **25**, as proposed in Scheme 14.



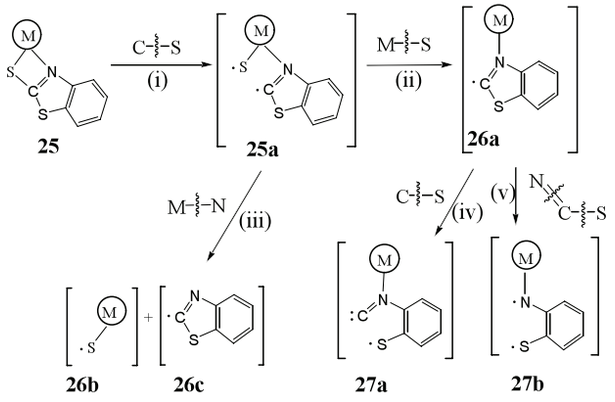

Molecular structure of $Cp_5Cr_6S_4(SN(C_6H_4\ (SNC_2–(C_6H_4))$ (**27**) [In the cube, Cr–Cr = 2.7065(6)–2.8972(7) Å. Others Cr(2)–Cr(3) = 2.9137(7), Cr(6)–Cr(2) = 2.8264(7) and Cr(6)–Cr(3) = 2.7933(7) Å]




Molecular structure of $Cp_6Cr_8S_4(OH)$-$(SN(C_6H_4))_2$ $(SNC_2(C_6H_4))_2$ (**28**) [Cr–Cr = 3.079(1), 3.087(1) Å in the two independent molecules in the unit cell In the cube, Cr–Cr = 2.6611(17) – 2.8211(18) Å]

**Scheme 14**

[ Ⓜ = $CpCr(CO)_2$. ⦚ = bond cleavage ]



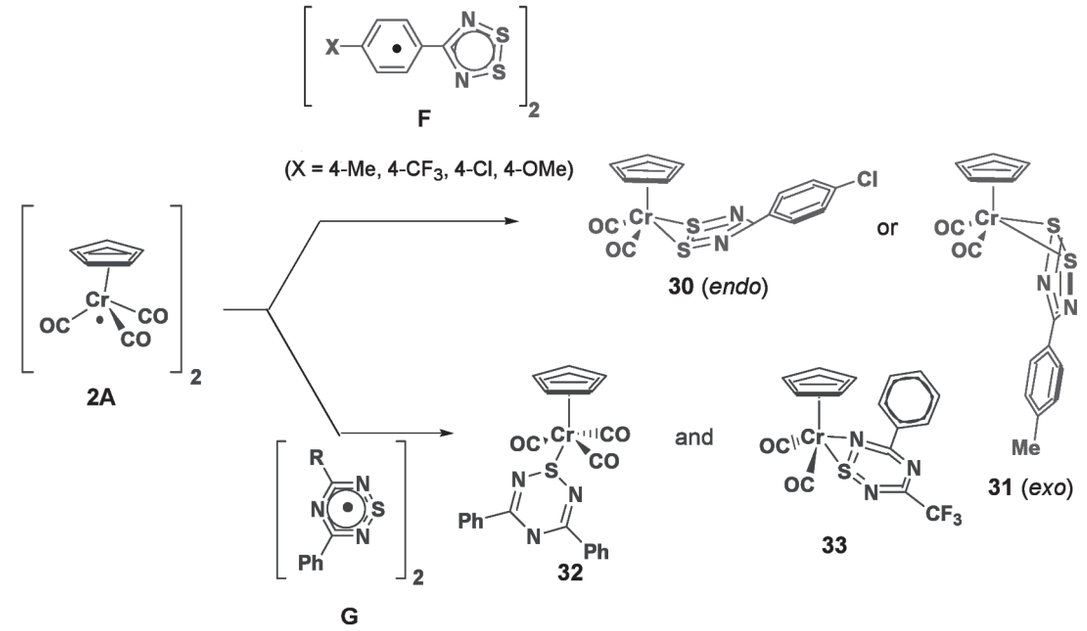

## REACTIONS WITH MAIN GROUP HETEROCYCLIC RADICALS (F and G)

The interaction between **2A** and dithiadiazolyl dimers $[S_2N_2CNR]_2$ (R = substituted aryl rings) has resulted in the isolation of a series of the first π organometallic complexes of C,N,S-heterocyclic compounds. Thus, the coupling of **2** and heterocyclic dithiadiazolyl radical **F** led to the formation of unique diamagnetic *endo* (**30**) and *exo* (**31**) isomers, in which the C,N,S rings are $\pi$:$\eta^2$-S,S'–coordinated to the metal. (Scheme 15). These isomers inter-convert in solution and remain redox-active through ligand-centered reductions.[24a,c] A similar reaction with **G** gave the complexes **32** and **33**, in which heterocyclic ring is S-bonded to Cr and $\eta^2$-N,S–coordinated, respectively.[24b] This study is particularly significant for providing the first examples of π-complexes of any thiazyl heterocycles and opens up a largely unexplored coordination chemistry of unsaturated C-N-S heterocyclic free radicals with paramagnetic organometallic species.

## SUMMARY

The 17-electron organometallic radical $[CpCr(CO)_3]$ (**2**) displays a remarkable capability in the scission of S–S, P–P and P–S bonds in organic substrates, forming radical-coupled products containing cyclopentadienyl chromium. By virtue of its high reactivity as a radical species and an avid thiophile, **2**

**Scheme 15**




further effects efficient cleavage of C–N, C–S, P–S, Cr–E (E = C, N, P and S) bonds in the CpCr complexes, generating radical species, which aggregate to yield a variety of new compounds, incorporating C–C and P–P bond formation in some cases. These findings suggest that fruitful results may be obtained from further investigations of the reactivity of **2**/**2A** towards radical or radical-like species from main group or transition metal compounds, particularly those containing sulfur ligands.

**Acknowledgements** – This work was supported by the University of Malaya and the National University of Singapore. The author acknowledges the intellectual and experimental contributions of her co-workers and students, whose names are cited in the references.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 87-89

# Malaysian Butterfly Lizard *Leiolepis triploida* (Reptilia, Squamata: Leiolepidae) in Clearwater Sanctuary, Perak: geographical range extension in Peninsular Malaysia

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*Received 22-09-2011; accepted 29-09-2011*

**Abstract** The Malaysian Butterfly Lizard *Leiolepis triploida* is known from the inland areas of Perlis, Kedah and Seberang Perai (Penang) in the northwestern part of Peninsular Malaysia. The present finding of this butterfly lizard in Clearwater Sanctuary, Batu Gajah, Perak Darul Ridzuan has extended its known geographical range further south in Peninsular Malaysia. It remains to be established how far south it would spread, how widespread it is in Peninsular Malaysia, and whether it would displace the existing populations of the Common Butterfly Lizard *Leiolepis belliana*.

**Keywords** Triploid Butterfly Lizard – geographical range extension – parthenogenetic *Leiolepis* – Malaysia – tin mining area

## INTRODUCTION

The lizard fauna of Peninsular Malaysia consists of some 128 species in eight families – Agamidae, 7 genera 28 species; Dibamidae, 1 genus 2 species; Eublepharidae, 1 genus 1 species; Gekkonidae, 9 genera 52 species; Lacertidae, 1 genus 1 species; Leiolepidae, 1 genus 2 species; Scincidae, 5 genera 38 species; and Varanidae, 1 genus 4 species [1].

There are two species belonging to the family Leiolepidae in Malaysia – *Leiolepis belliana* (Hardwicken& Gray) and *Leiolepis triploida* Peters [1]. They are commonly known as butterfly lizards. At present, the Common Butterfly Lizard *L. belliana* occurs on both the east and west coasts of Peninsular Malaysia – on the east coast from Tumpat, Kelantan south to Mersing, Johor; and on the west coast from Dinding, Perak south to Melaka as well as the offshore islands Langkawi, Kedah and Pulau Besar, Melaka [1]. On the other hand, the Malaysian Butterfly Lizard *L. triploida* is confined to the inland areas of Perlis, Kedah and Seberang Perai, Penang [1, 2].

We report here the finding of the Malaysian Butterfly Lizard *L. triploida* in Clearwater Sancturay, Batu Gajah, Perak Darul Ridzuan, thus extending its known geographical range further south in Peninsular Malaysia.

## MATERIALS AND METHODS

The observation was done on a sunny day in Clearwater Sanctuary, Batu Gajah, Perak Darul Ridzuan, Peninsular Malaysia, some 20 km from Ipoh (Fig. 1). This location is a former tin mining area, now a nature resort with a golf course. The lizard (Fig. 2) was photographed in the field and identified using existing literature [1, 3]. No specimen was collected from the site of its occurrence.

## RESULTS AND DISCUSSION

The butterfly lizards (Leiolepidae) are represented by at least eight species, comprising four sexual and four asexual species [4]. The sexual species are: *Leiolepis belliana* (Hardwicke & Gray, 1827) – Common Butterfly Lizard, Bell's Butterfly Lizard; *Leiolepis guttata* (Cuvier, 1829) – Giant Butterfly Lizard, Spotted Butterfly Lizard; *Leiolepis peguensis*


**Figure 1**. Location of Batu Gajah, Perak Darul Ridzuan, Peninsular Malaysia.

Peters, 1971 – Burmese Butterfly Lizard, Pegu Butterfly Lizard; and *Leiolepis reevesii* (Gray, 1831) – Chinese Butterfly Lizard, Reeves' Butterfly Lizard. The asexual or parthenogenetic species are: *Leiolepis boehmei* Darevsky & Kupriyanova, 1993 – Böhme's Butterfly Lizard; *Leiolepis guentherpetersi* Darevsky & Kupriyanova, 1993 – Peters' Butterfly Lizard; *Leiolepis ngovantrii* Grismer & Grismer, 2010 – Ngo Van Tri's Lady Butterfly Lizard; and *Leiolepis triploida* Peters, 1971 – Thai Butterfly Lizard, Malaysian Butterfly Lizard, Triploid Butterfly Lizard.

Butterfly lizards are characterized by the possession of forelimbs, eyelids and round pupils; with anterior portion of the tail relatively wide and dorsoventrally compressed; and the dorsal scales of the body, limbs and tail very small, smooth and granular [1]. Of the two species present in Peninsular Malaysia, *L. triploida* is easily distinguished from *L. belliana* by the colour pattern on the flanks – with thin, yellowish to cream coloured bars in *L. triploida*, but with broad vertical orange and black bars in *L. belliana* [1, 3]. Phylogenetic inference based on 700 base pairs of the mitochondrial ND2 region indicates *L. boehmei* as the maternal ancestor of *L. triploida* [4] – *L. boehmei* is restricted to southern Thailand [1].

**Figure 2**. The parthenogenetic Malaysian Butterfly Lizard *Leiolepis triploida* at Clearwater Sanctuary, Batu Gajah, Perak Darul Ridzuan, Peninsular Malaysia. (photo: H. S. Yong)

During a recent on-going survey (September 2011) of insect fauna at the Clearwater Sanctuary, a single specimen of *L. triploida* was encountered in the early afternoon on the ground near the periphery of the golf course. When approached, the lizard ran away rather quickly and vanished into the bush nearby. It was however captured in pictures (Fig. 2) before it dashed away and disappeared completely from view. Two burrows, separated some distance from each other, were present not far away along the path.

Adult *L. triploida* may reach a snout-vent length of 148 mm [1, 3]. It was first described and named in 1971 [5], with the type locality as 'Malaysia-Thailand border of the Malay Peninsula'. Being parthenogenetic it is represented by females only; males do not exist in such asexual organisms. It inhabits disturbed, open areas with loose soil. It has been reported to be most common in agricultural areas throughout eastern Perlis and Kedah, especially in rubber plantations but also in oil palm plantations, paddy fields, orchards and abandoned mining areas [1]. Based on historical records for the occurrence of *L. belliana* in Kedah but is no longer present, it has been suggested that *L. triploida* has outcompeted and replaced *L. belliana* due to the conversion of forests into plantations [6].

Extension of georaphical range has also been recorded for the Tawny Coster *Acraea terpsicore* (Linnaeus, 1758), synonym *Acraea violae* (Fabricius, 1793), in Clearwater Sanctuary in 2000 (H. S. Yong, unpublished data). This nymphalid butterfly originated in India (and Sri Lanka) but spread through Myanmar and Thailand into Peninsular Malaysia. It is now established in Kuala Lumpur and other southern parts of Peninsular Malaysia.

Another instance of seemingly 'extension of geographical range', among others in Peninsular Malaysia, is the Forest Crested Lizard *Calotes emma* Gray, 1845. Earlier studies documented its occurrence in Peninsular Malaysia only in the northern states of Kedah and Perak [7], and "remains west of the Banjaran Titiwangsa" [1]. It has more recently been recorded in the east coast state of Kelantan [8].

The present finding of *L. triploida* in Perak has extended its known geographical range further south in Peninsular Malaysia. Studies are needed to determine how far south it has spread, how widespread it is, and whether it would displace the known populations of *L. belliana* on the west coast of Peninsular Malaysia.

**Acknowledgements** – This study received support from University of Malaya (Special Research Fund H-00000 5620009) and the Clearwater Sanctuary Golf Resort.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 105-108

# Microcantilever release process using micromachining technology

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*Received 14-02-2011; accepted 24-03-2011*

**Abstract** An isotropic deep reactive ion etching (DRIE) technique was used to release a suspended $SiO_2$ microcantilever from the substrate of SOI wafer using bulk micromachining technology. The dimension of the fabricated $SiO_2$microcantilever was 250 µm in length, 100 µm in width with thickness of 1 µm. Employing the plasma dry etching technique, the release of $SiO_2$ microcantilever from the frontside of the wafer was done using Plasmalab System 100.The etching parameters such as $SF_6$ flow, ICP power, RF power, temperature and $O_2$ flow were properly specified in order to obtain anisotropic condition with significant lateral etch rate for the microcantilever release. For optimum etching condition at maximum lateral etch rate, the chamber pressure was varied in the range of 10 to 30 mTorr. The optimum etching condition was realized at maximum chamber pressure of 30 mTorr which yielded lateral etch rate of 5 µm/min and vertical etch rate of 8 µm/min. In conclusion, by using an isotropic dry etching technique utilized from micromachining technology, a microcantilever release from the substrate of SOI wafer was successfully realized.

**Keywords** microcantilever release – MEMS – plasma isotropic etching – SOI wafer – DRIE system

## INTRODUCTION

The advances in MEMS devices development have been triggered by the improvement in fabrication techniques of micromachining technology. Among the MEMS devices that have been successfully fabricated are microneedles, microvalve, microtransformer and micropump [1-4]. The most critical process in the fabrication of microcantilever is releasing the suspended beam. Many fabrication techniques from the micromachining technology have been employed by researchers in obtaining the suspended microcantilever including dry and wet etching. Wet etching process such as KOH etching was commonly employed to release the cantilever structure from the substrate wafer by etching the backside of the wafer. However, this method always comes with additional preventive methods in avoiding the stiction problem encountered during the release and drying steps [5,6]. Because of these reasons, dry etching technique is preferable due to the fact that it offers simpler etching process sequences and excellent etching profile.

Deep reactive ion etching (DRIE) which falls under the dry etching technique is considered as an extension of RIE. It relies on the same etching mechanisms of ion bombardment and chemical etching as RIE. Compare to RIE, the DRIE enables the fabrication of deeper and narrower structures at higher etch rate. DRIE reactors are equipped with two power sources which are inductively coupled plasma (ICP) source and radio frequency (RF) source which employ sidewall passivation for process anisotropy. Recent microfabrication technology introduces anisotropic dry plasma etching technique from DRIE system for suspended microcantilever release from the backside of the wafer which offers higher etch rate, compatible with traditional IC processing and most importantly, higher Si: $SiO_2$ selectivity in releasing $SiO_2$ microcantilever beams from bulk silicon [7]. However, the removal of about 300-500 µm thick sacrificial silicon from the backside of the wafer in anisotropic dry etching can weaken the device structure. Apart from anisotropic etching of DRIE system, the isotropic etching process can also be conducted using the system. The isotropic profile of DRIE system has been utilized in the fabrication of thermal microbridge [8]. Similar studies of isotropic dry etching employing inductive coupled plasma (ICP) system have been reported [9]. However, the undercut rate and etch rate are difficult to control

because of the simplicity of the gas phase etching process in the ICP system [9].

In this paper, an isotropic dry plasma etching to release the suspended $SiO_2$microcantilever from the substrate of SOI wafer is developed. Employing the DRIE system, the frontside etching for the $SiO_2$ microcantilever release was done using Plasmalab System 100. The etching parameters such as $SF_6$ flow, ICP power, RF power, temperature and $O_2$ flow were properly specified in order to obtain anisotropic condition with significant lateral etch rate for the microcantilever release. For optimum etching in the microcantilever release, the chamber pressure was varied in the range of 10 to 30 mTorr. The effect of varying the chamber pressure on the lateral etch rate of the isotropic etching process was studied. This study also investigated the stability of photoresist and aluminum as etch masks in isotropic etching process.

## MATERIALS AND METHODS

SOI wafer was used in this study for its outstanding advantages in terms of process simplicity and uniform doping profile. The wafer consisted of about 300 µm thick susbstrate silicon, 1 µm thick buried oxide (BOX) layer and 2 µm thick silicon device layer. The BOX layer was utilized as the $SiO_2$ microcantilever beam while the silicon device layer was reserved as a piezoresistive layer that could be used for piezoresistive sensing in MEMS microcantilever sensor applications. The utilization of SOI wafer in microcantilever release process involved bulk micromachining technology where the suspended structure was realized by sequences of etching processes. The dimension of the fabricated $SiO_2$ microcantilever was 250 µm in length, 100 µm in width with thickness of 1 µm.

First, the investigation on the possibility of using photoresist as an etch mask in isotropic dry etching process was carried out. The etch mask was used to protect some parts of wafer while the unprotected parts were removed during etching process. The etch mask should be stable under the etching conditions. For this purpose, two types of etch masks, photoresist and aluminum were used for comparison. For photoresist mask, resist of AZ 4620 of about 7 µm thick was used. For aluminum mask, an aluminum layer of about 1.5 µm thick which was deposited using metal evaporation technique was used as the etch mask. The mask patterns were then transferred onto the wafer using optical lithography process. Using both mask types, isotropic dry etching was conducted on the patterned wafer for 20 min to observe the stability of both etch mask types.

The anisotropic DRIE process relies on inductively coupled $SF_6/O_2$ plasma at temperatures below -100ºC. The anisotropy of the etching process is enhanced by a thin passivation layer on sidewalls that prevents lateral etching. The thickness of the passivation layer can be adjusted by changing the process temperature and the $O_2$ flow. At higher temperatures and without $O_2$ flow, the passivation layer is not formed which results in isotropic etching profile. Therefore, for the isotropic etching using the DRIE system, the etching temperature was set at 20ºC with $SF_6$ flow only, no $O_2$ flow.The suitable etching parameters for the isotropic etching condition were specified as 70 sccm $SF_6$ flow, 2000 W ICP power, 3 W RF power, 20ºC etching temperature and 0 sccm $O_2$ flow. The chamber pressure was varied in the range of 10 to 30 mTorr to observe the effect of the chamber pressure on the lateral etch rate of the etching process.

## RESULTS AND DISCUSSION

The observation on the stability of photoresist as an etch mask showed that the resist could not sustain the plasma reactions in the isotropic etching condition. After about 20 min of isotropic etching under lateral etch rate of 5 µm/min, the photoresist etch mask had disappeared indicating that the photoresist was slowly consumed during the etching process (Fig. 1). After certain etch time, the photoresist had vanished and the silicon structure which was previously covered under the photoresist was exposed. The exposed structure was also etched away during the subsequent etching process (Fig. 1).

Consequently, the aluminum etch mask was found very stable under the isotropic etching condition (Fig. 2). The etch mask protected the microcantilever structure very well and could sustain the ion bombardment reaction throughout the etching process as it had better mechanical properties and selectivity than photoresist.

The observation on the effects of the chamber pressure showed that higher pressure resulted in higher etch rate (Table 1). For example, 10 mTorr chamber pressure yielded 2.46 µm/min lateral

etch rate while 30 mTorr pressure yielded higher lateral etch rate of 5.04 µm/min. The high process pressure contributed to an increment of the angular distribution of ions which indirectly increased the lateral etch rate of the etching process.

Employing the specified etching parameters of the isotropic etching, the $SiO_2$ suspended microcantilever release from SOI wafer was realized. Fig. 3 shows the lateral and vertical etching profile of the isotropic etching of partially released microcantilever while Fig. 4 shows an SEM image of the suspended microcantilever which had been completely released using the isotropic etching.

## CONCLUSION

By utilizing the isotropic profile of plasma dry etching from the DRIE system, the microcantilever release from the frontside of the SOI wafer has been successfully realized. The optimum etching condition of 0.63 isotropic ratio was obtained at 70 sccm $SF_6$ flow, 2000 W ICP power, 3 W RF power, 20ºC etching temperature and 0 sccm $O_2$ flow at maximum pressure chamber of 30 mTorr.

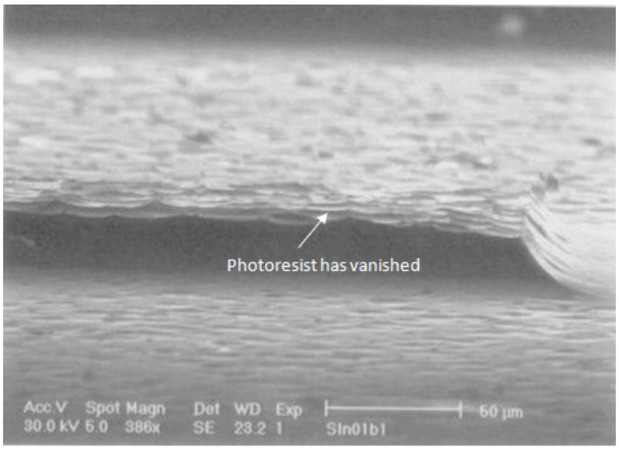


**Figure 1**. SEM image of microcantilever structure using photoresist etch mask after 20 min of isotropic dry etching.

**Table 1.** Etching parameters for microcantilever release.

| Parameter | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| $SF_6$ flow (sccm) | 70 | 70 | 70 |
| $O_2$ flow (sccm) | - | - | - |
| Pressure (mTorr) | 10 | 20 | 30 |
| ICP (W) | 2000 | 2000 | 2000 |
| RF (W) | 3 | 3 | 3 |
| Temperature (ºC) | 20 | 20 | 20 |
| Lateral etch rate (µm/min) | 2.46 | 2.84 | 5.04 |
| Vertical etch rate (µm/min) | 4.54 | 4.88 | 7.96 |
| Isotropic ratio | 0.54 | 0.58 | 0.63 |

**Acknowledgment** – The authors would like to thank the Malaysian Ministry of Science, Technology and Innovation under IRPA project (03-02-02-0015-SR003/07-01): MEMS Devices and Sensing Microstructure.

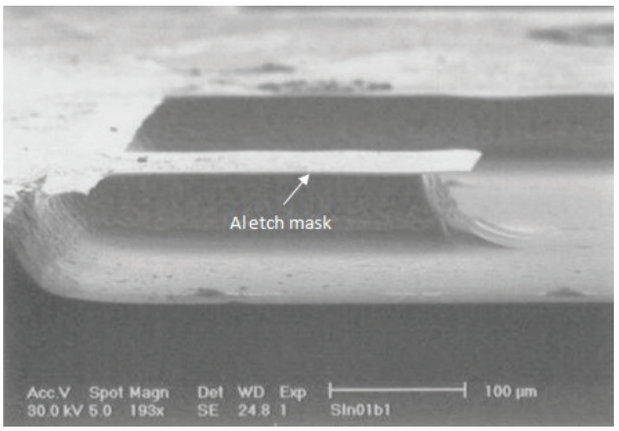


**Figure 2**. SEM image of microcantilever structure using aluminum etch mask after 20 min of isotropic dry etching.

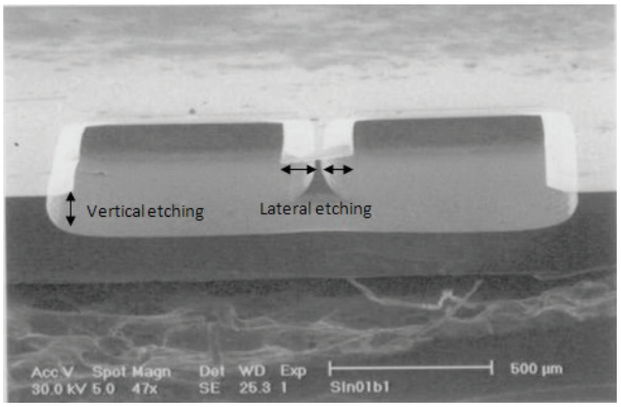


**Figure 3**. SEM image of partially released microcantilever showing lateral and vertical etching profiles.

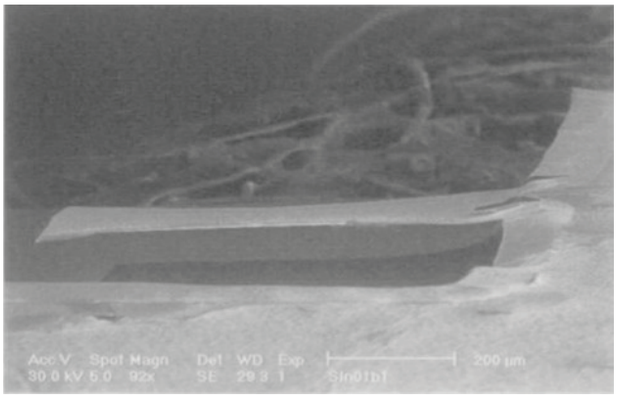


**Figure 4**. SEM image of 1 µm thick $SiO_2$microcantilever which was completely released.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 75-86

# Molecular phylogenetics and systematics of five genera of Malaysian murine rodents (*Maxomys*, *Sundamys*, *Leopoldamys*, *Niviventer* and *Rattus*) inferred from partial mitochondrial cytochrome *c* oxidase subunit I (COI) gene

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*Received 08-09-2011; accepted 26-09-2011*

**Abstract** Study on the taxonomy and systematic of Malaysian Murinae is very scarce especially due to the lack of material within the country. We provide an attempt to investigate the phylogenetic relationship and pattern thus identifying species within five genera comprising *Maxomys*, *Sundamys*, *Leopoldamys*, *Niviventer* and *Rattus*. We genetically analysed 50 specimens of Murinae from Peninsular Malaysia and Sarawak, assigned to 12 species. Phylogenetic analyses of partial mitochondrial cytochrome *c* oxidase subunit I (476 base pairs) using four methods, namely, neighbour-joining (NJ), maximum parsimony (MP), maximum-likelihood (ML) and Bayesian method resulted in similar statistically supported clades with minimal change in branching order. The analyses discovered that there were intermediate form of *Maxomys* species within *M. whiteheadi* and *M. ochraceiventer* populations. They display same external morphology as *M. whiteheadi* but genetically closer to *M. ochraceiventer*. Craniodental measurements showed significant differences between the three populations. *Rattus* and *Sundamys* appeared not fully resolved while *Leopoldamys* and *Niviventer* were steadily clustered. The intraspecific geographic variation in some species agrees with previous studies on the vicariance scenario and diversification of flora and fauna in Malaysia and Borneo.

**Keywords** Murinae – phylogenetics – COI – Genetic Species Concept – geographic structure

## INTRODUCTION

Traditionally, taxonomic status of Murinae was based on morphological characteristics. The classification was outdated due to the variation of morphological traits caused by rapid adaptation towards ecological habitats and high rate of evolution in Murinae. The variation of external features sometimes does not indicate the species to be in distinct taxa, at least not in Genetic Species Concept. As closely related species in the subfamily Murinae are morphologically similar to each other, the taxonomic status of Murinae is poorly resolved until recently. Many studies have been done using genetic data, morphology, immunology, albumin and karyotypic analyses but the information of Murinae in Malaysia is still lacking. Examining species boundaries using data from cytochrome *c* oxidase subunit I (COI) is an appropriate method to identify genetically isolated evolutionary units and the phylogenetic relationship estimation of Murinae.

In Peninsular Malaysia, the classical taxonomy of Murinae was based on their morphologies of external features [1-3]. Apart from the genera *Chiropodomys, Hapalomys, Pithecheir, Bandicota* and *Mus*, these authors placed all the remaining species of Murinae in the genus *Rattus*. Subsequent karyotypic and electrophoretic studies emphasised the distinctiveness of some species within the genus *Rattus* with great divergence [4, 5] as that between different genera from North American rodents [6, 7]. Later, the skins, skulls and dental morphology of these species were re-examined and some subgenera were elevated, new genera were named and described such as *Maxomys, Leopoldamys, Berylmys* and *Sundamys* [8, 9]. Splitting *Rattus* into well-defined genera gives better understanding of phylogenetic relationship among Murinae. Three genera of *Maxomys*, *Sundamys* and *Niviventer* that were previously included in *Rattus* were tested [10]. *Maxomys* was the basal group for these genera and *Rattus* was closely related and

monophyletic with *Sundamys* rather than the other genera.

Recently, the earlier classification was reviewed and challenged by molecular approaches [10-15], immunological experiments [16] and DNA hybridisation assays [17]. These studies had altered the classical classification made previously.

## MATERIALS AND METHODS

### Taxonomic sampling for molecular analyses

Sampling sites were chosen based on the distributions of subfamily Murinae in previous studies [18-24]. Twelve sampling sites throughout Peninsular Malaysia and Sarawak including three mountains were sampled for collecting fresh specimens (Appendix 1).

Specimens were collected from natural populations using baited cage traps and Sherman's traps. Voucher specimens were prepared either as skin and skeleton or as fluid-preserved specimens. Liver and muscle tissues were preserved in both lysis buffer and 95% ethanol. These materials were deposited at the UNIMAS Zoological Museum of Universiti Malaysia Sarawak. Museum vouchers or tissues and GenBank accession numbers for all specimens examined are listed in Appendix 2.

### Mitochondrial DNA sequencing

Total genomic DNA was extracted from muscle or liver tissues following 2X C-TAB protocol [25-27]. Partial length of 476 base pairs (bp) of COI gene was amplified using standard polymerase chain reaction procedures [28] using the GoTaq® Flexi DNA polymerase PCR kit (Promega Co.). Thermal cycle amplifications were performed using primers COIe (reverse) 5'–CCA GAG ATT AGA GGG AAT CAG TG–3' and COIf (forward) 5'–CCT GCA GGA GGA GGA GAY CC–3' [29] in a 25 µL reaction. The reaction included DNA product, 10 mM of each primer, 25 mM of $MgCl_2$, 10 mM of deoxynucleoside triphosphates, 5X reaction buffer and 1.25 U of *Taq* DNA polymerase. The thermal profile used was 93°C for 3 minutes, then amplification for 29 cycles of denaturation at 93°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 2 minutes, followed by 72°C for 5 minutes.

The amplified DNA products were purified by centrifugation using Promega Wizard SV Gel and PCR Clean Up System (Promega Co.) and sequenced at First Base Co. (Selangor, Malaysia) using the ABI PRISM® 377 DNA Sequencer with the BigDye® Terminator v3.0 Cycle Sequencing Kit. The sequencing product was run using ABI 3730 XL capillary DNA sequencer (50 cm capillary).

### Phylogenetic analyses

The CHROMAS (version 1.45) [30] software was used to observe and read nucleotide bases of DNA sequences before further analysis. The multiple alignments of the nucleotide sequences were done by using CLUSTAL X version 1.8 [31] program, later checked manually by eye. Phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 beta [32] software while Bayesian method was constructed in MrBayes [33]. For COI data set, neighbour-joining, maximum-parsimony, maximum-likelihood and Bayesian methods were used to infer phylogenies.

Out of 56 evolutionary models, Modeltest 3.7 [34] showed that general time reversible (GTR) models of substitution, with allowance for gamma distribution (G) of rate variation and for proportion of invariant sites (I), best fit the data. This model was used in maximum-likelihood and Bayesian method. Maximum-parsimony analysis was performed using heuristic searches, 10 random additions of taxa and tree-bisection-reconnection (TBR) as the branch-swapping algorithm. Pairwise genetic distances matrix between and within species were calculated using Kimura two-parameter (K2P) model [35] that was applied in Molecular Evolutionary Genetic Analysis (MEGA) 4.0 [36].

## RESULTS

### Phylogenetic analyses

The partial COI gene (GenBank JF343472–JF343519; Appendix 2) was sequenced for 50 specimens. Aligned sequences of 476 bp representing 95% of the total length of the partial mtDNA COI gene (≈500 bp) were used in the estimation of genetic distance and phylogenetic reconstruction. Alignment of sequences was unequivocal and without internal stop codons, resulting in 50 unique haplotypes. Out of the 476 bp nucleotides, 292 characters were invariant or conserved (50.8%), 184 characters showed variable sites (49.2%) with 43 variable characters being parsimony-uninformative sites and the remaining 141 characters of parsimony-informative sites. Including

the outgroup, 12 informative characters were at 1st codon positions, 2 characters at 2nd codon positions and 127 characters at 3rd codon positions. Parsimony analyses generated a single most parsimonious tree length of 682 with consistency index (CI) of 0.3959 and retention index (RI) of 0.7789. Maximum-likelihood analyses resulted in a single optimal tree (-Ln likelihood=3590.56574) while Bayesian analyses with 50% majority rule consensus resulted in statistically supported clades (Fig. 1).

Including the outgroup, the average base frequencies used in the analysis were thymine (T)

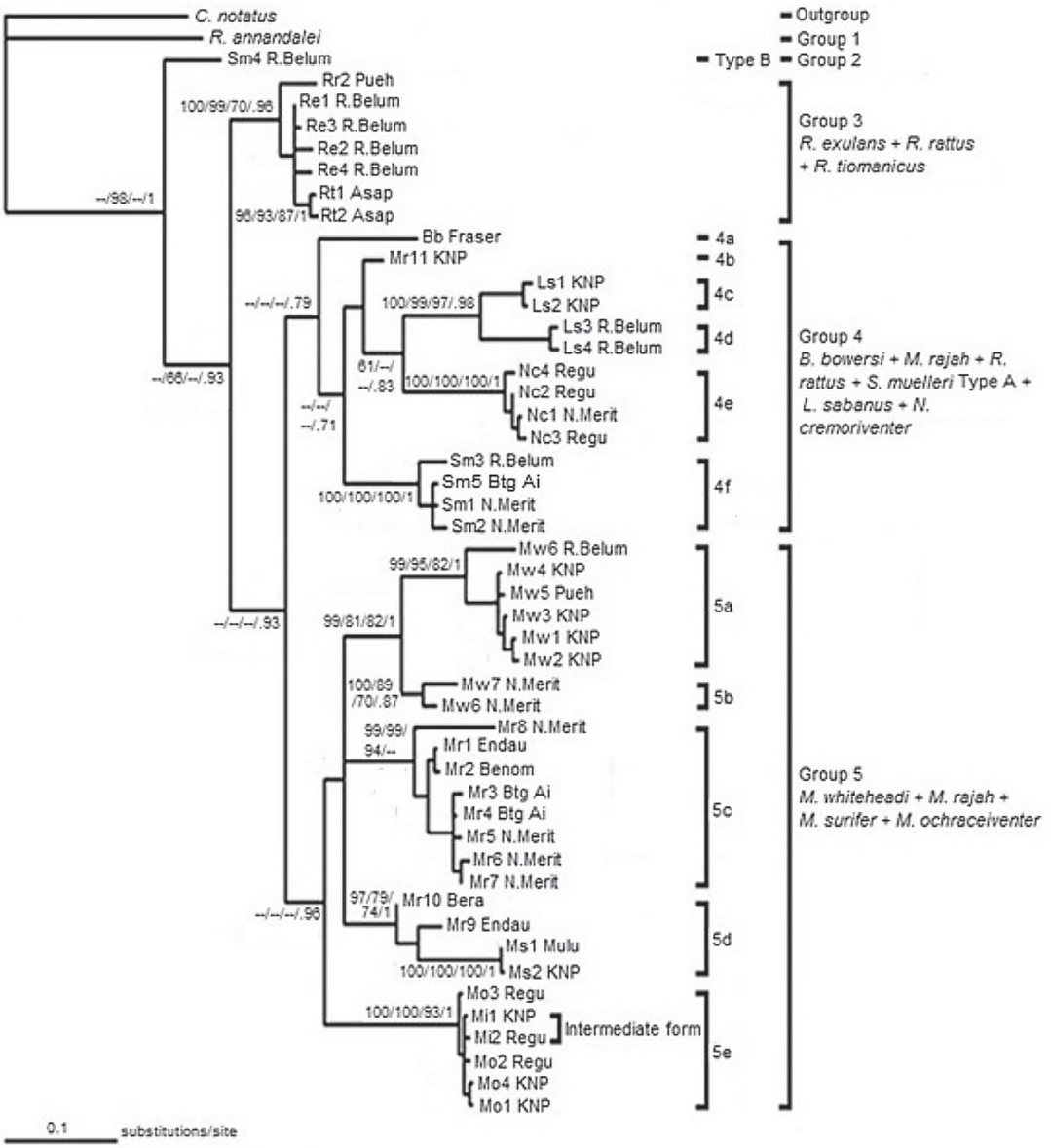


**Figure 1.** Bayesian phylogram of 50% majority-rule consensus tree inferred from aligned 476 bp partial COI gene sequences. Scores on the branches refer to bootstrap support values (1,000 iterations) from neighbour-joining (1st score), maximum parsimony (2nd score), maximum likelihood (3rd score) and Bayesian posterior probabilities (4tg score); -- = no support value. Specimens labelled by Sm = *S. muelleri*, Rr = *R. rattus*, Re = *R. exulans*, Rt = *R. tiomanicus*, Bb = *B. bowersi*, Ls = *L. sabanus*, Nc = *N. cremoriventer*, Mr = *M. rajah*, Mw = *M. whiteheadi*, Ms = *M. surifer*, Mo = *M. ochraceiventer* and Mi = intermediate form of *Maxomys*. Localities labelled by R.Belum = Royal Belum State Park, Pueh = Pueh Forest Reserve, Asap = Sungai Asap, Belaga, Fraser = Fraser's Hill Forest Reserve, KNP = Kubah National Park, Regu = Regu, Padawan, N. Merit = Nanga Merit, Kapit, Btg Ai = Batang Ai National Park, Endau = Endau-Kluang Forest Reserve, Benom = Krau Wildlife Reserve, Bera = Tasik Bera RAMSAR site, Mulu = Mulu National Park. Refer to Appendix 1 for the collecting region.

**Table 1.** Average percentage of Kimura two-parameter distance values within (boldface type along diagonal) and among species in subfamily Murinae from different clades based on COI gene sequences. n = sample size of each species. NA = not available.

| Taxon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Mi (n = 2) | **0.21** | | | | | | | | | | | | | | | |
| 2 Mw (n = 8) | 11.37 | **5.13** | | | | | | | | | | | | | | |
| 3 Mo (n = 4) | 0.32 | 11.21 | **0.35** | | | | | | | | | | | | | |
| 4 Mr11 (n = 1) | 14.03 | 10.22 | 13.83 | **NA** | | | | | | | | | | | | |
| 5 Mr Type A (n = 8) | 12.55 | 9.00 | 12.41 | 10.02 | **3.13** | | | | | | | | | | | |
| 6 Mr Type B (n = 2) | 11.39 | 8.34 | 11.26 | 9.06 | 7.14 | **4.80** | | | | | | | | | | |
| 7 Ms (n = 2) | 15.31 | 13.24 | 15.18 | 11.07 | 10.44 | 5.65 | **1.49** | | | | | | | | | |
| 8 Bb (n = 1) | 12.94 | 11.83 | 12.88 | 8.27 | 13.61 | 11.61 | 13.62 | **NA** | | | | | | | | |
| 9 Rr (n = 1) | 15.77 | 12.12 | 15.71 | 10.66 | 12.05 | 10.31 | 13.57 | 13.27 | **NA** | | | | | | | |
| 10 Rt (n = 2) | 14.81 | 12.84 | 14.75 | 10.25 | 12.28 | 9.90 | 13.66 | 13.39 | 3.26 | **0.63** | | | | | | |
| 11 Re (n = 4) | 15.15 | 12.42 | 15.08 | 9.65 | 11.41 | 9.74 | 12.76 | 12.17 | 3.27 | 1.19 | **1.17** | | | | | |
| 12 Ra (n = 1) | 21.41 | 21.93 | 27.19 | 22.78 | 21.03 | 18.76 | 23.53 | 24.52 | 24.30 | 23.22 | 22.49 | **NA** | | | | |
| 13 Sm Type A (n = 4) | 13.33 | 9.82 | 13.15 | 7.12 | 11.35 | 10.60 | 12.21 | 12.28 | 9.33 | 9.95 | 9.74 | 23.64 | **2.15** | | | |
| 14 Sm Type B (n = 1) | 16.89 | 12.29 | 16.62 | 13.91 | 13.41 | 11.92 | 17.81 | 16.14 | 10.88 | 10.96 | 9.82 | 20.76 | 12.69 | **NA** | | |
| 15 Ls (n = 4) | 13.31 | 10.08 | 13.25 | 8.79 | 11.76 | 8.25 | 11.60 | 12.34 | 13.33 | 12.27 | 11.93 | 20.27 | 9.02 | 14.47 | **5.53** | |
| 16 Nc (n = 4) | 16.88 | 12.02 | 16.89 | 10.08 | 12.06 | 9.23 | 14.49 | 13.92 | 13.44 | 14.10 | 14.01 | 24.39 | 11.72 | 14.44 | 9.75 | **0.74** |
| 17 Cn (n = 1) | 21.96 | 18.34 | 21.89 | 22.19 | 21.94 | 19.19 | 23.57 | 23.61 | 22.19 | 21.47 | 20.97 | 21.87 | 21.97 | 20.21 | 19.12 | 23.83 |

with 32.6%, cytosine (C) with 21.6%, adenine (A) with 28.4%, and guanine (G) with 17.5%. The highest frequency of nucleotide found in COI gene for these species including the outgroup was T nucleotide, ranging from 27.9% (*R. annandalei*) to 34.2% (*M. whiteheadi*) whereas G nucleotide had the lowest frequency, which showed the characteristic of anti-G bias ranging from 16.6% (*M. rajah* 11) to 18.5% (*S. muelleri* Type B). Anti-G bias sequences were one of the characters of mitochondrial gene [37, 38].

All the phylogenetic topologies revealed five strongly supported monophyletic clades with slightly different topologies and groupings. Genetic distance within and among each lineage was calculated by Kimura two-parameter [35] according to the groupings assignment in phylogenetic trees (Table 1). For distance character, NJ gave the most resolved topologies while the Bayesian phylogenetic tree was the most reliable for character based method observed by the higher bootstrap and bpp values (>50%) on each branch compared to the other character based phylogenetic trees (MP and ML).

## DISCUSSION

### *Maxomys* Clade

Genetic divergence between genera of 15.02% separated the lineage of *Maxomys* from the other Murinae lineages in this study. The separation was well supported by studies using mtDNA and nucDNA data [13], observing external morphologies, skull measurements and dental morphologies [8, 39], assessing microcomplement fixation of albumin [16] and chromosomal evidence [40]. Besides, the separation was also congruent with the external morphological differences of having short bicolour tail, being dark brown or black above and white beneath, separated by a sharp line [52] and obvious spiny fur with very stiff and prominent spines, which are lacking in other genera in this study [8, 19, 23].

In all four methods of phylogenetic analyses, intermediate form of *Maxomys* (Mi) was identified within *Maxomys* division. They exhibit external morphological characteristics similar to *M. whiteheadi* but they were excluded from the remaining *M. whiteheadi* population and formed monophyletic group with *M. ochraceiventer* lineage. High genetic divergence (mean = 11.37%) between Mi with *M. whiteheadi* population suggested that the taxa should be treated as different lineages or species and not as *M. whiteheadi* following the Genetic Species Concept [41]. Genetic distance >11% indicated a species recognition [41].

The close genetic relationship between Mi and *M. ochraceiventer* (mean = 0.32%) suggested that the intermediate form showed high probability of conspecific populations to *M. ochraceiventer*. Genetic distance < 2% indicated intraspecific variation [41]. However, obvious differences in the skulls and dental features (Appendices 3, 4 and 5) between Mi, *M. ochraceiventer* and *M. whiteheadi* elucidated that the intermediate form was distinct from *M. whiteheadi* and *M. ochraceiventer*.

*Maxomys ochraceiventer* has the flattest skull and the longest greatest skull length (GSL) followed by Mi and *M. whiteheadi* (Appendix 3). Comparatively,

the skull of *M. whiteheadi* was broader compared to those of Mi and *M. ochraceiventer*. Moreover, between these three skulls, there were differences in the shape of the bony palate and incisive foramina (Appendix 4). *Maxomys whiteheadi* (A) and Mi (B) have straight shape but *M. whiteheadi* has rounder curve at the top of the bony palate and Mi was rather square. On the other hand, *M. ochraceiventer* has a narrower bony palate at the base that widens to the top with square shape as Mi. Next, the incisive foramina for *M. whiteheadi* was relatively broader compared to Mi and *M. ochraceiventer*. Between Mi and *M. ochraceventer*, Mi has shorter length of incisive foramina. The zygomatic plate of *M. whiteheadi* was comparatively small compared to Mi and *M. ochraceiventer* (Appendix 5). *Maxomys ochraceiventer* has longer and narrower zygomatic plate compared to Mi.

Skull and dental measurements that indicate the variation among *M. whiteheadi*, *M. ochraceiventer* and intermediate form of *Maxomys* are shown in Appendix 6. The variance was calculated using Kruskal-Wallis test and the value $P < 0.05$ indicated significant difference between the skull and dental measurements. Larger dataset should be obtained and examined to further review the significant distinction.

These occurrences may suggest that Mi was a cryptic species in *Maxomys*. Recently, there were cryptic species recorded within the *Maxomys* population in Borneo that was closely related with *M. whiteheadi* and *M. ochraceiventer* [42, 43]. These *Maxomys* populations should be further investigated with highly evolving genes such as the control region to ensure the separation among the species clustering due to evolution and speciation. Furthermore, the intermediate form present was possibly due to hybridisation of *M. whiteheadi* and *M. ochraceiventer*. Hybrid species inherited similar genetic composition to the former but resembled the same morphologies of the latter species. The occurrence is possible as the two congeneric species are closely related. Thus, further studies need to be done on these complex taxa using several nucDNA to investigate the hybridisation.

Apart from *M. rajah* 11, *M. rajah* lineage was separated into two subgroups. *Maxomys rajah* (5c) was clustered among the same species while *M. rajah* (5d) clustered (two individuals) with *M. surifer* with genetic distance of 5.65% between the two species. The two subgroups of *M. rajah* were divided with genetic divergence of 7.14%. The genetic distances indicated that *M. rajah* 9 and 10 (5d) were genetically closer related to *M. surifer* rather than their own species. *Maxomys rajah* and *M. surifer* were once considered as conspecific [44]; however, genetic distance for conspecific value varies from 0.25 to 5% [45]. Thus, it was not supported that *M. rajah* and *M. surifer* were conspecific in this study. Although *M. rajah* and *M. surifer* proved difficult to distinguish [19, 23], there was no doubt that both *M. rajah* and *M. surifer* were distinct species by ecological observation, breeding behaviour, karyotype and serology [40]. Although they were found in the general habitat, they were not usually present together. Moreover, no attempts on mating were observed for the interspecific pairs under the prevalent animal-house condition [40]. Karyotypes of both species were also described and the chromosome numbers were distinctly different [4]. A study done using COI gene and morphological data analyses stated that the two congeneric taxa were not even closely related within *Maxomys* division [43]. This may suggest that there were high genetic variation within *M. rajah* population or *M. rajah* (5d) might be a subpopulation of *M. surifer* and should be treated with caution as there were no DNA sequences of *M. surifer* from Peninsular Malaysia for comparison. Larger dataset of this particular species is needed to make a conclusion of the clustering.

*Maxomys rajah* 11 phylogenetic relationship remains unresolved as it was independently clustered in phylogenetic trees. The partial fragment (≈500 bp) sequences of the COI gene might have insufficient informative sites for the analyses and this might explain the ambiguous clustering of *M. rajah* 11 discussed above. Only 33% of the complete length of COI gene (1500 bp) was used in this study. The primers might have sequenced any partial fragments in the COI gene and the polymorphic sites of the genetic composition that can signify different species might actually lie outside the fragments analysed.

Similar phylogeographic structuring among *M. whiteheadi* (5a and 5b) and *M. rajah* (5c) reflected a consistent pattern of vicariance scenario, in which *Maxomys* share a history of diversification resulting from barriers arising within their formerly continuous ranges that were distinguishable by levels of genetic divergence between Peninsular Malaysia and Sarawak. Species of *Maxomys* are non-commensal and forest dwellers. Thus, forest expansion and

contraction events across the Sunda shelf during the last 3 million years might have fragmented the northeast Sarawak populations before Peninsular Malaysian and southwest Sarawak populations. This pattern was also observed in other rodents and bats studies, which suggested that speciation occurred due to preglacial vicariance [53, 54]. In *M. rajah* lineage (5c), northeast Sarawak populations were separated; one derived earlier and branched independently (following the pattern of vicariance scenario) while the others clustered with southwest Sarawak populations. The latter form has not yet diversified to form a distinct population from southwest Sarawak. Based on this occurrence, hypothesis can be proposed that northeast Sarawak (in this case Nanga Merit, Kapit) might be the transition region and contact zone for speciation.

***Rattus* and *Sundamys* clade**

Over the past 17 years, the genus *Rattus* has been studied intensively. Species from this genus had been separated as distinct genera (*Leopoldamys*, *Maxomys* and *Niviventer*) based on morphological ground [46]. Non-morphological techniques (DNA) [47] agreed with the separation but failed to resolve the relationships between *Rattus* and *Sundamys*. The relationship was still ambiguous and many unidentified groups have been discovered.

The phylogenetic trees illustrated topologies that were not fully resolved in *Rattus* and *Sundamys* taxa. *Sundamys muelleri* 4 (Type B) branched out independently and diverged from the remaining *S. muelleri* linage (Type A) with high genetic divergence of 12.69%. The value implied a new genetic species [48]. *Rattus annandalei* was also unresolved with high genetic distance between its genus. The justification was that the partial fragment (≈500 bp) sequences of the COI gene might have insufficient informative sites for the analyses. More genetic data are needed to clarify whether *S. muelleri* 4 and *R. annandalei* could be categorised as distinct lineages.

*Rattus rattus* was the basal clade in *Rattus* lineage followed by *R. exulans* and *R. tiomanicus*. Species of *Rattus* are commensal species that are always associated with humans and live sympatrically with one species or another. This might contribute to the occurrence of hybridisation that explained the close genetic divergence among species (< 4%). *Sundamys* lineage showed a pattern of vicariance scenario where the South China Sea might be a geographic barrier that resulted in allopatric populations between Peninsular Malaysia and Sarawak. This is consistent in other faunal study [53]. In this lineage, the northeast and southwest Sarawak populations were not separated.

***Niviventer and Leopoldamys* clade**

The close relationship between *Niviventer* and *Leopoldamys* was claimed based on morphological analyses [49]. These two genera formed a sister group with high bootstrap support in this study. The taxonomic status of these genera was ambiguous as they were classified in the genus *Rattus* in the early nomenclatural history [16, 23]. This study proved that they belong to distinct genera as they showed high genetic divergence of 9.75%.

The clustering of *L. sabanus* was separated according to the geographic region, Peninsular Malaysia and southwest Sarawak. *Niviventer cremoriventer* showed an absence of phylogeographic structuring within the specimens indicating a recent common ancestor of northeast and southwest populations of this species.

The COI gene is a marker that has the characteristic of a conserved gene. It consists of more conserved sites rather than variable sites [50]. This particular region evolves slowly within the mtDNA which makes it suitable to resolve interspecies level but cannot determine at best intraspecific relationship for the vertebrates [50]. However, COI gene has proven to be a good genetic marker for intraspecific variation of the lower vertebrates as seen in amphibian populations [51].

**Acknowledgements –** This study would not be possible without various administrative and financial supports from Universiti Malaysia Sarawak (UNIMAS). We thank the Sarawak Forestry Corporation and Sarawak Forestry Department for granting permission to conduct wildlife research in national parks in Sarawak under the State Wild Life Protection Rules 1998; for research permit number NPW.907.4.2(II)-5 and permit to enter park. Our gratitude also goes to the Department of Wildlife and National Parks for inviting our team to various field trips in Peninsular Malaysia. Identifications of certain species in this study were greatly aided by Dr Lim Boo Liat. We wish to express our sincere gratitude to the members in the Department of Zoology for their hard work and assistance throughout this study. Special thanks to Professor Yong Hoi Sen, Dr Ramlah Zainuddin, Earl of Cranbrook, Mr. Faisal Ali

Anwarali Khan and Mr. Jayaraj Vijaya Kumaran for the constructive comments during the process of writing this manuscript.NAMT postgraduate study was supported by UNIMAS Vice Chancellor Scholarship and part of the data collection and laboratory analyses using the UNIMAS Eco-Zoonosis Grant lead by MTA.

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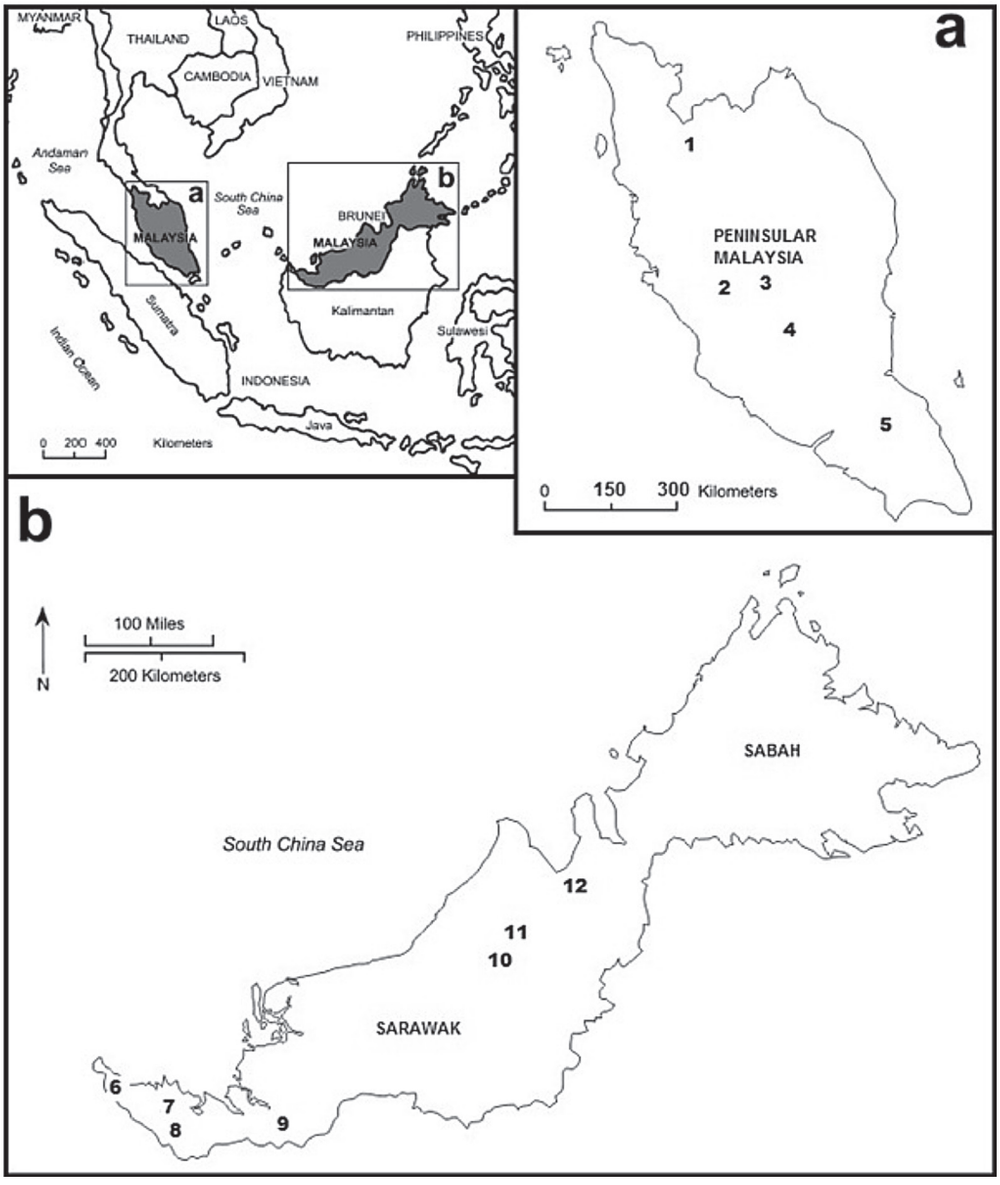
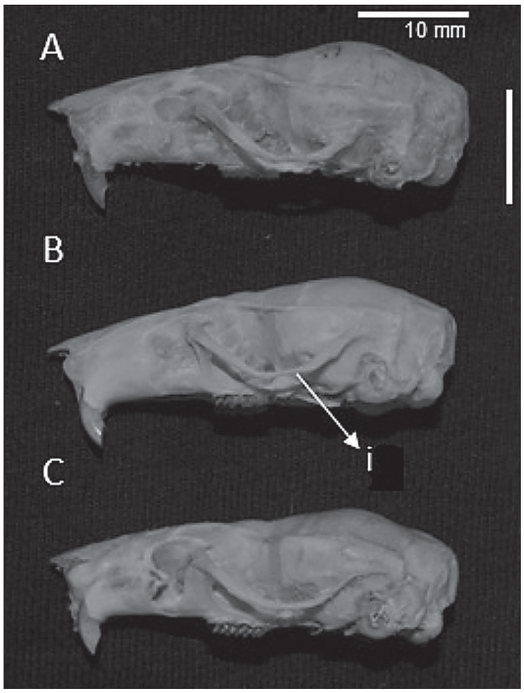
**Appendix 1.** Twelve study sites for this study. 1: Royal Belum State Park. 2: Fraser's Hill Wildlife Reserve. 3: Krau Wildlife Reserve. 4: Tasik Bera RAMSAR site. 5: Endau-Kluang Wildlife Reserve. 6: Pueh Forest Reserve. 7: Kubah National Park. 8: Regu, Padawan. 9: Batang Ai National Park. 10: Nanga Merit, Kapit. 11: Sungai Asap, Belaga. 12: Mulu National Park. Regions: Peninsular Malaysia (1-5), southwestern Sarawak (6-9) and eastern Sarawak (10-12).

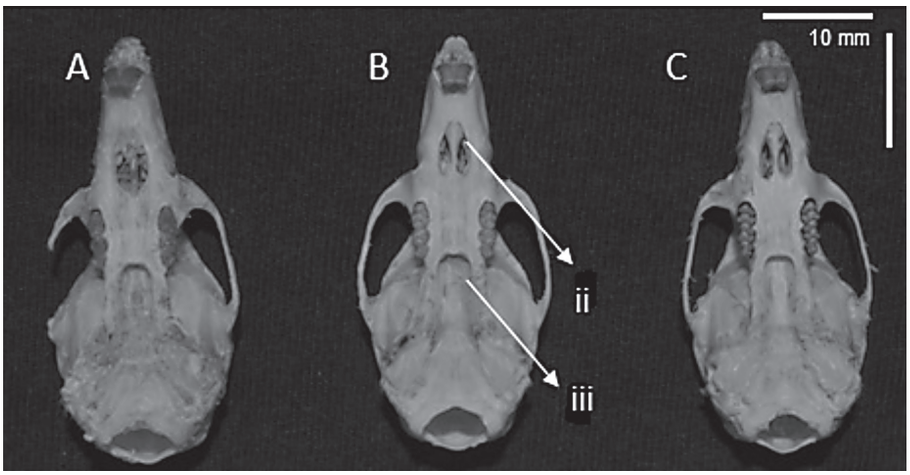


**Appendix 2.** Taxa, geographic localities, map point, tissue number, GenBank accession numbers of COIdata used for phylogenetic analysis. IP = in progress; Abbr. = abbreviation; NA = not available.

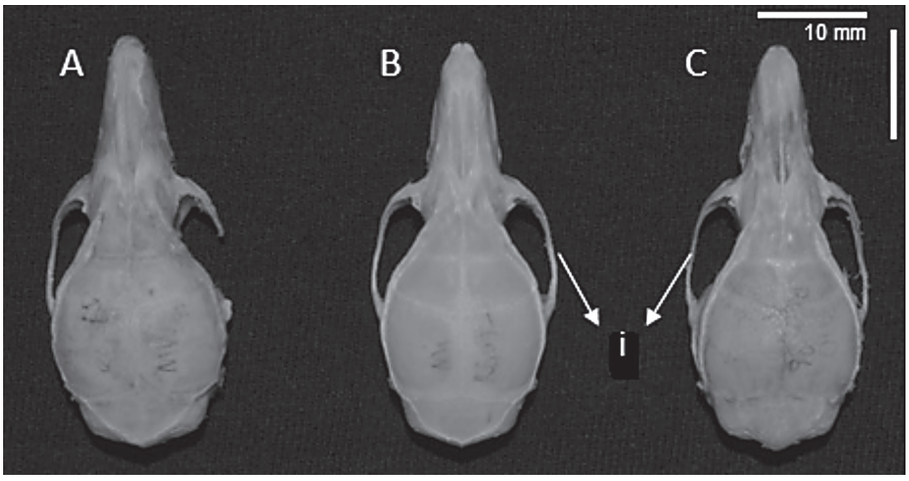
| Taxa | Abbr. | Tissue no. | Location | | Map point | GenBank no. |
|---|---|---|---|---|---|---|
| | | | Locality | Region | | COI |
| *M. whiteheadi* | Mw1 | TK152851 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343477 |
| *M. whiteheadi* | Mw2 | TK152854 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343478 |
| *M. whiteheadi* | Mw3 | TK152823 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343473 |
| *M. whiteheadi* | Mw4 | TK152846 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343474 |
| *M. whiteheadi* | Mw5 | Pueh006 | Pueh Forest Reserve, Sarawak | Southwest Sarawak | 7 | JF343482 |
| *M. whiteheadi* | Mw6 | TK156110 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343494 |
| *M. whiteheadi* | Mw7 | UNIMAS2083 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343493 |
| *Maxomys* sp. | Mi1 | TK152861 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343476 |
| *Maxomys* sp. | Mi2 | RG072 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343485 |
| *M. ochraceiventer* | Mo1 | TK152349 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343479 |
| *M. ochraceiventer* | Mo2 | RG092 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343487 |
| *M. ochraceiventer* | Mo3 | RG086 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343486 |
| *M. ochraceiventer* | Mo4 | KNP027 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343495 |
| *M. rajah* | Mr1 | EKS026 | Endau Kluang Forest Reserve, Johor | South Peninsular Malaysia | 6 | JF343480 |
| *M. rajah* | Mr2 | LB066 | Krau Wildlife Reserve, Pahang | Central Peninsular Malaysia | 4 | JF343516 |
| *M. rajah* | Mr3 | BTA007 | Batang Ai National Park, Sarawak | Southwest Sarawak | 10 | JF343511 |
| *M. rajah* | Mr4 | TK152348 | Batang Ai National Park, Sarawak | Southwest Sarawak | 10 | JF343515 |
| *M. rajah* | Mr5 | 2177 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343514 |
| *M. rajah* | Mr6 | 2176 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343513 |
| *M. rajah* | Mr7 | 2122 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343517 |
| *M. rajah* | Mr8 | UNIMAS2010 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343512 |
| *M. rajah* | Mr9 | EKS011 | Endau Kluang Forest Reserve, Johor | South Peninsular Malaysia | 6 | JF343500 |
| *M. rajah* | Mr10 | TB011 | TasikBera, Pahang | Central Peninsular Malaysia | 5 | JF343518 |
| *M. rajah* | Mr11 | 2192 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343519 |
| *M. surifer* | Ms1 | MM06 | Mulu National Park, Sarawak | East Sarawak | 13 | JF343502 |
| *M. surifer* | Ms2 | TK153614 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343504 |
| *R. rattus* | Rr2 | Pueh008 | Pueh Forest Reserve, Sarawak | Southwest Sarawak | 7 | JF343503 |
| *R. tiomanicus* | Rt1 | MPOB006 | Sungai Asap, Belaga, Sarawak | East Sarawak | 12 | JF343488 |
| *R. tiomanicus* | Rt2 | MPOB018 | Sungai Asap, Belaga, Sarawak | East Sarawak | 12 | IP |
| *R. exulans* | Re1 | TK156113 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343496 |
| *R. exulans* | Re2 | TK156111 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343489 |
| *R. exulans* | Re3 | TK156109 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343498 |
| *R. exulans* | Re4 | TK156125 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343499 |
| *R. annandalei* | NA | TG01 | Bukit Tagan, Perak | North Peninsular Malaysia | NA | IP |
| *S. muelleri* | Sm1 | UNIMAS2044 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343483 |
| *S. muelleri* | Sm2 | UNIMAS2050 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343508 |
| *S. muelleri* | Sm3 | TK156131 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343497 |
| *S. muelleri* | Sm4 | TK156119 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343509 |
| *S. muelleri* | Sm5 | BTA024 | Batang Ai National Park, Sarawak | Southwest Sarawak | 10 | JF343481 |
| *L. sabanus* | Ls1 | TK152824 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343475 |
| *L. sabanus* | Ls2 | TK152830 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343506 |
| *L. sabanus* | Ls3 | TK156130 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343501 |
| *L. sabanus* | Ls4 | TK152988 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343507 |
| *N. cremoriventer* | Nc1 | UNIMAS2082 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343490 |
| *N. cremoriventer* | Nc2 | RG067 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343491 |
| *N. cremoriventer* | Nc3 | RG076 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343492 |
| *N. cremoriventer* | Nc4 | RG033 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343484 |
| *B. bowersi* | Bb | FH036 | Fraser's Hill, Selangor | Central Peninsular Malaysia | 3 | JF343505 |
| *C. notatus* | NA | W02 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343472 |




**Appendix 3.** Lateral views of the skulls show variation between *M. whiteheadi* (A),intermediate form of *Maxomys* sp. (B) and *M. ochraceiventer* by observing the flatness of the braincase, the greatest skull length and the zygomatic plate (i).




**Appendix 4.** Ventral views of the skulls show variation between *M. whiteheadi* (A), intermediate form of *Maxomys* sp. (B) and *M. ochraceiventer* by observing the posterior edge of bony palate (ii) in relation to M3 and the palatal foramina (iii) in relation to M1.




**Appendix 5.** Dorsal views of skull show variation between *M. whiteheadi* (A), intermediate form of *Maxomys* sp. (B) and *M. ochraceiventer* by observing the broadness of the braincase and the features of the zygomatic plate (i).

**Appendix 6.** Skull and dental measurements among complex lineage in *Maxomys*. GSL = greatest skull length, CBL = condylobasal length, IOW = interorbital width, ZW = zygomatic width, Pm = premaxillary length and MT = maxillary toothrow. n = number of individuals.

| Character | Species | | | Kruskal-Wallis test | |
|---|---|---|---|---|---|
| | *M. whiteheadi* (n = 5) | Intermediate form (n = 2) | *M. ochraceiventer* (n = 2) | H | p |
| Skull (mm) | | | | | |
| GSL | 38.69 | 38.03 | 38.18 | 6.533 | P < 0.05 |
| CBL | 36.17 | 34.63 | 35.65 | 6.533 | P < 0.05 |
| ZW | 18.88 | 17.40 | 17.49 | 6.533 | P < 0.05 |
| Pm | 10.87 | 10.30 | 9.90 | 6.533 | P < 0.05 |
| IOW | 8.13 | 7.34 | 7.92 | 6.533 | P < 0.05 |
| Dental (mm) | | | | | |
| MT | 5.47 | 5.06 | 5.24 | 6.533 | P < 0.05 |

*Journal of Science and Technology in the Tropics* (2011) **7**: 69-74

# Producing energy from root crops in the humid tropics

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**Abstract** The world's depleting sources of fossil fuel and the burgeoning demand for animal protein in the new economies have created huge markets for alternate fuel and livestock feed. In the humid tropics, root crops such as cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*) have the potential of tapping into these markets. The question that often arises is which is a better crop choice? Both these food crops have advantages and disadvantages in terms of their botany, nutritional value and agronomic parameters. Chief among the shortcomings in cassava are its relatively long cropping cycle (and thus, its slow rate of returns), its proneness to lodging, its short post-harvest shelf life, and in Malaysia the scarcity of suitable land for its large-scale cultivation. In the case of sweet potato, its susceptibility to serious pests (weevil and vine borer) and disease (sweet potato virus disease) requires that it be cultivated in rotation with other short-term crops. In the final analysis, the choice depends on the intended end use of the crop, availability of suitable land, and the preferred cropping system and management.

**Keywords** biofuel – feedstuff – cassava – sweet potato – humid tropics

## INTRODUCTION

With the current worry over depleting fossil fuels in the world, several countries have embarked on the production of ethanol from plant carbohydrate sources to replace petrol. The most successful to date is Brazil, a country with more than 30 years' experience in producing ethanol from sugarcane as a biofuel. USA went the way of producing ethanol from corn, not the wisest of crop choices given the rather dismal net energy ratio (number of units of energy produced from one unit of energy expended) and reduction in greenhouse gas emissions *vis-a-vis* that of sugarcane (Table 1) [1-3]. Nevertheless, USA has had a long history of producing corn on a large scale and is good at it; thus, what is lacking in the conversion department is made up for by the sheer volume of production.

Similarly, a number of countries are giving attention to producing ethanol from cassava, a highly productive and vigorous root crop. Among these are Thailand, Australia and China. While cassava

**Table 1.** Net energy ratios and greenhouse gas emission for selected materials used in ethanol production. (Adapted from [1], [2], [3])

| Material | Net energy ratio | Greenhouse gas emission | |
|---|---|---|---|
| | | kg/L | Reduction |
| Sugarcane | 8.3 | 1.08 | 56% |
| Sugarcane (data from Thailand) | 9.3-10 | n/a | n/a |
| Sugarbeet | 1.9 | n/a | 35-56% |
| Corn | 1.35-1.5 | 1.94 | 22% |
| Corn (data from Thailand) | 4-5.2 | n/a | n/a |
| Cassava (data from Thailand) | 8-9.1 | 0.84 | 63% |
| Cellulose from corn stover | 4.39 | n/a | n/a |
| Cellulose from switchgrass | 8.3 | n/a | n/a |
| Cellulosic ethanol | 2-36 | 0.23 | 91% |
| Petrol | 1.0 | 2.44 | 0% |

grows well in the tropics between the latitudes 30°N and 30°S, it is very susceptible to frost. This latter characteristic has made it difficult for China (where there is a phenomenal growth in demand for petrol-driven vehicles) to embark at full steam on growing cassava for ethanol production. However, because the market for cassava in China is huge, there is increasing interest among Malaysian entrepreneurs to tap into this demand.

The world demand for energy-rich feed ingredients is also growing in tandem with the burgeoning appetite for meat in those countries showing strong economic growth. The chicken and pig industries have traditionally used corn as an energy source in feeds, but with substantial amounts being channelled towards biofuel production coupled with the unprecedented demand for poultry and pork in recent years in the emerging economies, there is unsurprisingly a shortfall. To fill this deficit, other carbohydrate sources are being used increasingly by feed millers.

In the Malaysian context, the chicken and pig production sectors have always depended on imported corn. In this equatorial country, the growing of grain corn faces many production problems, not the least of which is the unpredictability of rain – either too much or too little, and not coinciding with the needs of the crop at its different stages of growth [4, 5]. In contrast, carbohydrate-rich tropical root crops, such as cassava and sweet potato, grow very well in the humid tropics, and have been proven to be possible replacers of corn.

Thus, we are faced with the enviable situation of two fast-emerging markets – the biofuel market and the livestock feed market. Which then will be a better crop choice – cassava or sweet potato?

## BOTANY

Both cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* (Lam.) L.) are root crops, belonging to the families Euphorbiaceae and Convolvulaceae, respectively. Cassava has a semi-woody erect habit while sweet potato is a herbaceous creeper. Although both can persist perennially, they are cultivated as annuals. The storage organs of these crops are swollen adventitious roots; however, those of sweet potato form adventitous buds which allow the storage roots to be used as propagules. In the temperate zone, sweet potato roots are overwintered and sprouted in the spring to plant the new crop. More commonly in the tropics, stem cuttings are used as planting materials. In the case of cassava, the woody section of the stems is used, whereas shoot tip cuttings are preferred for sweet potato.

Having a cropping cycle of 9-14 months (9-10 months for the edible type; 12-14 months for the starch type), cassava is restricted in cultivation to the tropics and subtropics. In contrast, sweet potato is harvested after 3½ to 4 months, which means it can be planted in the temperate zone during the summer months.

## NUTRITIONAL VALUE

Sweet potato and cassava are starch crops, accumulating these complex carbohydrates in the storage roots (hereinafter to be referred to as 'roots' in this paper). The roots of both have low protein and lipid contents. Although sweet potato has generally a higher protein content than cassava, these crops are low in the sulphur-bearing amino acids such as cysteine and more importantly methionine, an essential amino acid (Table 2) [6]. Methionine has a detoxifying effect which is of particular relevance in cassava with its propensity to produce HCN (see below).

Cassava tissues contain an anti-nutritional factor in the form of cyanogenic glucosides, mainly linamarin. When the cells are ruptured, the inherent enzyme, linamarase, is released which reacts with linamarin to produce hydrogen cyanide (HCN), a highly toxic gas. Cultivars of cassava which have a high content of linamarin in the root pith (releasing >50 mg of HCN per kg fresh roots) are classified as the non-edible type, while those having a lower linamarin content are considered edible. High starch cultivars tend to be non-edible. Nevertheless, the leaves and root cortex of all cultivars have a high capacity of producing HCN. Being highly soluble and volatile, HCN is easily removed during starch extraction and processing, as well as when the roots are chipped and dried for use a livestock feedstuff.

The anti-nutritional factor in sweet potato is a trypsin-inhibitor which interferes with protein metabolism. Fortunately, this antitrypsin factor is heat labile and so can be effectively removed, as when the roots are boiled. Furthermore, it is possible to breed for cultivars which contain less trypsin-inhibitor.

Highly coloured sweet potato cultivars have

**Table 2.** Nutrient composition of cassava and sweet potato (dry weight basis). (Source: [6])

| Nutrient | Unit (per 100g) | Cassava[1] | Sweet potato[1] |
|---|---|---|---|
| **Proximates** | | | |
| Water | g | 12.0 | 12.0 |
| Energy | kcal | 349 | 333 |
| Protein | g | 2.97 | 6.08 |
| Total fat | g | 0.61 | 0.19 |
| Ash | g | 1.35 | 3.83 |
| Carbohydrate, by difference | g | 83.07 | 77.93 |
| Dietary fibre, total | g | 3.9 | 11.6 |
| **Minerals** | | | |
| Calcium, Ca | mg | 35 | 116 |
| Iron, Fe | mg | 0.59 | 2.36 |
| Magnesium, Mg | mg | 46 | 97 |
| Phosphorus, P | mg | 59 | 182 |
| Potassium, K | mg | 592 | 1305 |
| Sodium, Na | mg | 31 | 213 |
| Zinc, Zn | mg | 0.74 | 1.16 |
| Copper, Cu | mg | 0.218 | 0.585 |
| Manganese, Mn | mg | 0.838 | 0.999 |
| Selenium, Se | mcg | 1.5 | 2.3 |
| **Vitamins** | | | |
| Vitamin C | mg | 45.0 | 9.3 |
| Thiamin | mg | 0.190 | 0.302 |
| Riboflavin | mg | 0.105 | 0.236 |
| Niacin | mg | 1.864 | 2.157 |
| Panthotenic acid | mg | 0.234 | 3.099 |
| Vitamin B-6 | mg | 0.192 | 0.810 |
| Folate, total | mcg | 59 | 43 |
| Vitamin B-12 | mcg | 0.00 | 0.00 |
| Vitamin A, IU | IU | 28 | 54950 |
| Vitamin A, RAE | mcg_RAE | 2 | 2746 |
| Vitamin E | mg | 0.42 | 1.01 |
| Vitamin K | mcg | 4.2 | 7.0 |
| **Amino acids** | | | |
| Tryptophan | g | 0.042 | 0.120 |
| Threonine | g | 0.061 | 0.322 |
| Isoleucine | g | 0.059 | 0.213 |
| Leucine | g | 0.085 | 0.356 |
| Lysine | g | 0.096 | 0.256 |
| Methionine | g | 0.024 | 0.112 |
| Cysteine | g | 0.061 | 0.085 |
| Phenylalanine | g | 0.057 | 0.345 |
| Tyrosine | g | 0.037 | 0.132 |
| Valine | g | 0.076 | 0.333 |
| Arginie | g | 0.299 | 0.213 |
| Histidine | g | 0.044 | 0.120 |
| Alanine | g | 0.083 | 0.298 |
| Aspartic acid | g | 0.172 | 1.480 |
| Glutamic acid | g | 0.450 | 0.600 |
| Glycine | g | 0.061 | 0.244 |
| Proline | g | 0.072 | 0.201 |
| Serine | g | 0.072 | 0.341 |
| **Others** | | | |
| ß-carotene | mcg | 18 | 32,957 |

[1]Converted to 12% moisture content from values for the fresh roots.

desired anti-oxidant properties; the purple-fleshed cultivars contain anthocyanin, while the orange ones are high in ß-carotene.

## AGRONOMY

The pros and cons of planting cassava or sweet potato can best be appreciated when their agronomic parameters are tabulated (Table 3) [7-9].

## CASSAVA OR SWEET POTATO?

Agronomically speaking, cassava is a crop which is easier to manage. In Malaysia, it has relatively fewer pests and diseases, which seldom cause significant yield loss. Indeed, mammalian pests such as rats (and monkeys, wild boar and elephants in fields adjoining jungle) can cause more damage. This means it is possible to grow cassava year in year out without loss in yield, provided that the correct amount and type of fertilizers (according to the soil type) are applied for every crop.

One of the major shortcomings of cassava is its long cropping cycle despite its average root yield (see also Table 4 [10]) being only slightly better than sweet potato, resulting in a slower rate of returns. The latter can be offset by intercropping cassava with a shorter term crop such as sweet corn or groundnut [11]. The longer cropping cycle also excludes its cultivation in areas affected by annual floods during the months of the north-east monsoon (mainly the East Coast of Peninsular Malaysia). Having an erect plant habit (growing up to 2.4 metres or more in height), cassava is also prone to lodging in windy areas.

In the humid tropics, it is possible to crop the year round, thus ensuring a constant supply of roots to the processing plant. To keep a small starch factory in constant operation and running a single 8-hour shift, it has been estimated that 1500-2000 ha of land are required for staggered planting and harvests throughout the year [12]. However, suitable land for cassava in such large expanses seems to be no longer available in Peninsular Malaysia. It should also be remembered that the starch cultivars fetch only half the price of the edible cultivars, but then the market for starch (and feedstuff) is far larger.

Sweet potato is plagued by several important insect pests and viruses. While insect pests can be managed to some extent by chemical means, viruses can only be kept under control by the use of virus-free

**Table 3.** A comparison of the agronomic parameters of cassava and sweetpotato in the humid tropics.

| | Cassava[1] | Sweet potato[2] | Remarks |
|---|---|---|---|
| Preferred soil type | Sandy loam to loam | Sandy loam to loam | Sweet potato performs very well in sandy soils (e.g. *bris*[3] and ex tin-mining land), with adequate fertilizer inputs |
| Topography | Less than 6% gradient | Less than 6% gradient | Soil disturbance during the harvest of root crops causes severe soil erosion on a slope |
| Land preparation | Two rounds of ploughing, one round of rotortilling | Two rounds of ploughing, one round of rotor-ridging | Planting on ridges/beds is essential for storage root development in sweet potato |
| Planting materials | Mature woody stems stacked vertically in the shade can remain viable up to one month | Vine cuttings need to be planted within a few days | Vegetative planting materials are particularly prone to harbouring pests and diseases. Caution should be exercised when introducing planting materials from other countries. |
| Nursery preparation | No need; cuttings can be taken straight from the harvested crop | Required to raise good quality planting materials; cuttings taken from nursery at 2-2½ months | |
| Irrigation | Not required generally | Important in the first 2 weeks after planting | If planted on sandy soils, sweet potato requires irrigation throughout the crop duration |
| Weed control | Pre-emergence<br>Post-emergence at late stage and near root harvest | Pre-emergence | Once established sweet potato vines quickly cover the beds completely, precluding further weed control. Cassava canopy thins down at the later crop stage, allowing light through to the soil surface, resulting in weed growth. |
| Growing cycle | 9-10 months for edible cvs.<br>12-14 months for starch cvs. | 3½ - 4 months | Faster returns from sweet potato |
| Sensitivity to floods | Very sensitive | Very sensitive, but problem is somewhat alleviated by planting on beds | Generally, root crops are sensitive to waterlogged conditions which cause root rot due to lack of oxygen |
| Common pests of economic importance | Leaf-eating caterpillars (e.g. *Tiracola plagiata*)<br>Rats | Sweet potato weevil (*Cylas formicarius*); white grubs; vine borer (*Omphisa anastomosalis*); various leaf eaters (e.g. *Spodoptera litura*)<br>Rats | No serious pests in cassava. Look out for pink mealy bug which in recent years has devastated cassava yields in Thailand [9]. |
| Common diseases of economic importance | *Cercospora* brown and white leafspots (*C. henningsii*, *C. caribeae*); cassava bacterial blight (*Xanthomonas manihotis*); white root disease (*Rigidoporus lignosus*) | Sweet potato virus disease or SPVD (virus complex comprising the whitefly-borne sweet potato feathery mottle virus, SPFMV, sweet potato chlorotic stunt virus, SPCSV, and other viruses) | Leaf diseases of cassava cause little yield loss. White root disease can be managed by proper field sanitation. No means of control for SPVD. |
| Cropping system | Monocropping or intercropping | Rotational cropping (with sweet corn, groundnut, yambean, tobacco, etc.) | Rotational cropping is crucial for the management of pests and diseases in sweet potato |
| Root yield (t/ha) | On average, 30-35 | On average, 25-30 | |
| Post-harvest root shelf-life | Short shelf-life of 1-2 days | If uninfested, roots can keep till one month | |
| Price | RM0.70-R1.00 per kg edible roots; roots for starch extraction usually half the price | RM1.00-RM1.20 per kg; purple- and orange-fleshed cvs. can fetch higher prices | More profitable to grow edible cvs. of cassava |

[1]Much of the information is from [7]
[1]Much of the information is from [8]
[1]Beach ridges interspersed with swales: alternating parallel sandy beach ridges and low depressional areas,

**Table 4.** Performance of MARDI cassava starch cultivars *vs.* commercial cultivar, Black Twig. Values in the same column bearing the same letter are not significantly different from one another at $p = 0.05$ according to Duncan's multiple range test. (Source: [10])

| Cultivar | Fresh root yield (t/ha) | Root starch content (%) |
|---|---|---|
| Sri Kanji 1 | 37.6ab | 26.7a |
| Sri Kanji 2 | 32.2abc | 26.9a |
| MM 92 | 36.4abc | 20.9d |
| Perintis | 40.9a | 22.8c |
| Black Twig | 28.0cd | 25.2b |

planting materials. Virus-free vine cuttings can be produced by tissue culture but this is too expensive and impractical for commercial cultivation. The best alternatives are to practice crop rotation to prevent the build-up of pests and diseases over time, and to produce healthy shoot cuttings from a nursery, i.e. not to take cuttings straight from a harvested field to plant for production. Rotational cropping will require the management of two completely different crops, and stringent crop scheduling.

So far, in Malaysia, sweet potato is grown only for human consumption; thus, there is no price structure for roots to be used for industrial purposes (i.e. starch or feedstuff). Nonetheless, it may be expected that the same price differentiation as encountered by the starch cultivars of cassava will apply.

Nevertheless, sweet potato has several advantages over cassava. Its ability to yield well on sandy soils (with proper fertilizer management) makes it easier to locate land in Malaysia for its cultivation. Fresh root yields of 41 t/ha have been recorded on *bris* soil in Kelantan, compared with 28 t/ha on upland mineral soils (Table 5) [13]. There are still large tracts of *bris* soil and tin-tailings (considered marginal soils) which remain to be exploited for crop production. Furthermore, being a ground creeper, sweet potato is not at all prone to strong winds; indeed, it can even withstand typhoons which flatten most standing crops [14].

The nutritionally more superior purple- or orange-fleshed sweet potato cultivars can be capitalized for producing a wide range of food products – made either straight from the fresh roots, or after preprocessing into puree or flour [15]. If the target market is food-grade starch, sweet potato is at a disadvantage. The presence of more protein than cassava in the roots and the content of anthocyanin or carotenoids result in starch which is less than pure white. Industry specifications for food-grade starch require that the colour be uniform, white and free from pigments [16]. The colour specifications for non-food grade starch are less stringent.

## CONCLUSION

The question of whether cassava or sweet potato would be a better choice can only be answered when the following are taken into consideration:

1. End use – for industrial purposes (starch, feedstuff) or for human food?
2. Availability of suitable land – including edaphic and climatic conditions, and

**Table 5.** Yields (t/ha) and ranking of top five sweet potato cultivars at six sites in four agro-ecologies in Malaysia (after [13]). Site means bearing the same letter are not significantly different from one another at $p = 0.05$ according to Duncan's multiple range test.

| Cultivar | Upland mineral | | *Bris* | | Tin-tailings | Acid sulphate |
|---|---|---|---|---|---|---|
| | Serdang[1] | Bertam[2] | Telong[3] | Kandis[3] | K. Bikam[4] | K. Linggi[5] |
| VitAto | 28.2 (1)* | 16.2 (1) | 41.1 (1) | 39.2 (1) | 23.8 (1) | 36.0 (1) |
| Tainung No. 64 | 18.6 (4) | | 16.0 (3) | | | 28.4 (2) |
| Guan | 23.5 (3) | 6.5 (2) | 18.9 (2) | 36.6 (2) | 17.5 (2) | |
| Caromex | | | | 30.5 (3) | 12.3 (4) | 23.2 (3) |
| Gendut | 25.0 (2) | | | 21.2 (5) | 12.5 (3) | 18.3 (4) |
| Kuala Bikam 2 | 13.4 (5) | | | | | |
| W-154 | | | 14.9 (5) | | | |
| Travis | | 1.9 (5) | 16.0 (4) | | | |
| Julian | | | | | | 18.3 (5) |
| Benihayato | | | | | 10.5 (5) | |
| W-219 | | 5.3 (3) | | 22.7 (4) | | |
| Bugs Bunny | | 3.1 (4) | | | | |
| Site mean | 13.4b | 2.7d | 13.7b | 19.2a | 8.4c | 14.0b |

*Figures in brackets denote ranking.

[1]Serdang in Selangor; [2]Bertam in Seberang Perai; [3]Telong and Kandis in Kelantan; [4]Kuala Bikam in Perak; [5]Kuala Linggi in Melaka.

3. Preferred form of management – monocropping or rotational cropping.

Factor no. 2 should not signify too great a hindrance if the possibility of off-shore investment presents itself. Neighbouring countries (e.g. Vietnam, Cambodia and Myanmar) with tropical climate and abundant land resources (better still, with competitively priced labour) should be considered. Let Malaysia continue with the cultivation of crops for which she has a competitive edge, notably, oil palm and rubber, while other crops with good marketing potential can be produced elsewhere with Malaysian investment.

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# Think Malaysian Act Global: the Autobiography of Academician Dato' Ir. Lee Yee Cheong

ISBN 978-983-9445-47-3
Publisher: Academy of Sciences Malaysia, 2010

*Think Malaysian Act Global* is a testimony of the character and wisdom of Academician Dato' Ir. Lee Yee Cheong, a Foundation and Senior Fellow of the Academy of Sciences Malaysia. It portrays the life of this 'giant of a man' in the Academy who advocates and practises Think Malaysian Act Global.

Lee's remarkable insight deserves attention. It springs from his compassion, his humanity and his own rich life experiences in the pursuit of knowledge; professional engineering career; involvement in local and global academies, institutions in the sciences, technology, history, religion, economics, education, information and communication; as well as accounts of his tough, challenging human nature and character. This classic piece of lieterary prose is a truthful reflection of the rich fabric of his remarkable life.

This 378-page autobiography chronicles the details about the past; dreams for the future on the aspiration of an individual. It narrates vividly the successful science career-path of an engineer and his life's journey in pursuit of excellence. It would make an interesting read particularly for the young who want to learn, experience and experiment with life, and contribute to science, technology and innovation for the betterment of society; nationally and globally.

In the words of the then President of Academy of Sciences Malaysia, Tan Sri Dr Yusof Basiron: "This exposé of experiences (of Dato' Lee) will provide a window for students and stakeholders of the science industry on the importance of science and technology as well as create an excitement to pursue a career in science. This is precisely one of the ideals of the Academy and is consistent with our aims and objectives."

Lee was born in Ipoh, Perak, Malaya (now Malaysia) on 11 May 1937 – a double bull, being a Taurus and in the Year of the Ox. His family is of the Hakka stock. He attended Chinese schools from 1946-1950 (Min Tak Primary School in the Kayin Association building in Belfield Street, Yuk Choy Primary School in Gopeng Road), and St. Michael's Institution, Ipoh 1951-1956. He was a Colombo Plan scholar at the University of Adelaide (1956-1961) where he graduated with a First Class Honours in engineering and winning the Electricity Trust of South Australia prize for electrical power engineering.

For more information about this publication, contact: Academy of Sciences Malaysia, 902-4 Jalan Tun Ismail, 50480 Kuala Lumpur, Malaysia; www.akademisains.gov.my.

*Journal of Science and Technology in the Tropics* (2011) **7**: 128



## Portrait Of A Thousand Smiles: Academician Tan Sri Dato' Seri Dr. Salleh Mohd Nor

ISBN 978-983-9445-55-8
Publisher: Academy of Sciences Malaysia, 2010

Born in 1940 to a poor family in Ulu Inas in Negeri Sembilan, Peninsular Malaysia, Salleh received his early education at Tuanku Muhammad School, Kuala Pilah and the Royal Military College, Port Dickson. The military training at RMC with the motto "Serve to Lead" laid the foundation that served him throughout his career, that is service to the nation with integrity and honesty.

He had his initial tertiary education in Adelaide and Canberra, Australia where he studied forestry and enjoyed himself tremendously doing many other things besides study. His friendly disposition led him to be 'adopted' as the Malaysian son of an Australian Forest worker's family.

Salleh's stint in the Netherlands opened his eyes to the achievements of a small nation and what science and research can do for economic development. His final educational training was in Michigan State University, USA where despite working in a number of positions and raising a family, Salleh managed to complete a Masters and PhD with distinction in four years.

Salleh's international experience covered the four corners of the globe through his involvement with IUFRO, Forest Trends, NATMANCOM, Tropenbos, the CGIAR system, the MPTS Network and APAFRI, among others.

At home, Salleh shares his chanllenges in undertaking the first national forestry inventory, spending months camping in the 'jungles' of Peninsular Malaysia; the trials and tribulations and success in the formation of the Forest Research Institute of Malaysia; the challenges as the longest serving President of the Malaysian Nature Society; being Chief Executive Officer to the World Endurance 2008, the "Olympics" of endurance riding; his brief stint with Transparency International; his experiences with various universities in different capacities; and his involvement in the Academy of Sciences Malaysia and other NGOs, and his limited venture into business.

*Portrait Of A Thousand Smiles* is Salleh's foray into science as science permeates in all that the autobiography presents.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 109-114

# Roto-dynamic faults investigation using acoustic emission technique

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**Abstract** This paper provides a method of acoustic emission (AE) technique to detect roto-dynamic faults of rotating machineries. An approach is to utilize dynamic envelope which was extracted from the raw time-domain signals and processed to its spectrum using Fourier transform. The transformed signals may contain unique characteristic features relating to the various types of bearing faults. The experiments on different operating conditions were investigated and they corresponded to (a) a balanced, and (b) an unbalanced with misaligned shaft. The diagnostic process will discriminate between different bearing conditions. The recognition rate achieved with the limited test sets was promising.

**Keywords** Acoustic emission – roto-dynamic – low speed – bearing

## INTRODUCTION

Bearings can be found on almost all machines. Their failure invariably has production consequences and sometimes even health and safety consequences. A reliable condition monitoring system is therefore highly desirable for it will alleviate the cost of these consequences and enhance the overall equipment effectiveness.

For bearing maintenance, two methods have been used, namely the statistical bearing life estimation and the bearing condition monitoring and diagnostics [1, 2]. The first method relies on a model of the bearing survival probability in terms of the dynamic load rating and the equivalent load to give a prediction of the fatigue life of a bearing [3-5]. However, since operating conditions can vary significantly from one machine to another, the prediction based on the assumption of normal duty on a bearing can be in serious error. The second method, in theory, is superior to the first if the signals monitored have useful features that can reliably indicate a potential failure well ahead of the occurrence of the corresponding functional failure [6-8]. Signals that have been studied for bearing condition monitoring include time and frequency domain of acoustic emission signal.

In this paper, a frequency-domain technique, namely the Fast Fourier Transform (FFT) was assessed in terms of its ability to discriminate between different types of signals that had transient features in them. These signals were collected from different bearing conditions: normal; unbalanced and misaligned shaft.

The objective of this research is to demonstrate that a condition-based monitoring using acoustic emission (AE) can provide not only timely detection of low speed bearing but also the fault identification so that maintenance or replacement can be performed prior to the loss of safety function. Therefore the use of acoustic emission method has been proposed for low speed machineries monitoring instead of the conventional method.

## THE PROPOSED APPROACH

Figure 1 shows a block diagram of the proposed bearing condition monitoring procedure. The acquired AE signals are first filtered and amplified to remove noise and then processed in order to obtain AE envelope signal. Then, the Fast Fourier Transform was used to produce its frequency domain pattern. The frequency domain has a horizontal axis representing frequency and a vertical axis representing the intensity of the frequency component.

The pre-processed parameter of the dynamic envelope of the AE signal can be obtained from AE Ultraspan (Holroyd Instruments, UK) (Fig. 2). The AE Ultraspan is the wireless sensor which provides

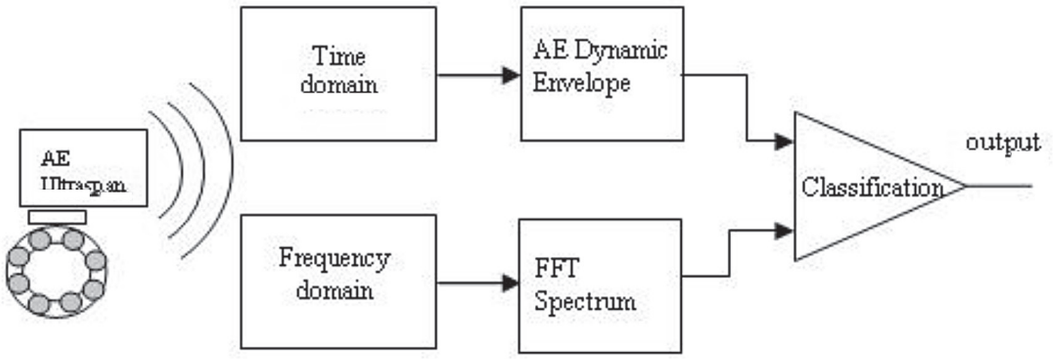


**Figure 1**. The proposed bearing monitoring block diagram.

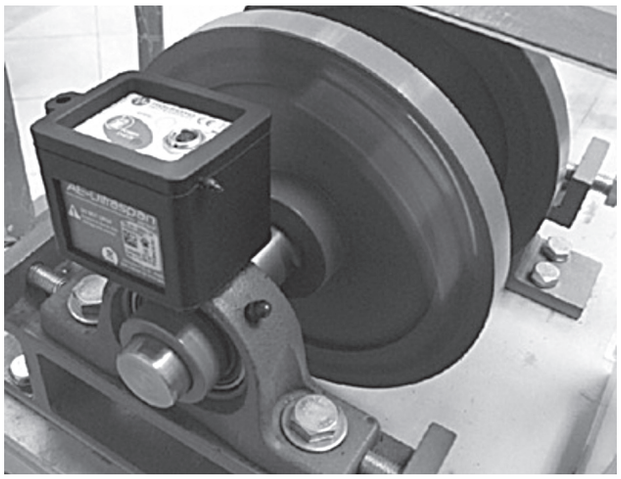

**Figure 2**. AE Ultraspan wireless sensor (Holroyd Instruments, UK).

many opportunities for diagnosing the nature of faults. The time dependence of the AE signal can reveal the occurrence and timing of subtle actions within machineries.

The AE dynamic envelope is suitable for both burst type and continuous AE signals. Effect of signal averaging is to improve statistical significance but distorts shape of activity giving time decays and reduced peak value. It is also represented in logarithmic scale of AE signals since it allows a greater signal range to be simultaneously observed with its unit in dB and is a ratio with respect to a reference voltage [9].

$$AE\ Log\ Magnitude in\ dB = 20\log_{10}\left(V_{sig}/V_{ref}\right)$$

To identify the roto-dynamic faults of low speed machineries, the Fast Fourier Transform is used in this study. The Fast Fourier Transform (FFT) does exactly that [10]. The Fourier transform of a signal x(t) is defined as:

$$F\left[x(t)\right] = X(f) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} x(t)e^{-j2\pi f t}dt$$

For Equation to be valid, the signal x(t) being transformed must be stationary, which means that its amplitude distribution does not depend on absolute time. In other words, the moments of the distribution – for example, mean, variance, and so on – are stationary.

## EXPERIMENTAL APPLICATION OF THE PROPOSED METHOD

The experimental setup consisted of roto-dynamic test rig which can produce multi fault operating conditions (Fig. 3a). Its schematic diagram of the test rig is shown in Figure 3b. The spindle was driven by a variable speed motor running at 1430 rpm. The two bearings were SKF 60062Z deep groove single row ball bearing. They were mounted in bearing housings which in turn were attached to a base plate. The test rig provided facilities to produce the different machine operating conditions characterized by: (1) the rotating shaft dynamically balanced (referred to as 'balanced shaft'); and (2) the rotating shaft dynamically unbalanced in one plane to the extent of 70 x $10^{-5}$ kg.m at roto-disc with misalignment achieved from moving one bearing laterally by 1 mm relative to the other (referred to as 'unbalanced and misaligned shaft').

Acoustic emission signal at the bearing was measured using AE Ultraspan sensor manufactured by Holroyd Instruments, UK (on top of both end and non-drive end housing). The AE sensor had resonance frequency at 100 kHz. The acquired AE signals, having been band-pass filtered at 20 kHz to

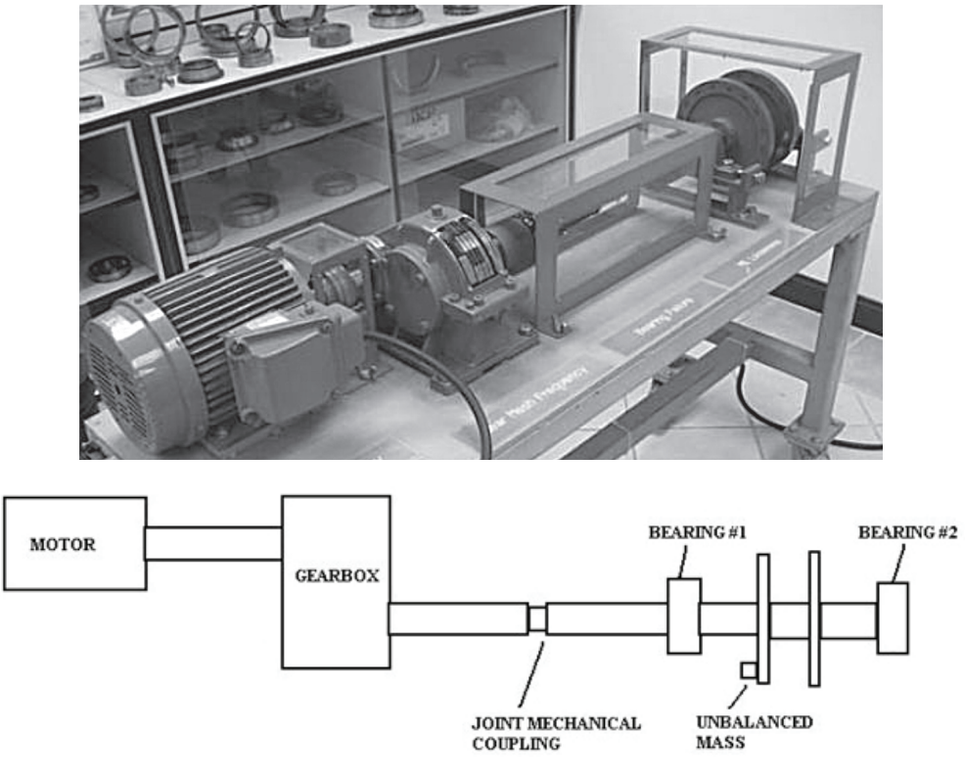


**Figure 3**. Test rig set (top) and test rig schematic diagram (bottom).

500 MHz for noise-removal and amplified to 60 dB, were sampled into a data acquisition card.

Measurements were obtained from different machine conditions: normal, unbalanced and misaligned shaft. For each condition, five signals were collected as for data processing.

## EXPERIMENTAL RESULTS AND DISCUSSION

Figure 4 shows the signal measured on bearing housing from different operating conditions – balanced, unbalanced shaft and misaligned shaft.

In the experiment, the AE dynamic envelope collected from a balanced shaft yielded mean of logarithm intensity about 13 dB whilst the intensity obtained from the unbalanced and misaligned shaft was about 20 dB. With machine fault condition resulting in greater energy release rates they produced higher continuous signal levels (such continuous signals result from the overlapping of many small transient signals).

For unbalanced shaft, the mass unbalanced ($70 \times 10^{-5}$ kg.mm.r product) occurred when the shaft center line and the mass center of the rotor did not coincide. Therefore the unbalanced is a once-per-revolution fault – then it occurs at the frequency of shaft speed (23.80 Hz) – and is sometimes difficult to distinguish from misaligned shaft. However, unbalanced shaft causes a rotating force; the force of misalignment is directional. Mass unbalance has a fixed phase angle with respect to a reference mark on the shaft. The spectrum has low-amplitude higher-order frequency components. In contrast to normal condition when motions are sinusoidal, nonlinear behavior of a bearing in the presence of excessive mass unbalance can cause truncated motions that introduce higher order (2x, 3x) (Fig. 5b).

For misalignment shaft, it can cause a rotating preload in the bearings, shaft and external couplings at the frequency of shaft speed. The magnitude of the resulting wave motion is dependent of the radial stiffness of the components in the system. Severe misalignment can cause nonlinear bearing behavior in one or both directions, depending on asymmetry in the bearing and the stiffness of the base-plate. The nonlinear behavior causes truncated waveforms and/or nonlinearly-generated second and higher orders of shaft frequency (Fig. 5b). The second order component of frequency in cases of severe misalignment can exceed the first order.

The experiments indicated that both AE dynamic envelope and its FFT spectrum of acoustic emission

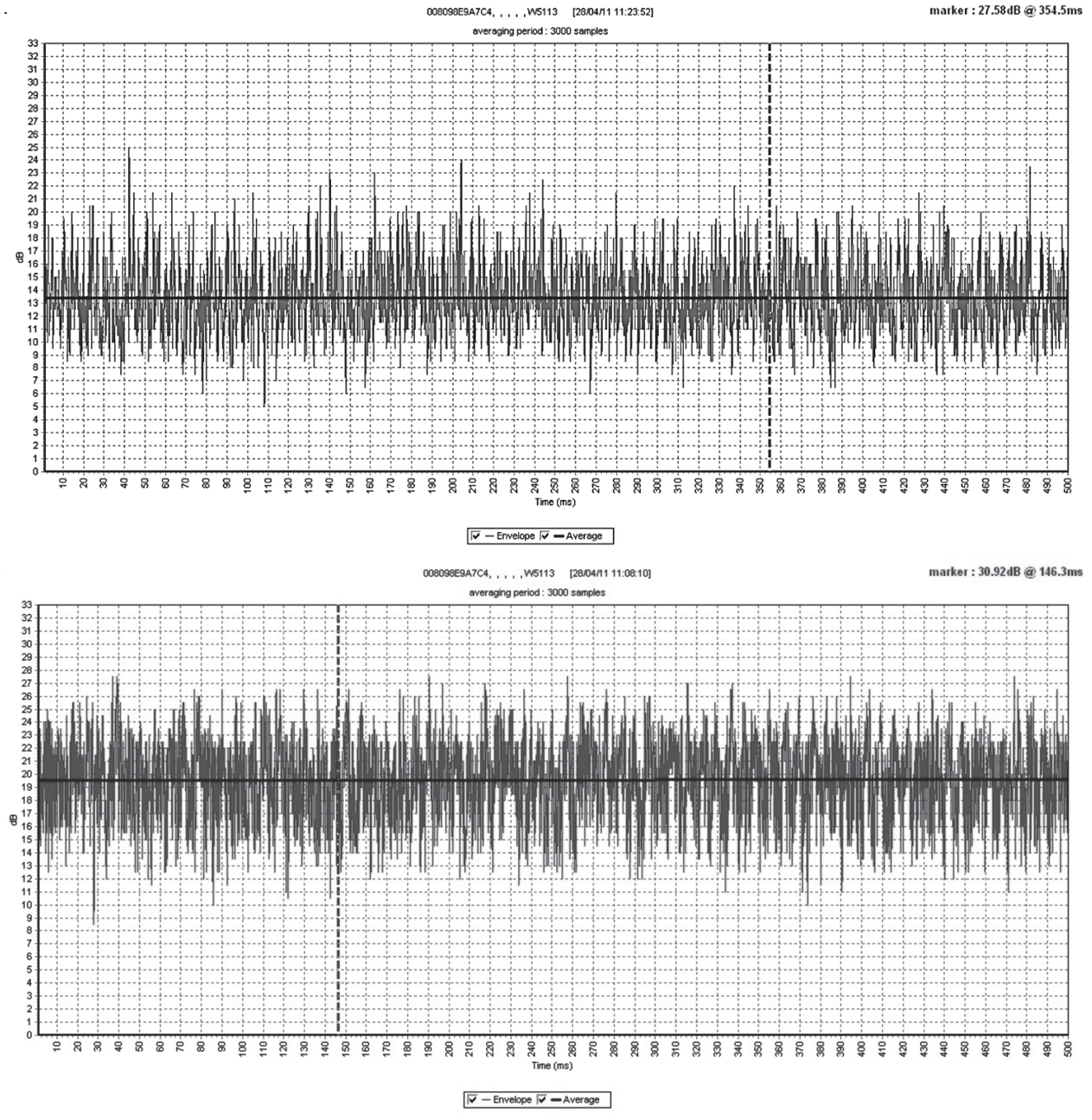


**Figure 4**. Results of AE dynamic envelope from balanced shaft (top), and unbalanced and misaligned shaft (bottom).

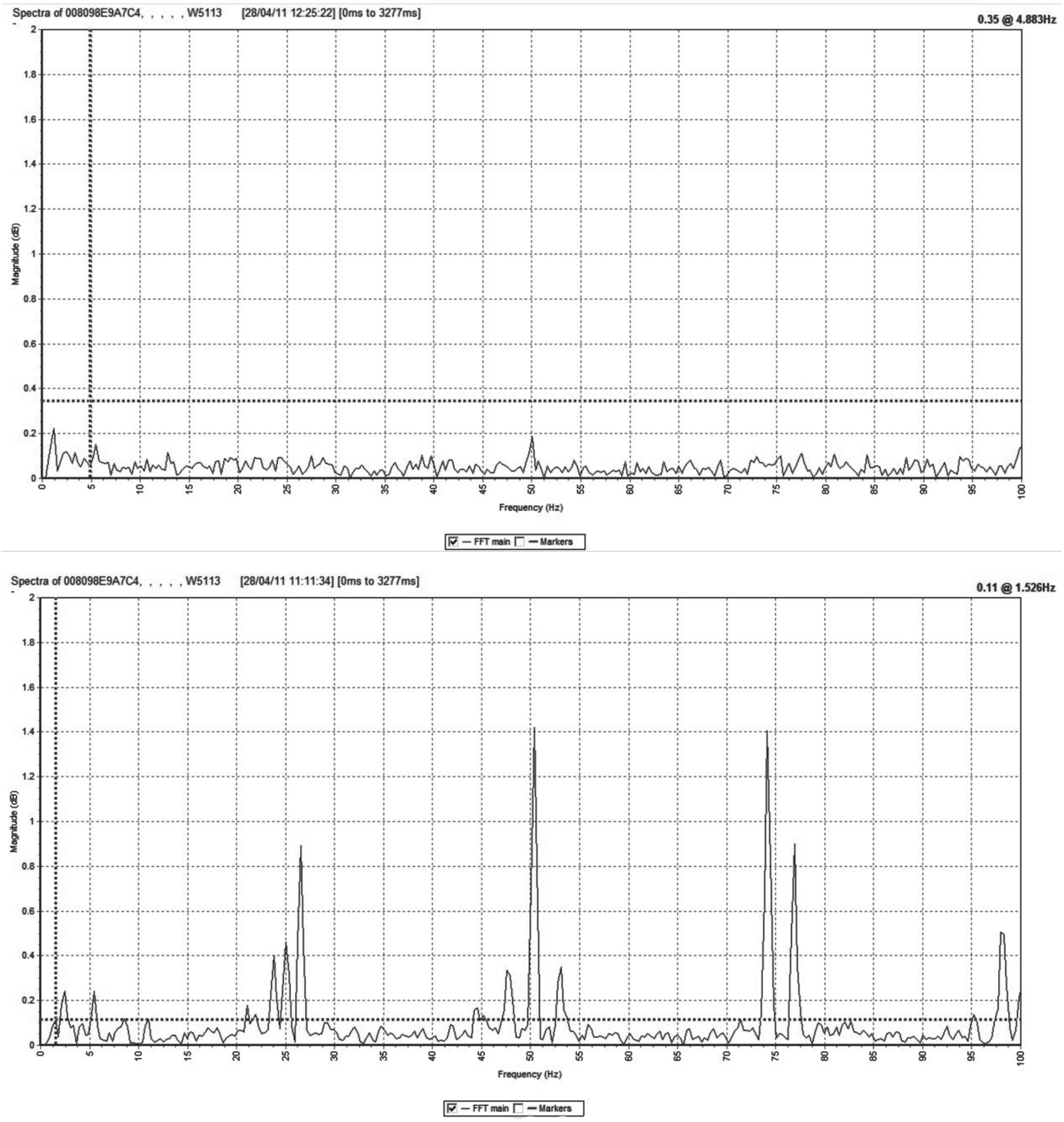


**Figure 5**. Results of spectrum from balanced shaft (top), and unbalanced and misaligned shaft (bottom).

signals from different roto-dynamic machine conditions – normal, unbalanced and misaligned bearing – produced distinct pattern of signals that could be discriminated by using its spectrum. The result was promising with 100 % recognition rate.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 121-125

# Structure activity relationship of flavonoid derivatives from two *Artocarpus* species as inhibitors of leukemia and breast cancer cells

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*Received 29-09-2011; accepted 3-10-2011*

**Abstract** Structure-activity relationship of a series of flavonoid compounds from Artocarpus kemando and Artocarpus odoratissimus were studied to clarify the structural requirement for inhibition of HL-60 and MCF-7 cancer cell lines by these compounds. The flavonoids are cycloartobiloxanthone (**1**), artomandin (**2**), artonol B (**3**) and artosimmin (**4**). Comparisons of these related flavonoids indicated that the prenyl and hydroxyl substituent groups, as well as the type of substitution pattern at A-ring and B-ring of tricyclic flavonoid skeleton were crucial for the inhibition of these cancer cells in MTT assays.

**Keywords** flavonoids – *Artocarpus kemando* – *Artocarpus odoratissimus* – cytotoxicity – HL-60 – MCF-7

## INTRODUCTION

Flavonoid constituents are one of the most abundant and diverse groups of natural products. They play an important role in the chemotaxonomy of the higher plant families (Coradin *et al*., 1985). The various classes of flavonoids differ in their level of substitution of the C ring of the basic benzopyrone structure [1]. Depending on the substitution of ring C, they are classified into six major subclasses: flavones, flavanols, flavanones, chalcones, isoflavones and anthocyanidins.

Previous studies on flavonoids from this plant and other species of *Artocarpus* have revealed them to exhibit a wide range of pharmacological activities such as anti-inflammatory [2], antioxidative [3], antiplatelet aggregation [4], 5α-reductase activities [5], inhibition of cathepsin K [6], and cytotoxicity [7]. Moraceae species have been reported to be rich in flavonoid derivatives [8]. Our research on *Artocarpus kemando* and *Artocarpus odoratissimus* has successfully led to the isolation of two furanocycloartobiloxanthone **(1-2)**, one xanthonolide **(3)** and a prenylated pyranoflavone **(4)**. These compounds were tested for their cytotoxic activities using HL-60 and MCF-7 cell lines. Until now, there is no information available on the pharmacological activities of flavonoids **1-4** on both types of cancer cells.

## MATERIALS AND METHOD

### Plant material

The stem bark of *A. kemando* and *A. odoratissimus* were collected from Sri Aman and Bintulu, Sarawak, Malaysia respectively.

### General

Infrared spectra were measured in KBr/NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500MHz NMR/JEOL 400MHz FT NMR spectrometer using tetramethylsilane

(TMS) as internal standard. Ultra violet spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer.

**Extraction and isolation**

The milled, air-dried stem bark (4.7 kg) of *A. kemando* was defatted with n-hexane and extracted exhaustively using ethanol, acetone and then methanol for more than 48 hours at room temperature. This gave 23.3 g, 50.2 g, 98.6 g and 198.5 g of hexane, ethanol, acetone, and methanol extracts respectively. The methanol extract was partitioned using chloroform to afford a 20.0 g extract. Solvent extractions on the dried powdered stem bark of *A. odoratissimus* (3.5 kg) gave hexane (6.0 g), chloroform (15.0 g), ethyl acetate (42.5 g) and ethanol (12.8 g) extracts. The ethanol extract of *A. kemando* was chromatographed using silica gel vacuum column chromatography using a stepwise gradient system (hexane/chloroform, chloroform / ethyl acetate, ethyl acetate /acetone and methanol) to give 20 fractions (250 mL). Fractions with similar profile on TLC were combined to give six major fractions. A portion of fraction 4 (2.57 g) was repeatedly chromatographed to give 19 subfractions. This resulted in cycloartobiloxanthone (**1**). Similarly, several fractionations of the acetone extract (45.0 g) of *A. kemando* by silica gel column and radial chromatography gave artomandin **(2)**. Similar column chromatographic separation as above on the chloroform extract (20.0 g) of *A. kemando* over silica gel yielded artonol B **(3).** The ethyl acetate extract (42.5 g) of *A. odoratissimus* gave artosimmin **(4).**

**Cycloartobiloxanthone (1)**

Dark yellow solid; mp 283-285 $^oC$ (Lit. 285-287 $^oC$ [9]; UV (MeOH) $\lambda_{max}$ (log ε) nm: 229 (4.31), 281 (4.31), 392 (4.02); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3434 (OH br), 2976, 2932 (C-H stretching), 1650 (C=O chelating), 1560, 1476 (C=C aromatic), 1358 ($CH_3$ alkane bending), 1272, 1160 (C-O); EIMS *m/z* (rel. int.): 434 [$M^+$, $C_{25}H_{22}O_7$]; FABMS *m/z* 435 [$(M+H)^+$, $C_{25}H_{23}O_7$]. $^1H$ and $^{13}C$ NMR spectral data are in agreement with published data [9].

**Artomandin (2)**

Yellow solid; mp 288-290 $^oC$; UV (MeOH) $\lambda_{max}$ (log ε) nm: 206 (3.89), 230 (3.91), 283 (3.93), 393 (3.64); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3351 (OH br), 2916, 2849 (C-H stretching), 1646 (C=O chelating), 1556, 1465 (C=C aromatic), 1348 ($CH_3$ alkane bending), 1272, 1159, 1017 (C-O); EIMS *m/z* (rel. int.): 434 [$M^+$, $C_{25}H_{22}O_7$]. $^1H$ NMR and $^{13}C$ NMR data are in agreement with published data [10].

**Artonol B (3)**

Fine yellow solid; mp 189-194 $^oC$ (Lit. 189-196 $^oC$ [11]; UV (MeOH) $\lambda_{max}$ (log ε) nm: 233 (2.39), 278 (2.35), 359 (2.24); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3423 (OH), 2969, 2921, 2851 (CH stretching), 1770 (C=O acetoxyl), 1717 (C=O cyclic), 1651 (conjugated C=O), 1606, 1581, 1479 (C=C aromatic), 1361, 1109 (C-O) ; EIMS *m/z* (rel. int.): 420 [$M^+$, $C_{24}H_{20}O_7$]. $^1H$ and $^{13}C$ NMR spectral data are in agreement with published data [11].

**Artosimmin (4)**

Yellow solid (95 % chloroform/ 5 % methanol); mp 213-215 $^oC$; UV (MeOH) $\lambda_{max}$ (log ε) nm: 213 (4.16), 271 (3.72), 340 (3.42); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3527 (OH), 2853 (C-H stretching), 1734 (C=C unsaturated), 1653 (conjugated C=O), 1598, 1567, 1513, 1488, 1447 (C=C aromatic), 1306, 1239 (C-O); EIMS *m/z* (rel. int.): 436 [$M^+$, $C_{25}H_{24}O_7$]; $^1H$ NMR and $^{13}C$ NMR spectral data are in agreement with published data [12].

**Cancer cell-lines culture**

Two human cancer cell lines were used. They were HL-60 and the IRM-32 cell lines. Both of the cell lines were obtained from the National Cancer Institute, Maryland, USA. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5 % Fetal Bovine Serum (FBS), 100 IU $mL^{-1}$ penicillin and 100g/mL streptomycin by using 25 $cm^2$ flask in a 37 $^oC$ incubator with 5% $CO_2$.

**Cytotoxicity and MTT assay**

Cytotoxic assay was carried out using the microculture 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay as described previously [13]. The exponentially growing cells were performed by suspending the cells in 100 µL of stock culture ($1 \times 10^5$ cells $mL^{-1}$) per well which were plated in a 96-well plate and incubated for 24 hours at 37 $^oC$ under humidified 5% $CO_2$. The stock solution was prepared by dissolving the sample in absolute ethanol (EtOH) to a final concentration of 1 mg/mL. Serial dilutions of the stock solution in the growth medium to produce seven sample solutions at concentrations of 0.47, 0.94, 1.88, 3.75, 7.50, 15.00,

30.00 µg/mL. The growth medium was removed from the wells. The cells in each well were treated with 100 µL of varying concentrations of test specimens in their respective medium. The positive control was made containing only untreated cell population in 100 µL of growth medium. Each concentration of sample was tested in triplicate and allowed to proceed for 72 hours at 37 °C in 90% humidified 5% $CO_2$ atmosphere. Then, 100 µL of MTT stock solution (5 mg in 1 mL PBS) was added to each well to determine the fraction of adherent cells relative to the untreated cell population. The plates were further incubated for 4 hours at 37 °C. 100 µL of EtOH was added to each well to dissolve the water-insoluble purple formazan crystal. After 30 minutes, the absorbance (OD) of the samples and the reference was measured by using ELISA spectrophotometer microplate reader at wavelength 550 nm. The inhibition concentration of 50% reduction (killed cells) in cell number, $IC_{50}$ was appraised visually to determine the absorbance (OD) against concentration curve.

## RESULTS AND DISCUSSION

Flavonoids which are derived from the tricyclic flavones are a relatively homogeneous group of constituents in the *Artocarpus* species [8]. In this report, the flavonoids tested include modified flavonoid and rearranged flavonoid groups. They reveal an interesting trend of cytotoxic effects on HL-60 and MCF-7 cell lines with $IC_{50}$ values between 1.0 and 8.0 µg/mL and between 3.0 and 14.0 µg/mL respectively. However, these cytotoxic results show significant dissimilarity in inhibition effect probably due to the nature of the substituents and the substitution pattern at A-ring and B-ring of the tricyclic flavonoid skeleton. Structurally, these include 2',4'-dioxygenated and 3',4'-dioxygenated furanodihydroxanthone **(1-2)**, 3',4'-dioxygenated pyranoflavone **(4)**, as well as xanthonolide **(3)** flavonoids. The screening results of these compounds are summarized in Table 1.

Of the compounds tested, it is interesting to note that artosimmin **(4)**, cycloartobiloxanthone **(1)** and artomandin **(2)** are strongly active cytotoxic compounds towards HL-60 (1.1, 5.7 and 2.4 µg/mL) and MCF-7 (3.4, 13.4 and 3.1 µg/mL) cell lines, respectively. The activity might be due to the ortho or meta positions of the dihydroxyl moieties at the B-ring. The assays also indicated both artosimmin **(4)** and artomandin **(2)** which are 3',4'-dioxygenated at the B-ring of the structure exhibited a stronger cytotoxic activity when compared to that of **1** which has 2',4'-dioxygenated substitution. Hence, it is deduced that flavonoids with the presence of 2',4'- and 3',4'- positions of free hydroxyl moieties at B ring is inclined to exhibit a prominent inhibitory activity. Furthermore, the only example of a 5'-(3-methylbut-2-enyl) pyranoflavone type compound tested which is artosimmin **(4)** also demonstrates strong cytotoxic properties. From the structure-activity relationship study, compounds such as cycloartobiloxanthone **(1)**, artomandin **(2)** and artosimmin **(4)** consisting of C-3 isoprenyl unit substitution, gave a good degree of inhibition towards HL-60 cell line.

The C-3 isoprenyl unit substitution always tends to form a carbocyclic ring or an oxygen-bearing fused B-ring and C-ring, respectively [14]. Thus, it is suggested that the 3-prenyl flavone skeleton is a necessary requirement for the cytotoxic activity towards HL-60 and MCF-7 cell lines. In addition, it is observed that the modified and totally opened B-ring of flavonoids such as artonol B **(3)** tend to reduce the degree of inhibition effect towards the MCF-7

**Table 1**: Cytotoxic activities of **1-4** against HL-60 and MCF-7 cell lines.

| Compound | Type | HL-60 | MCF-7 |
|---|---|---|---|
| | | $IC_{50}$ ± SD (µg/mL) | $IC_{50}$ ± SD (µg/mL) |
| **1** | Furanodihydrobenzoxanthone | 5.7 ± 1.7 | 13.4 ± 2.4 |
| **2** | Furanodihydrobenzoxanthone | 2.4 ± 0.5 | 3.1 ± 0.4 |
| **3** | Xanthonolide | 7.2 ± 1.6 | > 30.0 |
| **4** | Pyranoflavone | 1.1 ± 0.1 | 3.4 ± 0.3 |
| [1]Goniothalamin | | 1.4 | - |
| [2]Tamoxifen | | - | 3.1 |

[1]positive control of HL-60 cell line; [2]positive control of MCF-7 cell line; - not tested.
Note:*$IC_{50}$ < 5.0 µg/mL = strong inhibition activity. *5.0 ≤ $IC_{50}$ ≤ 25.0 µg/mL = moderate inhibition activity. *$IC_{50}$ > 25.0 µg/mL = weak inhibition activity. [13]

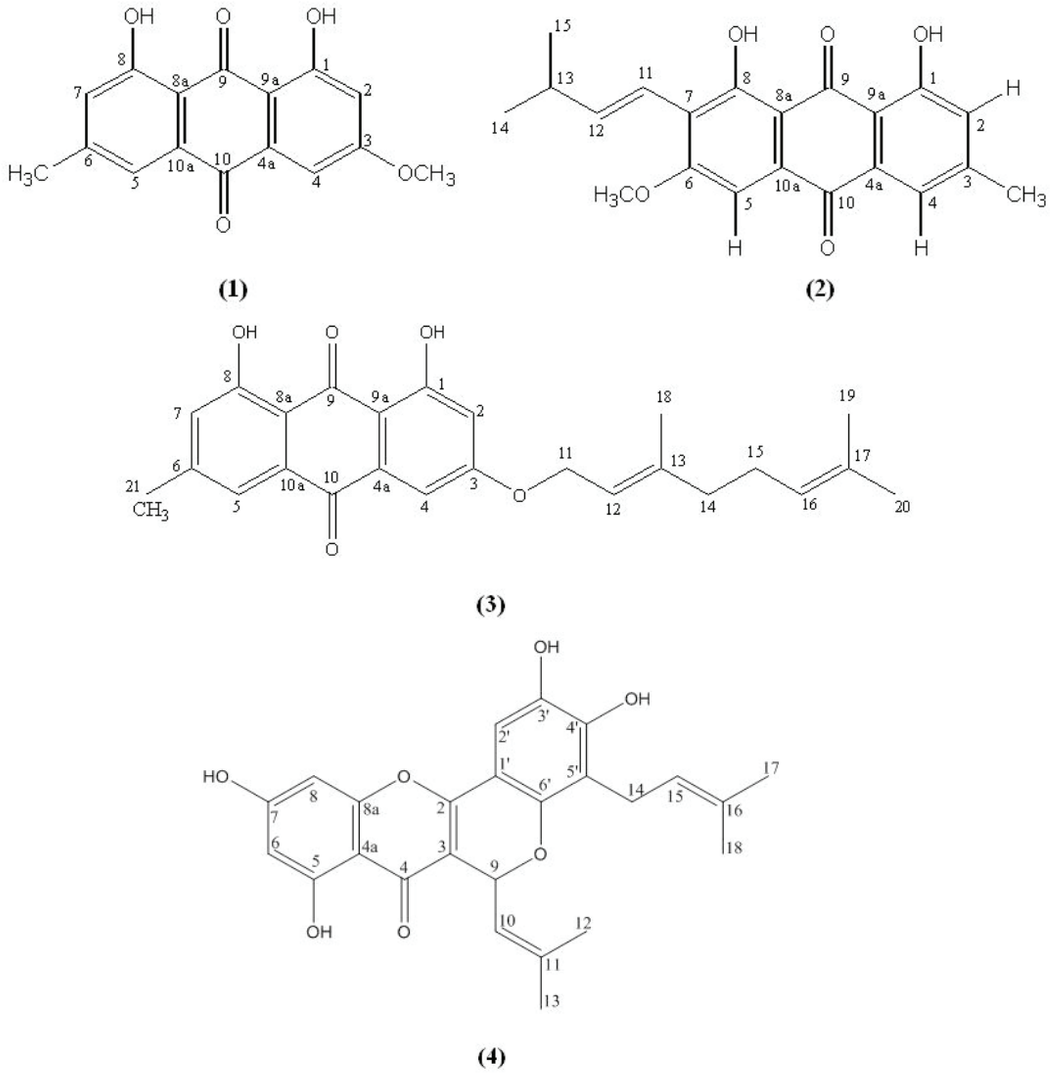


**Figure 1**. Structures of flavonoids.

cell line. However, **3** exhibited moderate cytotoxic activities towards HL-60 cell line with $IC_{50}$ values less than 10.0 µg/mL.

In summary, the unsaturated prenyl side chain (3-methylbut-2-enyl) at C-3/ C-5' and the presence of the 2',4'- and 3',4'- positions of free hydroxyl moieties at B-ring of flavonoid derivatives contribute to the prominent inhibitory activity. Hence, it can be concluded that flavonoids with 3, 5'-prenylated skeleton and are 2',4'- or 3',4'-dioxygenated might be lead compounds for HL-60 and MCF-7 cancer cell lines, respectively.

**Acknowledgements** – We thank the Malaysian FRGS grant for financial support, Assoc. Prof. Dr Jegak Uli for collection of plant samples and Mr Johadi Iskander for recording NMR spectra.

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December 2011
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Journal of Science & Technology in the Tropics Volume 7 Number 2 December 2011

JOSTT
DEDICATED TO THE ADVANCEMENT OF SCIENCE AND TECHNOLOGY RELATED TO THE TROPICS

Journal of Science & Technology in the Tropics

AKADEMI SAINS MALAYSIA

COSTAM

Volume 7 Number 2
December 2011
ISSN 1823-5034
9 771823 503009

Journal of
Science &
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in the Tropics Volume 7 Number 2 Dec 2011

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2. Beveridge W.I.B. (1961) *The Art of Scientific Investigation*. Mercury Book, London.
3. Berryman A.A. (1987) The theory and classification of outbreaks. In Barbosa P. and Schultz J.C. (eds.) *Insect outbreaks* pp. 3-30. Academic Press, San Diego.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 67

# EDITORIAL

## Mahathir Science Award

The Academy of Sciences Malaysia (ASM) at its 53rd meeting in February 2004 approved the establishment of the Mahathir Science Award to encourage scientists to address problems of the tropics using science and technology. This award was in appreciation of Y.A.Bhg. Tun Dr. Mahathir and his contribution in the establishment of ASM and his support to S&T in the country during his tenure as Prime Minister. The award was established and launched in conjunction with ASM's tenth Anniversary celebrations on the 17th August 2004. The award is to be bestowed on any scientist, institution or organization worldwide in recognition of contributions to solving problems in the tropics through science and technology. It was the hope of ASM Council that this award would set a new benchmark for scientific research in the country.

There are four categories to the award, viz. tropical medicine, tropical agriculture, tropical architecture and engineering, and tropical natural resources. One award is conferred each year covering any of the four categories. The prize is RM100,000.00, a gold medal and a certificate. The scientific criteria for the award are scientific breakthrough, impact of that breakthrough and solving problems of the tropics. A stringent vetting process by an evaluation committee is held comprising Fellows of the ASM, an international panel of experts and invited Nobel Prize winners. Submissions close in March each year.

The recipients of the award to-date are: **2005** – Professor John Sheppard Mackenzie of Australia "for his contribution in solving the problems related to Japanese encephalitis virus" in the field of tropical medicine; **2006** – Faculty of Medicine, University of Malaya "for outstanding contribution to the understanding and treatment of the Nipah virus" in the field of tropical medicine; **2007** – Professor Joseph S.M. Peiris of Sri Lanka "for his discovery of the aetiological agent causing SARS leading to the understanding of pathogenesis and epidemiology of the disease in 2003" in the field of tropical medicine; **2008** – Professor Gurdev Singh Khush from India "for his perseverance, leadership, commitment and revolutionary work in systematically directing and developing several rice varieties that have overwhelmingly contributed towards reducing global hunger" in the field of tropical agriculture; **2009** – Forest Research Institute Malaysia "for technology and development of the rubberwood furniture industry in Malaysia and globally" in the field of tropical natural resources; **2010** – no winner; and **2011** – Professor Yuan Long-Ping from China in recognition "of his courage in independent thinking in rice breeding resulting in the innovative development of the hybrid rice, that has revolutionized rice production and sustainability" in the field of tropical agriculture.

It is thus hoped that the ASM Award for Scientific Excellence in Honour of Tun Dr. Mahathir will help spur Malaysian scientists in particular to achieve excellence in R&D and in so doing help develop the scientific research culture in the country.

**Salleh Mohd. Nor and Ong Eng Long**
Co-Chairman, JOSTT

*Journal of Science and Technology in the Tropics* (2011) **7**: 69-74

# Producing energy from root crops in the humid tropics

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**Abstract** The world's depleting sources of fossil fuel and the burgeoning demand for animal protein in the new economies have created huge markets for alternate fuel and livestock feed. In the humid tropics, root crops such as cassava (*Manihot esculenta*) and sweet potato (*Ipomoea batatas*) have the potential of tapping into these markets. The question that often arises is which is a better crop choice? Both these food crops have advantages and disadvantages in terms of their botany, nutritional value and agronomic parameters. Chief among the shortcomings in cassava are its relatively long cropping cycle (and thus, its slow rate of returns), its proneness to lodging, its short post-harvest shelf life, and in Malaysia the scarcity of suitable land for its large-scale cultivation. In the case of sweet potato, its susceptibility to serious pests (weevil and vine borer) and disease (sweet potato virus disease) requires that it be cultivated in rotation with other short-term crops. In the final analysis, the choice depends on the intended end use of the crop, availability of suitable land, and the preferred cropping system and management.

**Keywords** biofuel – feedstuff – cassava – sweet potato – humid tropics

## INTRODUCTION

With the current worry over depleting fossil fuels in the world, several countries have embarked on the production of ethanol from plant carbohydrate sources to replace petrol. The most successful to date is Brazil, a country with more than 30 years' experience in producing ethanol from sugarcane as a biofuel. USA went the way of producing ethanol from corn, not the wisest of crop choices given the rather dismal net energy ratio (number of units of energy produced from one unit of energy expended) and reduction in greenhouse gas emissions *vis-a-vis* that of sugarcane (Table 1) [1-3]. Nevertheless, USA has had a long history of producing corn on a large scale and is good at it; thus, what is lacking in the conversion department is made up for by the sheer volume of production.

Similarly, a number of countries are giving attention to producing ethanol from cassava, a highly productive and vigorous root crop. Among these are Thailand, Australia and China. While cassava

**Table 1.** Net energy ratios and greenhouse gas emission for selected materials used in ethanol production. (Adapted from [1], [2], [3])

| Material | Net energy ratio | Greenhouse gas emission | |
|---|---|---|---|
| | | kg/L | Reduction |
| Sugarcane | 8.3 | 1.08 | 56% |
| Sugarcane (data from Thailand) | 9.3-10 | n/a | n/a |
| Sugarbeet | 1.9 | n/a | 35-56% |
| Corn | 1.35-1.5 | 1.94 | 22% |
| Corn (data from Thailand) | 4-5.2 | n/a | n/a |
| Cassava (data from Thailand) | 8-9.1 | 0.84 | 63% |
| Cellulose from corn stover | 4.39 | n/a | n/a |
| Cellulose from switchgrass | 8.3 | n/a | n/a |
| Cellulosic ethanol | 2-36 | 0.23 | 91% |
| Petrol | 1.0 | 2.44 | 0% |

grows well in the tropics between the latitudes 30°N and 30°S, it is very susceptible to frost. This latter characteristic has made it difficult for China (where there is a phenomenal growth in demand for petrol-driven vehicles) to embark at full steam on growing cassava for ethanol production. However, because the market for cassava in China is huge, there is increasing interest among Malaysian entrepreneurs to tap into this demand.

The world demand for energy-rich feed ingredients is also growing in tandem with the burgeoning appetite for meat in those countries showing strong economic growth. The chicken and pig industries have traditionally used corn as an energy source in feeds, but with substantial amounts being channelled towards biofuel production coupled with the unprecedented demand for poultry and pork in recent years in the emerging economies, there is unsurprisingly a shortfall. To fill this deficit, other carbohydrate sources are being used increasingly by feed millers.

In the Malaysian context, the chicken and pig production sectors have always depended on imported corn. In this equatorial country, the growing of grain corn faces many production problems, not the least of which is the unpredictability of rain – either too much or too little, and not coinciding with the needs of the crop at its different stages of growth [4, 5]. In contrast, carbohydrate-rich tropical root crops, such as cassava and sweet potato, grow very well in the humid tropics, and have been proven to be possible replacers of corn.

Thus, we are faced with the enviable situation of two fast-emerging markets – the biofuel market and the livestock feed market. Which then will be a better crop choice – cassava or sweet potato?

## BOTANY

Both cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* (Lam.) L.) are root crops, belonging to the families Euphorbiaceae and Convolvulaceae, respectively. Cassava has a semi-woody erect habit while sweet potato is a herbaceous creeper. Although both can persist perennially, they are cultivated as annuals. The storage organs of these crops are swollen adventitious roots; however, those of sweet potato form adventitous buds which allow the storage roots to be used as propagules. In the temperate zone, sweet potato roots are overwintered and sprouted in the spring to plant the new crop. More commonly in the tropics, stem cuttings are used as planting materials. In the case of cassava, the woody section of the stems is used, whereas shoot tip cuttings are preferred for sweet potato.

Having a cropping cycle of 9-14 months (9-10 months for the edible type; 12-14 months for the starch type), cassava is restricted in cultivation to the tropics and subtropics. In contrast, sweet potato is harvested after 3½ to 4 months, which means it can be planted in the temperate zone during the summer months.

## NUTRITIONAL VALUE

Sweet potato and cassava are starch crops, accumulating these complex carbohydrates in the storage roots (hereinafter to be referred to as 'roots' in this paper). The roots of both have low protein and lipid contents. Although sweet potato has generally a higher protein content than cassava, these crops are low in the sulphur-bearing amino acids such as cysteine and more importantly methionine, an essential amino acid (Table 2) [6]. Methionine has a detoxifying effect which is of particular relevance in cassava with its propensity to produce HCN (see below).

Cassava tissues contain an anti-nutritional factor in the form of cyanogenic glucosides, mainly linamarin. When the cells are ruptured, the inherent enzyme, linamarase, is released which reacts with linamarin to produce hydrogen cyanide (HCN), a highly toxic gas. Cultivars of cassava which have a high content of linamarin in the root pith (releasing >50 mg of HCN per kg fresh roots) are classified as the non-edible type, while those having a lower linamarin content are considered edible. High starch cultivars tend to be non-edible. Nevertheless, the leaves and root cortex of all cultivars have a high capacity of producing HCN. Being highly soluble and volatile, HCN is easily removed during starch extraction and processing, as well as when the roots are chipped and dried for use a livestock feedstuff.

The anti-nutritional factor in sweet potato is a trypsin-inhibitor which interferes with protein metabolism. Fortunately, this antitrypsin factor is heat labile and so can be effectively removed, as when the roots are boiled. Furthermore, it is possible to breed for cultivars which contain less trypsin-inhibitor.

Highly coloured sweet potato cultivars have

**Table 2.** Nutrient composition of cassava and sweet potato (dry weight basis). (Source: [6])

| Nutrient | Unit (per 100g) | Cassava[1] | Sweet potato[1] |
|---|---|---|---|
| **Proximates** | | | |
| Water | g | 12.0 | 12.0 |
| Energy | kcal | 349 | 333 |
| Protein | g | 2.97 | 6.08 |
| Total fat | g | 0.61 | 0.19 |
| Ash | g | 1.35 | 3.83 |
| Carbohydrate, by difference | g | 83.07 | 77.93 |
| Dietary fibre, total | g | 3.9 | 11.6 |
| **Minerals** | | | |
| Calcium, Ca | mg | 35 | 116 |
| Iron, Fe | mg | 0.59 | 2.36 |
| Magnesium, Mg | mg | 46 | 97 |
| Phosphorus, P | mg | 59 | 182 |
| Potassium, K | mg | 592 | 1305 |
| Sodium, Na | mg | 31 | 213 |
| Zinc, Zn | mg | 0.74 | 1.16 |
| Copper, Cu | mg | 0.218 | 0.585 |
| Manganese, Mn | mg | 0.838 | 0.999 |
| Selenium, Se | mcg | 1.5 | 2.3 |
| **Vitamins** | | | |
| Vitamin C | mg | 45.0 | 9.3 |
| Thiamin | mg | 0.190 | 0.302 |
| Riboflavin | mg | 0.105 | 0.236 |
| Niacin | mg | 1.864 | 2.157 |
| Panthotenic acid | mg | 0.234 | 3.099 |
| Vitamin B-6 | mg | 0.192 | 0.810 |
| Folate, total | mcg | 59 | 43 |
| Vitamin B-12 | mcg | 0.00 | 0.00 |
| Vitamin A, IU | IU | 28 | 54950 |
| Vitamin A, RAE | mcg_RAE | 2 | 2746 |
| Vitamin E | mg | 0.42 | 1.01 |
| Vitamin K | mcg | 4.2 | 7.0 |
| **Amino acids** | | | |
| Tryptophan | g | 0.042 | 0.120 |
| Threonine | g | 0.061 | 0.322 |
| Isoleucine | g | 0.059 | 0.213 |
| Leucine | g | 0.085 | 0.356 |
| Lysine | g | 0.096 | 0.256 |
| Methionine | g | 0.024 | 0.112 |
| Cysteine | g | 0.061 | 0.085 |
| Phenylalanine | g | 0.057 | 0.345 |
| Tyrosine | g | 0.037 | 0.132 |
| Valine | g | 0.076 | 0.333 |
| Arginie | g | 0.299 | 0.213 |
| Histidine | g | 0.044 | 0.120 |
| Alanine | g | 0.083 | 0.298 |
| Aspartic acid | g | 0.172 | 1.480 |
| Glutamic acid | g | 0.450 | 0.600 |
| Glycine | g | 0.061 | 0.244 |
| Proline | g | 0.072 | 0.201 |
| Serine | g | 0.072 | 0.341 |
| **Others** | | | |
| ß-carotene | mcg | 18 | 32,957 |

[1]Converted to 12% moisture content from values for the fresh roots.

desired anti-oxidant properties; the purple-fleshed cultivars contain anthocyanin, while the orange ones are high in ß-carotene.

## AGRONOMY

The pros and cons of planting cassava or sweet potato can best be appreciated when their agronomic parameters are tabulated (Table 3) [7-9].

## CASSAVA OR SWEET POTATO?

Agronomically speaking, cassava is a crop which is easier to manage. In Malaysia, it has relatively fewer pests and diseases, which seldom cause significant yield loss. Indeed, mammalian pests such as rats (and monkeys, wild boar and elephants in fields adjoining jungle) can cause more damage. This means it is possible to grow cassava year in year out without loss in yield, provided that the correct amount and type of fertilizers (according to the soil type) are applied for every crop.

One of the major shortcomings of cassava is its long cropping cycle despite its average root yield (see also Table 4 [10]) being only slightly better than sweet potato, resulting in a slower rate of returns. The latter can be offset by intercropping cassava with a shorter term crop such as sweet corn or groundnut [11]. The longer cropping cycle also excludes its cultivation in areas affected by annual floods during the months of the north-east monsoon (mainly the East Coast of Peninsular Malaysia). Having an erect plant habit (growing up to 2.4 metres or more in height), cassava is also prone to lodging in windy areas.

In the humid tropics, it is possible to crop the year round, thus ensuring a constant supply of roots to the processing plant. To keep a small starch factory in constant operation and running a single 8-hour shift, it has been estimated that 1500-2000 ha of land are required for staggered planting and harvests throughout the year [12]. However, suitable land for cassava in such large expanses seems to be no longer available in Peninsular Malaysia. It should also be remembered that the starch cultivars fetch only half the price of the edible cultivars, but then the market for starch (and feedstuff) is far larger.

Sweet potato is plagued by several important insect pests and viruses. While insect pests can be managed to some extent by chemical means, viruses can only be kept under control by the use of virus-free

**Table 3.** A comparison of the agronomic parameters of cassava and sweetpotato in the humid tropics.

| | Cassava[1] | Sweet potato[2] | Remarks |
|---|---|---|---|
| Preferred soil type | Sandy loam to loam | Sandy loam to loam | Sweet potato performs very well in sandy soils (e.g. *bris*[3] and ex tin-mining land), with adequate fertilizer inputs |
| Topography | Less than 6% gradient | Less than 6% gradient | Soil disturbance during the harvest of root crops causes severe soil erosion on a slope |
| Land preparation | Two rounds of ploughing, one round of rotortilling | Two rounds of ploughing, one round of rotor-ridging | Planting on ridges/beds is essential for storage root development in sweet potato |
| Planting materials | Mature woody stems stacked vertically in the shade can remain viable up to one month | Vine cuttings need to be planted within a few days | Vegetative planting materials are particularly prone to harbouring pests and diseases. Caution should be exercised when introducing planting materials from other countries. |
| Nursery preparation | No need; cuttings can be taken straight from the harvested crop | Required to raise good quality planting materials; cuttings taken from nursery at 2-2½ months | |
| Irrigation | Not required generally | Important in the first 2 weeks after planting | If planted on sandy soils, sweet potato requires irrigation throughout the crop duration |
| Weed control | Pre-emergence<br>Post-emergence at late stage and near root harvest | Pre-emergence | Once established sweet potato vines quickly cover the beds completely, precluding further weed control. Cassava canopy thins down at the later crop stage, allowing light through to the soil surface, resulting in weed growth. |
| Growing cycle | 9-10 months for edible cvs.<br>12-14 months for starch cvs. | 3½ - 4 months | Faster returns from sweet potato |
| Sensitivity to floods | Very sensitive | Very sensitive, but problem is somewhat alleviated by planting on beds | Generally, root crops are sensitive to waterlogged conditions which cause root rot due to lack of oxygen |
| Common pests of economic importance | Leaf-eating caterpillars (e.g. *Tiracola plagiata*)<br>Rats | Sweet potato weevil (*Cylas formicarius*); white grubs; vine borer (*Omphisa anastomosalis*); various leaf eaters (e.g. *Spodoptera litura*)<br>Rats | No serious pests in cassava. Look out for pink mealy bug which in recent years has devastated cassava yields in Thailand [9]. |
| Common diseases of economic importance | *Cercospora* brown and white leafspots (*C. henningsii*, *C. caribeae*); cassava bacterial blight (*Xanthomonas manihotis*); white root disease (*Rigidoporus lignosus*) | Sweet potato virus disease or SPVD (virus complex comprising the whitefly-borne sweet potato feathery mottle virus, SPFMV, sweet potato chlorotic stunt virus, SPCSV, and other viruses) | Leaf diseases of cassava cause little yield loss. White root disease can be managed by proper field sanitation. No means of control for SPVD. |
| Cropping system | Monocropping or intercropping | Rotational cropping (with sweet corn, groundnut, yambean, tobacco, etc.) | Rotational cropping is crucial for the management of pests and diseases in sweet potato |
| Root yield (t/ha) | On average, 30-35 | On average, 25-30 | |
| Post-harvest root shelf-life | Short shelf-life of 1-2 days | If uninfested, roots can keep till one month | |
| Price | RM0.70-R1.00 per kg edible roots; roots for starch extraction usually half the price | RM1.00-RM1.20 per kg; purple- and orange-fleshed cvs. can fetch higher prices | More profitable to grow edible cvs. of cassava |

[1]Much of the information is from [7]
[1]Much of the information is from [8]
[1]Beach ridges interspersed with swales: alternating parallel sandy beach ridges and low depressional areas,

**Table 4.** Performance of MARDI cassava starch cultivars *vs.* commercial cultivar, Black Twig. Values in the same column bearing the same letter are not significantly different from one another at $p = 0.05$ according to Duncan's multiple range test. (Source: [10])

| Cultivar | Fresh root yield (t/ha) | Root starch content (%) |
|---|---|---|
| Sri Kanji 1 | 37.6ab | 26.7a |
| Sri Kanji 2 | 32.2abc | 26.9a |
| MM 92 | 36.4abc | 20.9d |
| Perintis | 40.9a | 22.8c |
| Black Twig | 28.0cd | 25.2b |

planting materials. Virus-free vine cuttings can be produced by tissue culture but this is too expensive and impractical for commercial cultivation. The best alternatives are to practice crop rotation to prevent the build-up of pests and diseases over time, and to produce healthy shoot cuttings from a nursery, i.e. not to take cuttings straight from a harvested field to plant for production. Rotational cropping will require the management of two completely different crops, and stringent crop scheduling.

So far, in Malaysia, sweet potato is grown only for human consumption; thus, there is no price structure for roots to be used for industrial purposes (i.e. starch or feedstuff). Nonetheless, it may be expected that the same price differentiation as encountered by the starch cultivars of cassava will apply.

Nevertheless, sweet potato has several advantages over cassava. Its ability to yield well on sandy soils (with proper fertilizer management) makes it easier to locate land in Malaysia for its cultivation. Fresh root yields of 41 t/ha have been recorded on *bris* soil in Kelantan, compared with 28 t/ha on upland mineral soils (Table 5) [13]. There are still large tracts of *bris* soil and tin-tailings (considered marginal soils) which remain to be exploited for crop production. Furthermore, being a ground creeper, sweet potato is not at all prone to strong winds; indeed, it can even withstand typhoons which flatten most standing crops [14].

The nutritionally more superior purple- or orange-fleshed sweet potato cultivars can be capitalized for producing a wide range of food products – made either straight from the fresh roots, or after preprocessing into puree or flour [15]. If the target market is food-grade starch, sweet potato is at a disadvantage. The presence of more protein than cassava in the roots and the content of anthocyanin or carotenoids result in starch which is less than pure white. Industry specifications for food-grade starch require that the colour be uniform, white and free from pigments [16]. The colour specifications for non-food grade starch are less stringent.

## CONCLUSION

The question of whether cassava or sweet potato would be a better choice can only be answered when the following are taken into consideration:

1. End use – for industrial purposes (starch, feedstuff) or for human food?
2. Availability of suitable land – including edaphic and climatic conditions, and

**Table 5.** Yields (t/ha) and ranking of top five sweet potato cultivars at six sites in four agro-ecologies in Malaysia (after [13]). Site means bearing the same letter are not significantly different from one another at $p = 0.05$ according to Duncan's multiple range test.

| Cultivar | Upland mineral | | *Bris* | | Tin-tailings | Acid sulphate |
|---|---|---|---|---|---|---|
| | Serdang[1] | Bertam[2] | Telong[3] | Kandis[3] | K. Bikam[4] | K. Linggi[5] |
| VitAto | 28.2 (1)* | 16.2 (1) | 41.1 (1) | 39.2 (1) | 23.8 (1) | 36.0 (1) |
| Tainung No. 64 | 18.6 (4) | | 16.0 (3) | | | 28.4 (2) |
| Guan | 23.5 (3) | 6.5 (2) | 18.9 (2) | 36.6 (2) | 17.5 (2) | |
| Caromex | | | | 30.5 (3) | 12.3 (4) | 23.2 (3) |
| Gendut | 25.0 (2) | | | 21.2 (5) | 12.5 (3) | 18.3 (4) |
| Kuala Bikam 2 | 13.4 (5) | | | | | |
| W-154 | | | 14.9 (5) | | | |
| Travis | | 1.9 (5) | 16.0 (4) | | | |
| Julian | | | | | | 18.3 (5) |
| Benihayato | | | | | 10.5 (5) | |
| W-219 | | 5.3 (3) | | 22.7 (4) | | |
| Bugs Bunny | | 3.1 (4) | | | | |
| Site mean | 13.4b | 2.7d | 13.7b | 19.2a | 8.4c | 14.0b |

*Figures in brackets denote ranking.

[1]Serdang in Selangor; [2]Bertam in Seberang Perai; [3]Telong and Kandis in Kelantan; [4]Kuala Bikam in Perak; [5]Kuala Linggi in Melaka.

3. Preferred form of management – monocropping or rotational cropping.

Factor no. 2 should not signify too great a hindrance if the possibility of off-shore investment presents itself. Neighbouring countries (e.g. Vietnam, Cambodia and Myanmar) with tropical climate and abundant land resources (better still, with competitively priced labour) should be considered. Let Malaysia continue with the cultivation of crops for which she has a competitive edge, notably, oil palm and rubber, while other crops with good marketing potential can be produced elsewhere with Malaysian investment.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 75-86

# Molecular phylogenetics and systematics of five genera of Malaysian murine rodents (*Maxomys*, *Sundamys*, *Leopoldamys*, *Niviventer* and *Rattus*) inferred from partial mitochondrial cytochrome *c* oxidase subunit I (COI) gene

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*Received 08-09-2011; accepted 26-09-2011*

**Abstract** Study on the taxonomy and systematic of Malaysian Murinae is very scarce especially due to the lack of material within the country. We provide an attempt to investigate the phylogenetic relationship and pattern thus identifying species within five genera comprising *Maxomys*, *Sundamys*, *Leopoldamys*, *Niviventer* and *Rattus*. We genetically analysed 50 specimens of Murinae from Peninsular Malaysia and Sarawak, assigned to 12 species. Phylogenetic analyses of partial mitochondrial cytochrome *c* oxidase subunit I (476 base pairs) using four methods, namely, neighbour-joining (NJ), maximum parsimony (MP), maximum-likelihood (ML) and Bayesian method resulted in similar statistically supported clades with minimal change in branching order. The analyses discovered that there were intermediate form of *Maxomys* species within *M. whiteheadi* and *M. ochraceiventer* populations. They display same external morphology as *M. whiteheadi* but genetically closer to *M. ochraceiventer*. Craniodental measurements showed significant differences between the three populations. *Rattus* and *Sundamys* appeared not fully resolved while *Leopoldamys* and *Niviventer* were steadily clustered. The intraspecific geographic variation in some species agrees with previous studies on the vicariance scenario and diversification of flora and fauna in Malaysia and Borneo.

**Keywords** Murinae – phylogenetics – COI – Genetic Species Concept – geographic structure

## INTRODUCTION

Traditionally, taxonomic status of Murinae was based on morphological characteristics. The classification was outdated due to the variation of morphological traits caused by rapid adaptation towards ecological habitats and high rate of evolution in Murinae. The variation of external features sometimes does not indicate the species to be in distinct taxa, at least not in Genetic Species Concept. As closely related species in the subfamily Murinae are morphologically similar to each other, the taxonomic status of Murinae is poorly resolved until recently. Many studies have been done using genetic data, morphology, immunology, albumin and karyotypic analyses but the information of Murinae in Malaysia is still lacking. Examining species boundaries using data from cytochrome *c* oxidase subunit I (COI) is an appropriate method to identify genetically isolated evolutionary units and the phylogenetic relationship estimation of Murinae.

In Peninsular Malaysia, the classical taxonomy of Murinae was based on their morphologies of external features [1-3]. Apart from the genera *Chiropodomys, Hapalomys, Pithecheir, Bandicota* and *Mus*, these authors placed all the remaining species of Murinae in the genus *Rattus*. Subsequent karyotypic and electrophoretic studies emphasised the distinctiveness of some species within the genus *Rattus* with great divergence [4, 5] as that between different genera from North American rodents [6, 7]. Later, the skins, skulls and dental morphology of these species were re-examined and some subgenera were elevated, new genera were named and described such as *Maxomys, Leopoldamys, Berylmys* and *Sundamys* [8, 9]. Splitting *Rattus* into well-defined genera gives better understanding of phylogenetic relationship among Murinae. Three genera of *Maxomys*, *Sundamys* and *Niviventer* that were previously included in *Rattus* were tested [10]. *Maxomys* was the basal group for these genera and *Rattus* was closely related and

monophyletic with *Sundamys* rather than the other genera.

Recently, the earlier classification was reviewed and challenged by molecular approaches [10-15], immunological experiments [16] and DNA hybridisation assays [17]. These studies had altered the classical classification made previously.

## MATERIALS AND METHODS

### Taxonomic sampling for molecular analyses

Sampling sites were chosen based on the distributions of subfamily Murinae in previous studies [18-24]. Twelve sampling sites throughout Peninsular Malaysia and Sarawak including three mountains were sampled for collecting fresh specimens (Appendix 1).

Specimens were collected from natural populations using baited cage traps and Sherman's traps. Voucher specimens were prepared either as skin and skeleton or as fluid-preserved specimens. Liver and muscle tissues were preserved in both lysis buffer and 95% ethanol. These materials were deposited at the UNIMAS Zoological Museum of Universiti Malaysia Sarawak. Museum vouchers or tissues and GenBank accession numbers for all specimens examined are listed in Appendix 2.

### Mitochondrial DNA sequencing

Total genomic DNA was extracted from muscle or liver tissues following 2X C-TAB protocol [25-27]. Partial length of 476 base pairs (bp) of COI gene was amplified using standard polymerase chain reaction procedures [28] using the GoTaq® Flexi DNA polymerase PCR kit (Promega Co.). Thermal cycle amplifications were performed using primers COIe (reverse) 5'–CCA GAG ATT AGA GGG AAT CAG TG–3' and COIf (forward) 5'–CCT GCA GGA GGA GGA GAY CC–3' [29] in a 25 µL reaction. The reaction included DNA product, 10 mM of each primer, 25 mM of $MgCl_2$, 10 mM of deoxynucleoside triphosphates, 5X reaction buffer and 1.25 U of *Taq* DNA polymerase. The thermal profile used was 93°C for 3 minutes, then amplification for 29 cycles of denaturation at 93°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 2 minutes, followed by 72°C for 5 minutes.

The amplified DNA products were purified by centrifugation using Promega Wizard SV Gel and PCR Clean Up System (Promega Co.) and sequenced at First Base Co. (Selangor, Malaysia) using the ABI PRISM® 377 DNA Sequencer with the BigDye® Terminator v3.0 Cycle Sequencing Kit. The sequencing product was run using ABI 3730 XL capillary DNA sequencer (50 cm capillary).

### Phylogenetic analyses

The CHROMAS (version 1.45) [30] software was used to observe and read nucleotide bases of DNA sequences before further analysis. The multiple alignments of the nucleotide sequences were done by using CLUSTAL X version 1.8 [31] program, later checked manually by eye. Phylogenetic analyses were performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 beta [32] software while Bayesian method was constructed in MrBayes [33]. For COI data set, neighbour-joining, maximum-parsimony, maximum-likelihood and Bayesian methods were used to infer phylogenies.

Out of 56 evolutionary models, Modeltest 3.7 [34] showed that general time reversible (GTR) models of substitution, with allowance for gamma distribution (G) of rate variation and for proportion of invariant sites (I), best fit the data. This model was used in maximum-likelihood and Bayesian method. Maximum-parsimony analysis was performed using heuristic searches, 10 random additions of taxa and tree-bisection-reconnection (TBR) as the branch-swapping algorithm. Pairwise genetic distances matrix between and within species were calculated using Kimura two-parameter (K2P) model [35] that was applied in Molecular Evolutionary Genetic Analysis (MEGA) 4.0 [36].

## RESULTS

### Phylogenetic analyses

The partial COI gene (GenBank JF343472–JF343519; Appendix 2) was sequenced for 50 specimens. Aligned sequences of 476 bp representing 95% of the total length of the partial mtDNA COI gene (≈500 bp) were used in the estimation of genetic distance and phylogenetic reconstruction. Alignment of sequences was unequivocal and without internal stop codons, resulting in 50 unique haplotypes. Out of the 476 bp nucleotides, 292 characters were invariant or conserved (50.8%), 184 characters showed variable sites (49.2%) with 43 variable characters being parsimony-uninformative sites and the remaining 141 characters of parsimony-informative sites. Including

the outgroup, 12 informative characters were at 1st codon positions, 2 characters at 2nd codon positions and 127 characters at 3rd codon positions. Parsimony analyses generated a single most parsimonious tree length of 682 with consistency index (CI) of 0.3959 and retention index (RI) of 0.7789. Maximum-likelihood analyses resulted in a single optimal tree (-Ln likelihood=3590.56574) while Bayesian analyses with 50% majority rule consensus resulted in statistically supported clades (Fig. 1).

Including the outgroup, the average base frequencies used in the analysis were thymine (T)

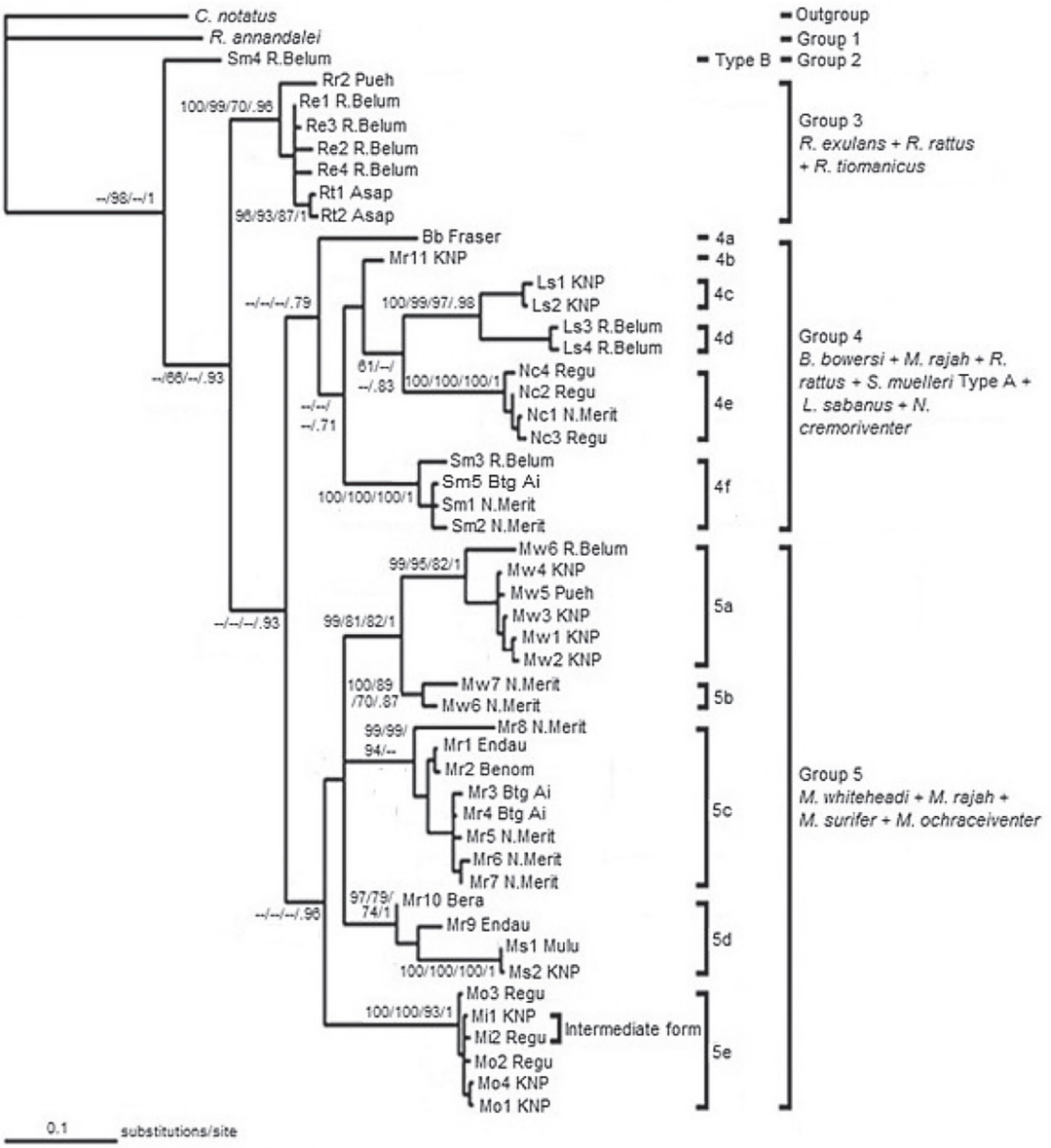


**Figure 1.** Bayesian phylogram of 50% majority-rule consensus tree inferred from aligned 476 bp partial COI gene sequences. Scores on the branches refer to bootstrap support values (1,000 iterations) from neighbour-joining (1st score), maximum parsimony (2nd score), maximum likelihood (3rd score) and Bayesian posterior probabilities (4tg score); -- = no support value. Specimens labelled by Sm = *S. muelleri*, Rr = *R. rattus*, Re = *R. exulans*, Rt = *R. tiomanicus*, Bb = *B. bowersi*, Ls = *L. sabanus*, Nc = *N. cremoriventer*, Mr = *M. rajah*, Mw = *M. whiteheadi*, Ms = *M. surifer*, Mo = *M. ochraceiventer* and Mi = intermediate form of *Maxomys*. Localities labelled by R.Belum = Royal Belum State Park, Pueh = Pueh Forest Reserve, Asap = Sungai Asap, Belaga, Fraser = Fraser's Hill Forest Reserve, KNP = Kubah National Park, Regu = Regu, Padawan, N. Merit = Nanga Merit, Kapit, Btg Ai = Batang Ai National Park, Endau = Endau-Kluang Forest Reserve, Benom = Krau Wildlife Reserve, Bera = Tasik Bera RAMSAR site, Mulu = Mulu National Park. Refer to Appendix 1 for the collecting region.

**Table 1.** Average percentage of Kimura two-parameter distance values within (boldface type along diagonal) and among species in subfamily Murinae from different clades based on COI gene sequences. n = sample size of each species. NA = not available.

| Taxon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Mi (n = 2) | **0.21** | | | | | | | | | | | | | | | |
| 2 Mw (n = 8) | 11.37 | **5.13** | | | | | | | | | | | | | | |
| 3 Mo (n = 4) | 0.32 | 11.21 | **0.35** | | | | | | | | | | | | | |
| 4 Mr11 (n = 1) | 14.03 | 10.22 | 13.83 | **NA** | | | | | | | | | | | | |
| 5 Mr Type A (n = 8) | 12.55 | 9.00 | 12.41 | 10.02 | **3.13** | | | | | | | | | | | |
| 6 Mr Type B (n = 2) | 11.39 | 8.34 | 11.26 | 9.06 | 7.14 | **4.80** | | | | | | | | | | |
| 7 Ms (n = 2) | 15.31 | 13.24 | 15.18 | 11.07 | 10.44 | 5.65 | **1.49** | | | | | | | | | |
| 8 Bb (n = 1) | 12.94 | 11.83 | 12.88 | 8.27 | 13.61 | 11.61 | 13.62 | **NA** | | | | | | | | |
| 9 Rr (n = 1) | 15.77 | 12.12 | 15.71 | 10.66 | 12.05 | 10.31 | 13.57 | 13.27 | **NA** | | | | | | | |
| 10 Rt (n = 2) | 14.81 | 12.84 | 14.75 | 10.25 | 12.28 | 9.90 | 13.66 | 13.39 | 3.26 | **0.63** | | | | | | |
| 11 Re (n = 4) | 15.15 | 12.42 | 15.08 | 9.65 | 11.41 | 9.74 | 12.76 | 12.17 | 3.27 | 1.19 | **1.17** | | | | | |
| 12 Ra (n = 1) | 21.41 | 21.93 | 27.19 | 22.78 | 21.03 | 18.76 | 23.53 | 24.52 | 24.30 | 23.22 | 22.49 | **NA** | | | | |
| 13 Sm Type A (n = 4) | 13.33 | 9.82 | 13.15 | 7.12 | 11.35 | 10.60 | 12.21 | 12.28 | 9.33 | 9.95 | 9.74 | 23.64 | **2.15** | | | |
| 14 Sm Type B (n = 1) | 16.89 | 12.29 | 16.62 | 13.91 | 13.41 | 11.92 | 17.81 | 16.14 | 10.88 | 10.96 | 9.82 | 20.76 | 12.69 | **NA** | | |
| 15 Ls (n = 4) | 13.31 | 10.08 | 13.25 | 8.79 | 11.76 | 8.25 | 11.60 | 12.34 | 13.33 | 12.27 | 11.93 | 20.27 | 9.02 | 14.47 | **5.53** | |
| 16 Nc (n = 4) | 16.88 | 12.02 | 16.89 | 10.08 | 12.06 | 9.23 | 14.49 | 13.92 | 13.44 | 14.10 | 14.01 | 24.39 | 11.72 | 14.44 | 9.75 | **0.74** |
| 17 Cn (n = 1) | 21.96 | 18.34 | 21.89 | 22.19 | 21.94 | 19.19 | 23.57 | 23.61 | 22.19 | 21.47 | 20.97 | 21.87 | 21.97 | 20.21 | 19.12 | 23.83 |

with 32.6%, cytosine (C) with 21.6%, adenine (A) with 28.4%, and guanine (G) with 17.5%. The highest frequency of nucleotide found in COI gene for these species including the outgroup was T nucleotide, ranging from 27.9% (*R. annandalei*) to 34.2% (*M. whiteheadi*) whereas G nucleotide had the lowest frequency, which showed the characteristic of anti-G bias ranging from 16.6% (*M. rajah* 11) to 18.5% (*S. muelleri* Type B). Anti-G bias sequences were one of the characters of mitochondrial gene [37, 38].

All the phylogenetic topologies revealed five strongly supported monophyletic clades with slightly different topologies and groupings. Genetic distance within and among each lineage was calculated by Kimura two-parameter [35] according to the groupings assignment in phylogenetic trees (Table 1). For distance character, NJ gave the most resolved topologies while the Bayesian phylogenetic tree was the most reliable for character based method observed by the higher bootstrap and bpp values (>50%) on each branch compared to the other character based phylogenetic trees (MP and ML).

## DISCUSSION

### *Maxomys* Clade

Genetic divergence between genera of 15.02% separated the lineage of *Maxomys* from the other Murinae lineages in this study. The separation was well supported by studies using mtDNA and nucDNA data [13], observing external morphologies, skull measurements and dental morphologies [8, 39], assessing microcomplement fixation of albumin [16] and chromosomal evidence [40]. Besides, the separation was also congruent with the external morphological differences of having short bicolour tail, being dark brown or black above and white beneath, separated by a sharp line [52] and obvious spiny fur with very stiff and prominent spines, which are lacking in other genera in this study [8, 19, 23].

In all four methods of phylogenetic analyses, intermediate form of *Maxomys* (Mi) was identified within *Maxomys* division. They exhibit external morphological characteristics similar to *M. whiteheadi* but they were excluded from the remaining *M. whiteheadi* population and formed monophyletic group with *M. ochraceiventer* lineage. High genetic divergence (mean = 11.37%) between Mi with *M. whiteheadi* population suggested that the taxa should be treated as different lineages or species and not as *M. whiteheadi* following the Genetic Species Concept [41]. Genetic distance >11% indicated a species recognition [41].

The close genetic relationship between Mi and *M. ochraceiventer* (mean = 0.32%) suggested that the intermediate form showed high probability of conspecific populations to *M. ochraceiventer*. Genetic distance < 2% indicated intraspecific variation [41]. However, obvious differences in the skulls and dental features (Appendices 3, 4 and 5) between Mi, *M. ochraceiventer* and *M. whiteheadi* elucidated that the intermediate form was distinct from *M. whiteheadi* and *M. ochraceiventer*.

*Maxomys ochraceiventer* has the flattest skull and the longest greatest skull length (GSL) followed by Mi and *M. whiteheadi* (Appendix 3). Comparatively,

the skull of *M. whiteheadi* was broader compared to those of Mi and *M. ochraceiventer*. Moreover, between these three skulls, there were differences in the shape of the bony palate and incisive foramina (Appendix 4). *Maxomys whiteheadi* (A) and Mi (B) have straight shape but *M. whiteheadi* has rounder curve at the top of the bony palate and Mi was rather square. On the other hand, *M. ochraceiventer* has a narrower bony palate at the base that widens to the top with square shape as Mi. Next, the incisive foramina for *M. whiteheadi* was relatively broader compared to Mi and *M. ochraceiventer*. Between Mi and *M. ochraceventer*, Mi has shorter length of incisive foramina. The zygomatic plate of *M. whiteheadi* was comparatively small compared to Mi and *M. ochraceiventer* (Appendix 5). *Maxomys ochraceiventer* has longer and narrower zygomatic plate compared to Mi.

Skull and dental measurements that indicate the variation among *M. whiteheadi*, *M. ochraceiventer* and intermediate form of *Maxomys* are shown in Appendix 6. The variance was calculated using Kruskal-Wallis test and the value $P < 0.05$ indicated significant difference between the skull and dental measurements. Larger dataset should be obtained and examined to further review the significant distinction.

These occurrences may suggest that Mi was a cryptic species in *Maxomys*. Recently, there were cryptic species recorded within the *Maxomys* population in Borneo that was closely related with *M. whiteheadi* and *M. ochraceiventer* [42, 43]. These *Maxomys* populations should be further investigated with highly evolving genes such as the control region to ensure the separation among the species clustering due to evolution and speciation. Furthermore, the intermediate form present was possibly due to hybridisation of *M. whiteheadi* and *M. ochraceiventer*. Hybrid species inherited similar genetic composition to the former but resembled the same morphologies of the latter species. The occurrence is possible as the two congeneric species are closely related. Thus, further studies need to be done on these complex taxa using several nucDNA to investigate the hybridisation.

Apart from *M. rajah* 11, *M. rajah* lineage was separated into two subgroups. *Maxomys rajah* (5c) was clustered among the same species while *M. rajah* (5d) clustered (two individuals) with *M. surifer* with genetic distance of 5.65% between the two species. The two subgroups of *M. rajah* were divided with genetic divergence of 7.14%. The genetic distances indicated that *M. rajah* 9 and 10 (5d) were genetically closer related to *M. surifer* rather than their own species. *Maxomys rajah* and *M. surifer* were once considered as conspecific [44]; however, genetic distance for conspecific value varies from 0.25 to 5% [45]. Thus, it was not supported that *M. rajah* and *M. surifer* were conspecific in this study. Although *M. rajah* and *M. surifer* proved difficult to distinguish [19, 23], there was no doubt that both *M. rajah* and *M. surifer* were distinct species by ecological observation, breeding behaviour, karyotype and serology [40]. Although they were found in the general habitat, they were not usually present together. Moreover, no attempts on mating were observed for the interspecific pairs under the prevalent animal-house condition [40]. Karyotypes of both species were also described and the chromosome numbers were distinctly different [4]. A study done using COI gene and morphological data analyses stated that the two congeneric taxa were not even closely related within *Maxomys* division [43]. This may suggest that there were high genetic variation within *M. rajah* population or *M. rajah* (5d) might be a subpopulation of *M. surifer* and should be treated with caution as there were no DNA sequences of *M. surifer* from Peninsular Malaysia for comparison. Larger dataset of this particular species is needed to make a conclusion of the clustering.

*Maxomys rajah* 11 phylogenetic relationship remains unresolved as it was independently clustered in phylogenetic trees. The partial fragment (≈500 bp) sequences of the COI gene might have insufficient informative sites for the analyses and this might explain the ambiguous clustering of *M. rajah* 11 discussed above. Only 33% of the complete length of COI gene (1500 bp) was used in this study. The primers might have sequenced any partial fragments in the COI gene and the polymorphic sites of the genetic composition that can signify different species might actually lie outside the fragments analysed.

Similar phylogeographic structuring among *M. whiteheadi* (5a and 5b) and *M. rajah* (5c) reflected a consistent pattern of vicariance scenario, in which *Maxomys* share a history of diversification resulting from barriers arising within their formerly continuous ranges that were distinguishable by levels of genetic divergence between Peninsular Malaysia and Sarawak. Species of *Maxomys* are non-commensal and forest dwellers. Thus, forest expansion and

contraction events across the Sunda shelf during the last 3 million years might have fragmented the northeast Sarawak populations before Peninsular Malaysian and southwest Sarawak populations. This pattern was also observed in other rodents and bats studies, which suggested that speciation occurred due to preglacial vicariance [53, 54]. In *M. rajah* lineage (5c), northeast Sarawak populations were separated; one derived earlier and branched independently (following the pattern of vicariance scenario) while the others clustered with southwest Sarawak populations. The latter form has not yet diversified to form a distinct population from southwest Sarawak. Based on this occurrence, hypothesis can be proposed that northeast Sarawak (in this case Nanga Merit, Kapit) might be the transition region and contact zone for speciation.

***Rattus* and *Sundamys* clade**

Over the past 17 years, the genus *Rattus* has been studied intensively. Species from this genus had been separated as distinct genera (*Leopoldamys*, *Maxomys* and *Niviventer*) based on morphological ground [46]. Non-morphological techniques (DNA) [47] agreed with the separation but failed to resolve the relationships between *Rattus* and *Sundamys*. The relationship was still ambiguous and many unidentified groups have been discovered.

The phylogenetic trees illustrated topologies that were not fully resolved in *Rattus* and *Sundamys* taxa. *Sundamys muelleri* 4 (Type B) branched out independently and diverged from the remaining *S. muelleri* linage (Type A) with high genetic divergence of 12.69%. The value implied a new genetic species [48]. *Rattus annandalei* was also unresolved with high genetic distance between its genus. The justification was that the partial fragment (≈500 bp) sequences of the COI gene might have insufficient informative sites for the analyses. More genetic data are needed to clarify whether *S. muelleri* 4 and *R. annandalei* could be categorised as distinct lineages.

*Rattus rattus* was the basal clade in *Rattus* lineage followed by *R. exulans* and *R. tiomanicus*. Species of *Rattus* are commensal species that are always associated with humans and live sympatrically with one species or another. This might contribute to the occurrence of hybridisation that explained the close genetic divergence among species (< 4%). *Sundamys* lineage showed a pattern of vicariance scenario where the South China Sea might be a geographic barrier that resulted in allopatric populations between Peninsular Malaysia and Sarawak. This is consistent in other faunal study [53]. In this lineage, the northeast and southwest Sarawak populations were not separated.

***Niviventer and Leopoldamys* clade**

The close relationship between *Niviventer* and *Leopoldamys* was claimed based on morphological analyses [49]. These two genera formed a sister group with high bootstrap support in this study. The taxonomic status of these genera was ambiguous as they were classified in the genus *Rattus* in the early nomenclatural history [16, 23]. This study proved that they belong to distinct genera as they showed high genetic divergence of 9.75%.

The clustering of *L. sabanus* was separated according to the geographic region, Peninsular Malaysia and southwest Sarawak. *Niviventer cremoriventer* showed an absence of phylogeographic structuring within the specimens indicating a recent common ancestor of northeast and southwest populations of this species.

The COI gene is a marker that has the characteristic of a conserved gene. It consists of more conserved sites rather than variable sites [50]. This particular region evolves slowly within the mtDNA which makes it suitable to resolve interspecies level but cannot determine at best intraspecific relationship for the vertebrates [50]. However, COI gene has proven to be a good genetic marker for intraspecific variation of the lower vertebrates as seen in amphibian populations [51].

**Acknowledgements –** This study would not be possible without various administrative and financial supports from Universiti Malaysia Sarawak (UNIMAS). We thank the Sarawak Forestry Corporation and Sarawak Forestry Department for granting permission to conduct wildlife research in national parks in Sarawak under the State Wild Life Protection Rules 1998; for research permit number NPW.907.4.2(II)-5 and permit to enter park. Our gratitude also goes to the Department of Wildlife and National Parks for inviting our team to various field trips in Peninsular Malaysia. Identifications of certain species in this study were greatly aided by Dr Lim Boo Liat. We wish to express our sincere gratitude to the members in the Department of Zoology for their hard work and assistance throughout this study. Special thanks to Professor Yong Hoi Sen, Dr Ramlah Zainuddin, Earl of Cranbrook, Mr. Faisal Ali

Anwarali Khan and Mr. Jayaraj Vijaya Kumaran for the constructive comments during the process of writing this manuscript.NAMT postgraduate study was supported by UNIMAS Vice Chancellor Scholarship and part of the data collection and laboratory analyses using the UNIMAS Eco-Zoonosis Grant lead by MTA.

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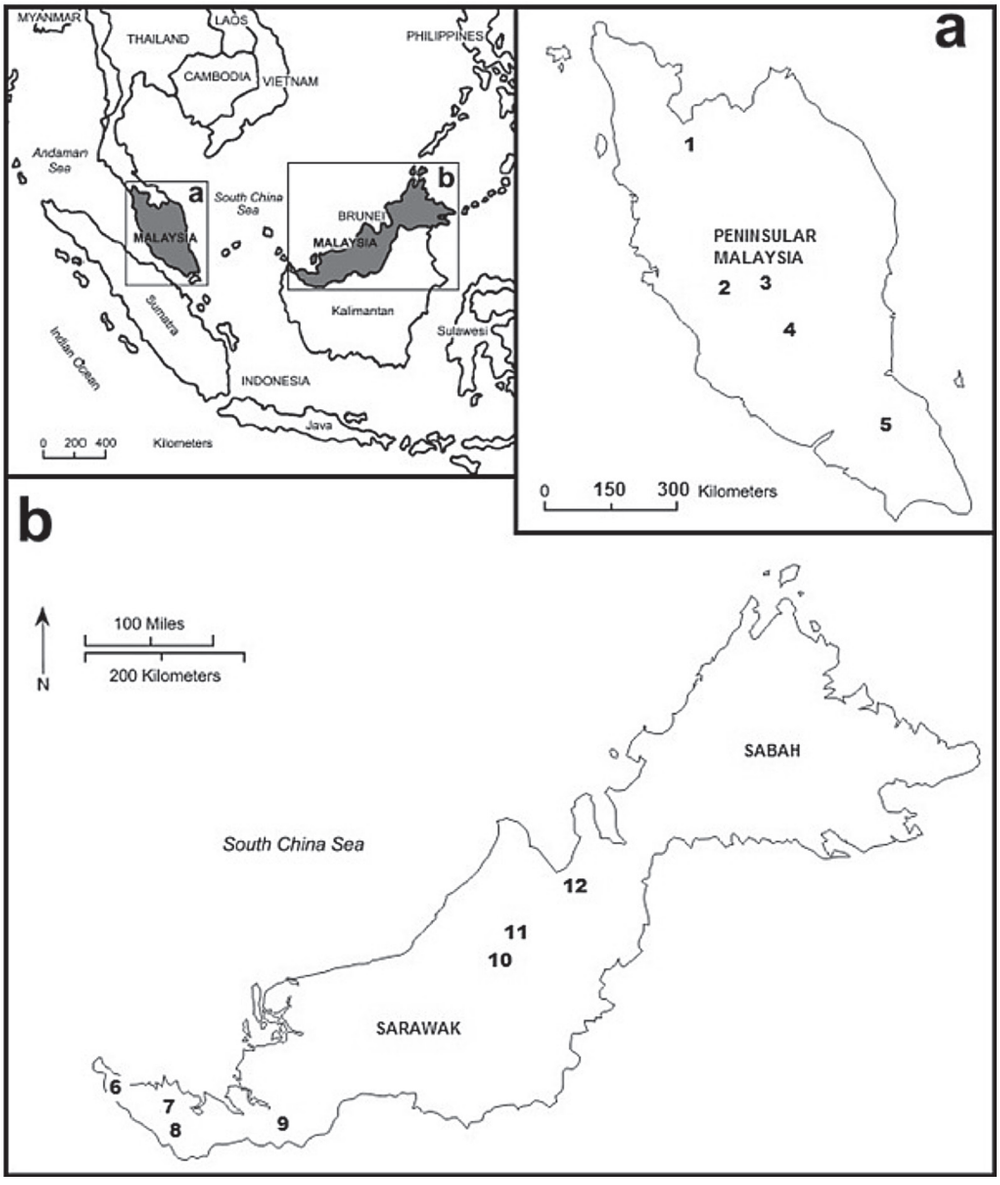
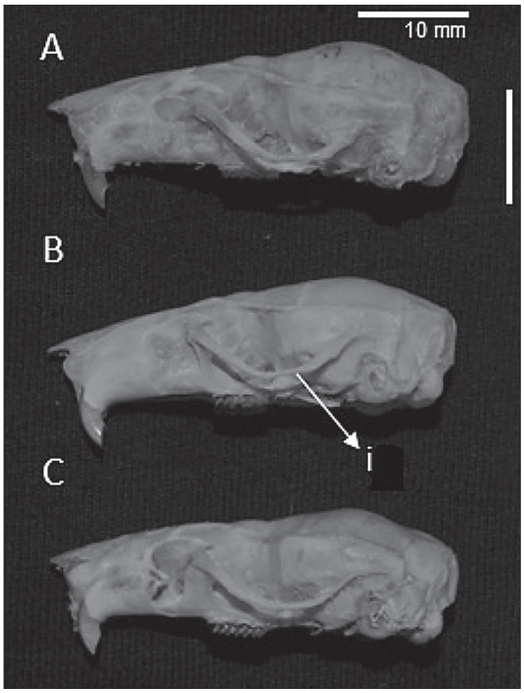
**Appendix 1.** Twelve study sites for this study. 1: Royal Belum State Park. 2: Fraser's Hill Wildlife Reserve. 3: Krau Wildlife Reserve. 4: Tasik Bera RAMSAR site. 5: Endau-Kluang Wildlife Reserve. 6: Pueh Forest Reserve. 7: Kubah National Park. 8: Regu, Padawan. 9: Batang Ai National Park. 10: Nanga Merit, Kapit. 11: Sungai Asap, Belaga. 12: Mulu National Park. Regions: Peninsular Malaysia (1-5), southwestern Sarawak (6-9) and eastern Sarawak (10-12).

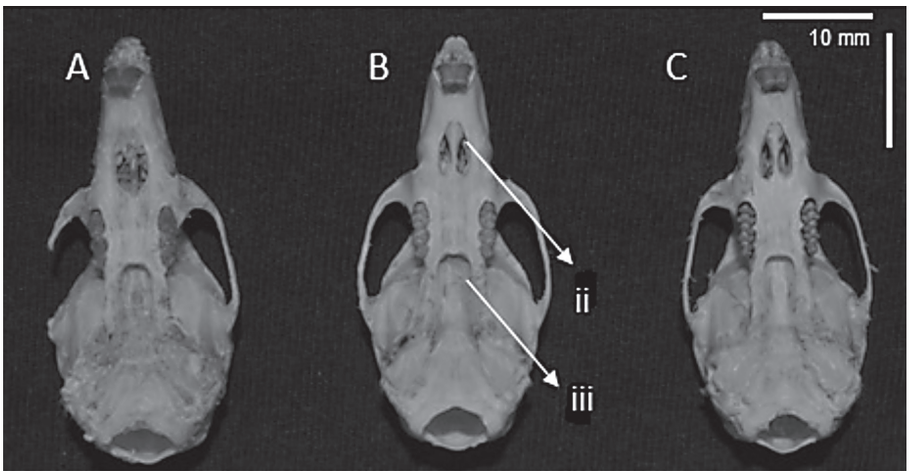


**Appendix 2.** Taxa, geographic localities, map point, tissue number, GenBank accession numbers of COIdata used for phylogenetic analysis. IP = in progress; Abbr. = abbreviation; NA = not available.

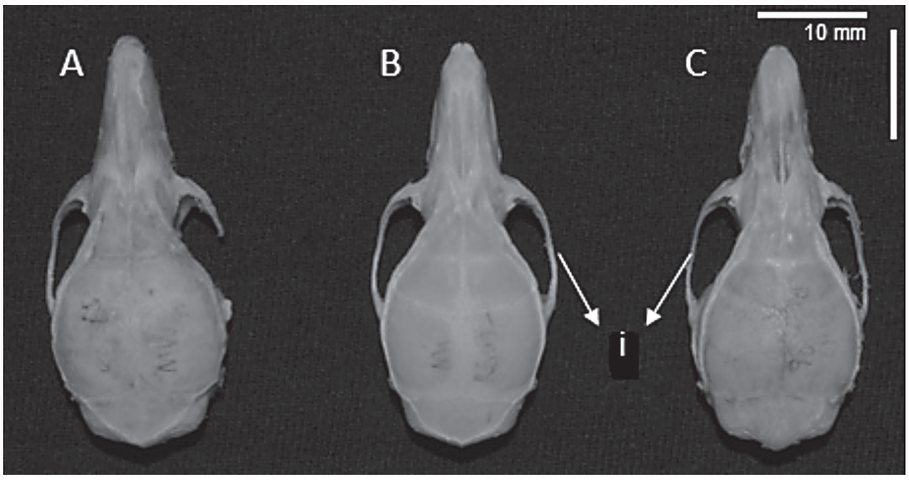
| Taxa | Abbr. | Tissue no. | Location | | Map point | GenBank no. |
|---|---|---|---|---|---|---|
| | | | Locality | Region | | COI |
| *M. whiteheadi* | Mw1 | TK152851 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343477 |
| *M. whiteheadi* | Mw2 | TK152854 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343478 |
| *M. whiteheadi* | Mw3 | TK152823 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343473 |
| *M. whiteheadi* | Mw4 | TK152846 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343474 |
| *M. whiteheadi* | Mw5 | Pueh006 | Pueh Forest Reserve, Sarawak | Southwest Sarawak | 7 | JF343482 |
| *M. whiteheadi* | Mw6 | TK156110 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343494 |
| *M. whiteheadi* | Mw7 | UNIMAS2083 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343493 |
| *Maxomys* sp. | Mi1 | TK152861 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343476 |
| *Maxomys* sp. | Mi2 | RG072 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343485 |
| *M. ochraceiventer* | Mo1 | TK152349 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343479 |
| *M. ochraceiventer* | Mo2 | RG092 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343487 |
| *M. ochraceiventer* | Mo3 | RG086 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343486 |
| *M. ochraceiventer* | Mo4 | KNP027 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343495 |
| *M. rajah* | Mr1 | EKS026 | Endau Kluang Forest Reserve, Johor | South Peninsular Malaysia | 6 | JF343480 |
| *M. rajah* | Mr2 | LB066 | Krau Wildlife Reserve, Pahang | Central Peninsular Malaysia | 4 | JF343516 |
| *M. rajah* | Mr3 | BTA007 | Batang Ai National Park, Sarawak | Southwest Sarawak | 10 | JF343511 |
| *M. rajah* | Mr4 | TK152348 | Batang Ai National Park, Sarawak | Southwest Sarawak | 10 | JF343515 |
| *M. rajah* | Mr5 | 2177 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343514 |
| *M. rajah* | Mr6 | 2176 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343513 |
| *M. rajah* | Mr7 | 2122 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343517 |
| *M. rajah* | Mr8 | UNIMAS2010 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343512 |
| *M. rajah* | Mr9 | EKS011 | Endau Kluang Forest Reserve, Johor | South Peninsular Malaysia | 6 | JF343500 |
| *M. rajah* | Mr10 | TB011 | TasikBera, Pahang | Central Peninsular Malaysia | 5 | JF343518 |
| *M. rajah* | Mr11 | 2192 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343519 |
| *M. surifer* | Ms1 | MM06 | Mulu National Park, Sarawak | East Sarawak | 13 | JF343502 |
| *M. surifer* | Ms2 | TK153614 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343504 |
| *R. rattus* | Rr2 | Pueh008 | Pueh Forest Reserve, Sarawak | Southwest Sarawak | 7 | JF343503 |
| *R. tiomanicus* | Rt1 | MPOB006 | Sungai Asap, Belaga, Sarawak | East Sarawak | 12 | JF343488 |
| *R. tiomanicus* | Rt2 | MPOB018 | Sungai Asap, Belaga, Sarawak | East Sarawak | 12 | IP |
| *R. exulans* | Re1 | TK156113 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343496 |
| *R. exulans* | Re2 | TK156111 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343489 |
| *R. exulans* | Re3 | TK156109 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343498 |
| *R. exulans* | Re4 | TK156125 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343499 |
| *R. annandalei* | NA | TG01 | Bukit Tagan, Perak | North Peninsular Malaysia | NA | IP |
| *S. muelleri* | Sm1 | UNIMAS2044 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343483 |
| *S. muelleri* | Sm2 | UNIMAS2050 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343508 |
| *S. muelleri* | Sm3 | TK156131 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343497 |
| *S. muelleri* | Sm4 | TK156119 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343509 |
| *S. muelleri* | Sm5 | BTA024 | Batang Ai National Park, Sarawak | Southwest Sarawak | 10 | JF343481 |
| *L. sabanus* | Ls1 | TK152824 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343475 |
| *L. sabanus* | Ls2 | TK152830 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343506 |
| *L. sabanus* | Ls3 | TK156130 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343501 |
| *L. sabanus* | Ls4 | TK152988 | Royal Belum State Park, Perak | North Peninsular Malaysia | 1 | JF343507 |
| *N. cremoriventer* | Nc1 | UNIMAS2082 | Nanga Merit, Kapit, Sarawak | East Sarawak | 11 | JF343490 |
| *N. cremoriventer* | Nc2 | RG067 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343491 |
| *N. cremoriventer* | Nc3 | RG076 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343492 |
| *N. cremoriventer* | Nc4 | RG033 | Regu, Padawan, Sarawak | Southwest Sarawak | 9 | JF343484 |
| *B. bowersi* | Bb | FH036 | Fraser's Hill, Selangor | Central Peninsular Malaysia | 3 | JF343505 |
| *C. notatus* | NA | W02 | Kubah National Park, Sarawak | Southwest Sarawak | 8 | JF343472 |




**Appendix 3.** Lateral views of the skulls show variation between *M. whiteheadi* (A),intermediate form of *Maxomys* sp. (B) and *M. ochraceiventer* by observing the flatness of the braincase, the greatest skull length and the zygomatic plate (i).




**Appendix 4.** Ventral views of the skulls show variation between *M. whiteheadi* (A), intermediate form of *Maxomys* sp. (B) and *M. ochraceiventer* by observing the posterior edge of bony palate (ii) in relation to M3 and the palatal foramina (iii) in relation to M1.




**Appendix 5.** Dorsal views of skull show variation between *M. whiteheadi* (A), intermediate form of *Maxomys* sp. (B) and *M. ochraceiventer* by observing the broadness of the braincase and the features of the zygomatic plate (i).

**Appendix 6.** Skull and dental measurements among complex lineage in *Maxomys*. GSL = greatest skull length, CBL = condylobasal length, IOW = interorbital width, ZW = zygomatic width, Pm = premaxillary length and MT = maxillary toothrow. n = number of individuals.

| Character | Species | | | Kruskal-Wallis test | |
|---|---|---|---|---|---|
| | *M. whiteheadi* (n = 5) | Intermediate form (n = 2) | *M. ochraceiventer* (n = 2) | H | p |
| Skull (mm) | | | | | |
| GSL | 38.69 | 38.03 | 38.18 | 6.533 | P < 0.05 |
| CBL | 36.17 | 34.63 | 35.65 | 6.533 | P < 0.05 |
| ZW | 18.88 | 17.40 | 17.49 | 6.533 | P < 0.05 |
| Pm | 10.87 | 10.30 | 9.90 | 6.533 | P < 0.05 |
| IOW | 8.13 | 7.34 | 7.92 | 6.533 | P < 0.05 |
| Dental (mm) | | | | | |
| MT | 5.47 | 5.06 | 5.24 | 6.533 | P < 0.05 |

*Journal of Science and Technology in the Tropics* (2011) **7**: 87-89

# Malaysian Butterfly Lizard *Leiolepis triploida* (Reptilia, Squamata: Leiolepidae) in Clearwater Sanctuary, Perak: geographical range extension in Peninsular Malaysia

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*Received 22-09-2011; accepted 29-09-2011*

**Abstract** The Malaysian Butterfly Lizard *Leiolepis triploida* is known from the inland areas of Perlis, Kedah and Seberang Perai (Penang) in the northwestern part of Peninsular Malaysia. The present finding of this butterfly lizard in Clearwater Sanctuary, Batu Gajah, Perak Darul Ridzuan has extended its known geographical range further south in Peninsular Malaysia. It remains to be established how far south it would spread, how widespread it is in Peninsular Malaysia, and whether it would displace the existing populations of the Common Butterfly Lizard *Leiolepis belliana*.

**Keywords** Triploid Butterfly Lizard – geographical range extension – parthenogenetic *Leiolepis* – Malaysia – tin mining area

## INTRODUCTION

The lizard fauna of Peninsular Malaysia consists of some 128 species in eight families – Agamidae, 7 genera 28 species; Dibamidae, 1 genus 2 species; Eublepharidae, 1 genus 1 species; Gekkonidae, 9 genera 52 species; Lacertidae, 1 genus 1 species; Leiolepidae, 1 genus 2 species; Scincidae, 5 genera 38 species; and Varanidae, 1 genus 4 species [1].

There are two species belonging to the family Leiolepidae in Malaysia – *Leiolepis belliana* (Hardwicken& Gray) and *Leiolepis triploida* Peters [1]. They are commonly known as butterfly lizards. At present, the Common Butterfly Lizard *L. belliana* occurs on both the east and west coasts of Peninsular Malaysia – on the east coast from Tumpat, Kelantan south to Mersing, Johor; and on the west coast from Dinding, Perak south to Melaka as well as the offshore islands Langkawi, Kedah and Pulau Besar, Melaka [1]. On the other hand, the Malaysian Butterfly Lizard *L. triploida* is confined to the inland areas of Perlis, Kedah and Seberang Perai, Penang [1, 2].

We report here the finding of the Malaysian Butterfly Lizard *L. triploida* in Clearwater Sancturay, Batu Gajah, Perak Darul Ridzuan, thus extending its known geographical range further south in Peninsular Malaysia.

## MATERIALS AND METHODS

The observation was done on a sunny day in Clearwater Sanctuary, Batu Gajah, Perak Darul Ridzuan, Peninsular Malaysia, some 20 km from Ipoh (Fig. 1). This location is a former tin mining area, now a nature resort with a golf course. The lizard (Fig. 2) was photographed in the field and identified using existing literature [1, 3]. No specimen was collected from the site of its occurrence.

## RESULTS AND DISCUSSION

The butterfly lizards (Leiolepidae) are represented by at least eight species, comprising four sexual and four asexual species [4]. The sexual species are: *Leiolepis belliana* (Hardwicke & Gray, 1827) – Common Butterfly Lizard, Bell's Butterfly Lizard; *Leiolepis guttata* (Cuvier, 1829) – Giant Butterfly Lizard, Spotted Butterfly Lizard; *Leiolepis peguensis*


**Figure 1**. Location of Batu Gajah, Perak Darul Ridzuan, Peninsular Malaysia.

Peters, 1971 – Burmese Butterfly Lizard, Pegu Butterfly Lizard; and *Leiolepis reevesii* (Gray, 1831) – Chinese Butterfly Lizard, Reeves' Butterfly Lizard. The asexual or parthenogenetic species are: *Leiolepis boehmei* Darevsky & Kupriyanova, 1993 – Böhme's Butterfly Lizard; *Leiolepis guentherpetersi* Darevsky & Kupriyanova, 1993 – Peters' Butterfly Lizard; *Leiolepis ngovantrii* Grismer & Grismer, 2010 – Ngo Van Tri's Lady Butterfly Lizard; and *Leiolepis triploida* Peters, 1971 – Thai Butterfly Lizard, Malaysian Butterfly Lizard, Triploid Butterfly Lizard.

Butterfly lizards are characterized by the possession of forelimbs, eyelids and round pupils; with anterior portion of the tail relatively wide and dorsoventrally compressed; and the dorsal scales of the body, limbs and tail very small, smooth and granular [1]. Of the two species present in Peninsular Malaysia, *L. triploida* is easily distinguished from *L. belliana* by the colour pattern on the flanks – with thin, yellowish to cream coloured bars in *L. triploida*, but with broad vertical orange and black bars in *L. belliana* [1, 3]. Phylogenetic inference based on 700 base pairs of the mitochondrial ND2 region indicates *L. boehmei* as the maternal ancestor of *L. triploida* [4] – *L. boehmei* is restricted to southern Thailand [1].

**Figure 2**. The parthenogenetic Malaysian Butterfly Lizard *Leiolepis triploida* at Clearwater Sanctuary, Batu Gajah, Perak Darul Ridzuan, Peninsular Malaysia. (photo: H. S. Yong)

During a recent on-going survey (September 2011) of insect fauna at the Clearwater Sanctuary, a single specimen of *L. triploida* was encountered in the early afternoon on the ground near the periphery of the golf course. When approached, the lizard ran away rather quickly and vanished into the bush nearby. It was however captured in pictures (Fig. 2) before it dashed away and disappeared completely from view. Two burrows, separated some distance from each other, were present not far away along the path.

Adult *L. triploida* may reach a snout-vent length of 148 mm [1, 3]. It was first described and named in 1971 [5], with the type locality as 'Malaysia-Thailand border of the Malay Peninsula'. Being parthenogenetic it is represented by females only; males do not exist in such asexual organisms. It inhabits disturbed, open areas with loose soil. It has been reported to be most common in agricultural areas throughout eastern Perlis and Kedah, especially in rubber plantations but also in oil palm plantations, paddy fields, orchards and abandoned mining areas [1]. Based on historical records for the occurrence of *L. belliana* in Kedah but is no longer present, it has been suggested that *L. triploida* has outcompeted and replaced *L. belliana* due to the conversion of forests into plantations [6].

Extension of georaphical range has also been recorded for the Tawny Coster *Acraea terpsicore* (Linnaeus, 1758), synonym *Acraea violae* (Fabricius, 1793), in Clearwater Sanctuary in 2000 (H. S. Yong, unpublished data). This nymphalid butterfly originated in India (and Sri Lanka) but spread through Myanmar and Thailand into Peninsular Malaysia. It is now established in Kuala Lumpur and other southern parts of Peninsular Malaysia.

Another instance of seemingly 'extension of geographical range', among others in Peninsular Malaysia, is the Forest Crested Lizard *Calotes emma* Gray, 1845. Earlier studies documented its occurrence in Peninsular Malaysia only in the northern states of Kedah and Perak [7], and "remains west of the Banjaran Titiwangsa" [1]. It has more recently been recorded in the east coast state of Kelantan [8].

The present finding of *L. triploida* in Perak has extended its known geographical range further south in Peninsular Malaysia. Studies are needed to determine how far south it has spread, how widespread it is, and whether it would displace the known populations of *L. belliana* on the west coast of Peninsular Malaysia.

**Acknowledgements** – This study received support from University of Malaya (Special Research Fund H-00000 5620009) and the Clearwater Sanctuary Golf Resort.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 91-104

# From organometallic radicals to complex molecules: structural and mechanistic studies

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**Abstract** The reactivity features of $[CpCr(CO)_3]_2$ (Cp = $\eta^5$-$C_5H_5$) (**2A**) towards (i) homo- and hetero-polyatomic aggregates of the nonmetal elements of Groups 15 and 16, (ii) several classes of organo- P-, S- and N-compounds, and (iii) heterocyclic dithiadiazolyl radicals, are described. The primary products obtained arise from facile cleavage of S–S, P–P and P–S bonds by the 17-electron species $[CpCr(CO)_3]$ (**2**). Further treatment of the product complexes with **2** under thermal activation results in cleavage of C–X (X = N, S), P–S and Cr–E (E = C, N, P, S) bonds, accompanied by C–C and P–P bond formation in some cases, generating new organometallic compounds, belonging to various classes and often incorporating interesting novel structures.

**Keywords** *organotransition-metal chemistry – radical-coupled products*

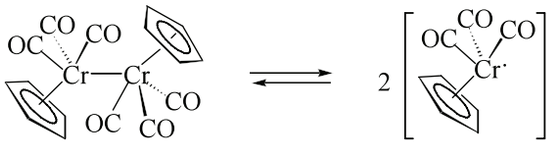
## INTRODUCTION

There is continuing interest in radical species as reactive intermediates in organotransition-metal chemistry [1]. The classical method for generating such radicals is the homolysis of metal-metal bonds under thermal or photochemical activation (eq i).

$$M_2L_{2n} \rightleftharpoons 2ML_n \qquad \text{(i)}$$

$$[Co_2(CN)_{10}]^{6-} \rightleftharpoons 2[Co(CN)_5]^{3-} \qquad \text{(ii)}$$

**1A** **1**



**2A** **2** (iii)

Notable examples are the 17-electron pentacyanocobalt(II)ate species **1** (eq ii) and the cyclopentadienylchromium tricarbonyl monomer $[CpCr(CO)_3]$ (Cp = $\eta^5$-$C_5H_5$) (**2**), readily formed via the facile dissociation of the unusually long Cr–Cr bond (3.281(1) Å) [2] in the dichromium species **2A** (eq iii). This phenomenon has been substantiated by various studies via NMR [3], ESR [4] and electronic spectral [5] and electrochemical [6] techniques.

Reactivity studies of **1A/1** antedated those of **2A/2** by more than two decades. The formation of the first organocobalt complexes was initiated by the facile abstraction of halogen from alkyl halides, as illustrated in eq. (iv) [7]

$$Co(CN)_5^{3-} + RX \longrightarrow R\cdot + XCo(CN)_5^{3-}$$
$$R\cdot + Co(CN)_5^{3-} \longrightarrow RCo(CN)_5^{3-}$$

(R = Me; n-py$CH_2$ (n = 2,3,4)) (iv)

$$CpCr(CO)_3\cdot + RX \longrightarrow R\cdot + CpCr(CO)_3X$$
$$R\cdot + CpCr(CO)_3\cdot \longrightarrow CpCr(CO)_3R$$

(R = Me; $CH_2$ = $CHCH_2$, $PhCH_2$, $CH_2CN$, and others containing α-H's) (v)

Some three decades later, it was established that a similar mechanism operates in the reaction of **2A** with alkyl halides, eq. v [8].)

Early indications of the high reactivity of **2A** came from our observation of a facile ligand substitution with trimethyl phosphite, yielding the derivative complex **3A** (Scheme 1) [9]. The extremely long Cr–Cr bond in **3A**, longer than that in **2A**, renders it highly susceptible to dissociation; the resulting radical species $[CpCr(CO)_2(P(OMe)_3]$ (**3**) is capable of cleaving the O–C bond in the methoxy group, generating a methyl derivative **3a** and a phosphonate complex **3b**.

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## REACTIONS WITH NONMETAL COMPOUNDS

Our subsequent investigations had demonstrated the facile reactivity of **2A** towards nonmetal-nonmetal bonds in the homo- and hetero-polynuclear molecules of the chalcogens (S and Se) and the pnicogens (P, As and Sb) (Chart 1). The cleavage of these bonds yielded organometallic derivatives of nonmetal elements of Groups 15 and 16, shown in Schemes 2 and 3. These results have been reviewed [10].

Clearly, the formation of polynuclear complexes like $[(CpCr(CO)_2]_5P_{10}$ (**5c**), $Cp_4Cr_4(CO)_9P_4X_3$ (X = S, Se) (**7a**) and $[(CpCr(CO)_3]_4(Sb_2S)$ (**8a**) in reactions with $P_4$ [11a,b], $P_4X_3$ (X = S, Se) [11c-e] and polymeric $Sb_2S_3$ [11f], respectively, has involved multiple bond cleavage in the nonmetal polynuclear molecules by **2**, followed by fragment aggregation. In an attempt to probe the generality of such phenomena in the chemistry of **2A**, we have pursued similar investigations with S–S, P–P and S–P bonds in organic substrates shown in Chart 2, including the reactivity of **2A** towards various Cr–E (E = C, N, P, S) bonds in the primary CpCr complexes formed.

## S–S, P–P and P–S BOND CLEAVAGE IN ORGANIC SUBSTRATES

### Reactions with bis(thiophosphinyl)disulfanes (A) and bis(thiophosphoryl)disulfanes (B)

$[CpCr(CO)_3]_2$ (**2A**) reacts readily with the disulfane **A** or **B** yielding $CpCr(CO)_2(S_2PR'_2)$ (R' = Ph, **9a**; R' = O$^i$Pr, **9b**) as the primary products [12-14], via an initial homolytic S–S bond cleavage of the disulfanes by **2,** accompanied by an incumbent coupling reaction (Scheme 4).

Under thermolytic conditions, complex **9a/9b** undergoes degradation via loss of CO ligands and/or

**Scheme 1**

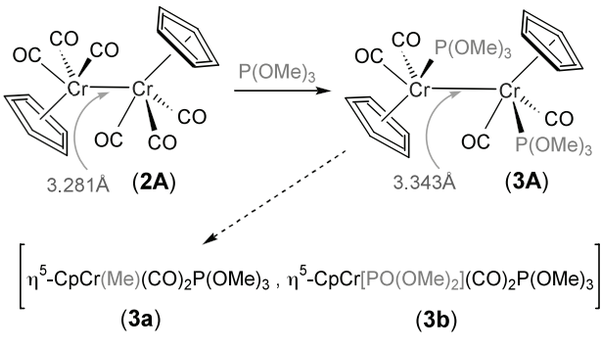
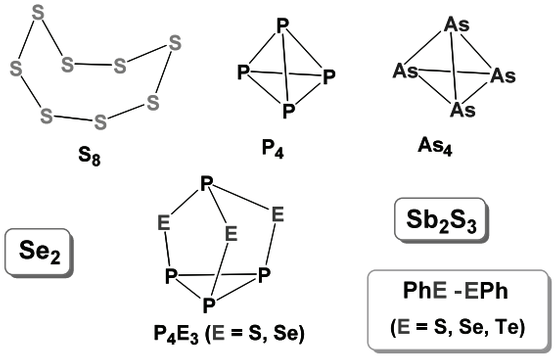
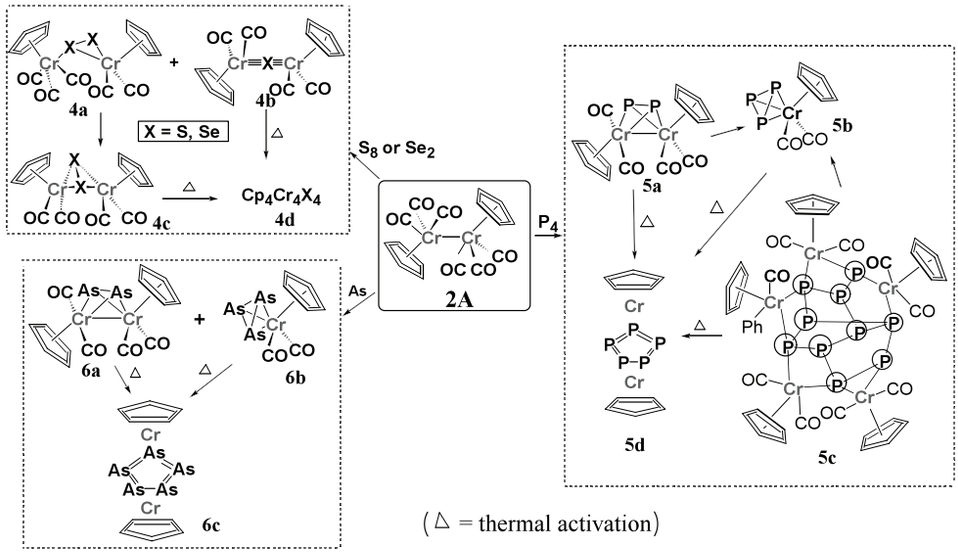



**Chart 1**




**Scheme 2**



**Scheme 3**

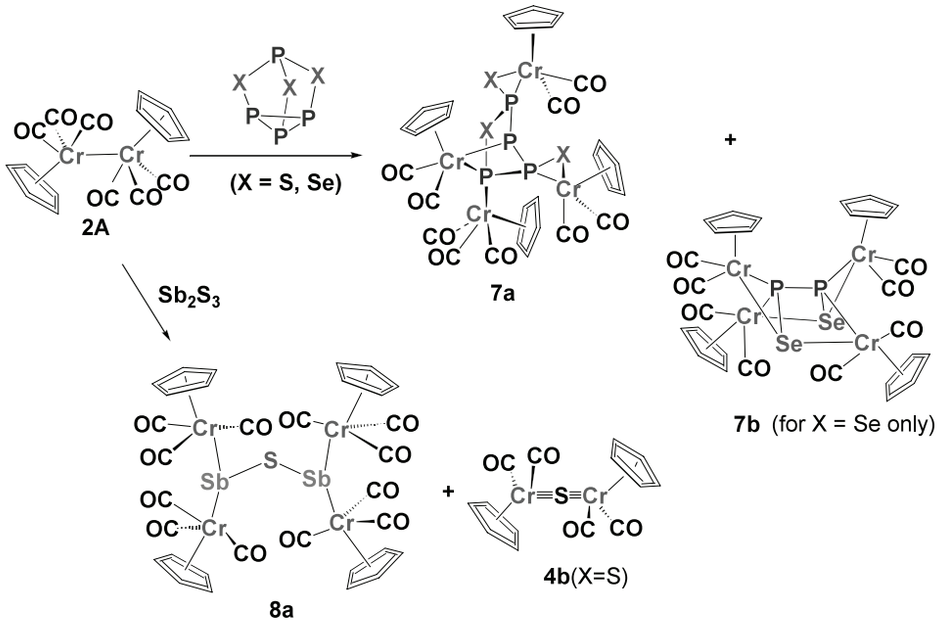
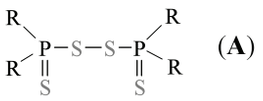
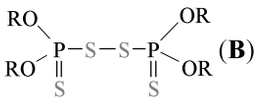


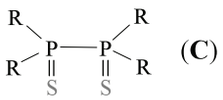

**Chart 2**


(**A**)

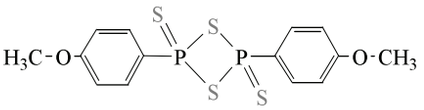
bis(thiophosphinyl)disulfanes


(**B**)

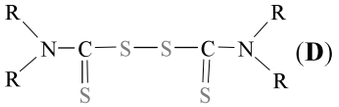
bis(thiophosphoryl)disulfanes


(**C**)

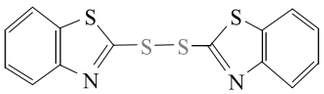
tetraalkyldiphosphine disulfides



Lawesson's Reagent (**LR**)


(**D**)

tetraalkylthiuram disulfides



dibenzothiazolyl disulfide (**E**)

sulfur atoms in the thiophosphinyl/phosphoryl ligands, with concomitant or subsequent intermolecular association to form the thiophosphinito complex $CpCr(CO)_2(SPPh_2)$ (**9c**), $CpCr(S_2PR'_2)_2$ (**9d**), the Cr≡S≡Cr compound $Cp_2Cr_2(CO)_4S$ (**4b**) [10, 15] and the cubane-like complex $Cp_4Cr_4S_4$ (**4d**), the ultimate thermolytic derivative of **4b** [10].

**Reactions with tetraalkyldiphosphine disulfides $R_2P(S)P(S)R_2$ (C)**

As previously found for the cleavage of P–P bonds in $P_4$ by **2A** [10, 11a,b], thermal activation is required for the reaction of **2A** with **C,** which yields the $\eta^2$-thiophosphinito complex $CpCr(CO)_2(SPR_2)$ (R= Me, Et, **10**) (Scheme 5) and desulfurized derivatives, *viz.* the hydrido-phosphido-bridged complexes $Cp_2Cr_2(CO)_4(\mu\text{-H})(\mu\text{-PR}_2)$ (**10a**), the bis(μ-phosphido) doubly metal-metal bonded complex $Cp_2Cr_2(CO)_2(\mu\text{-PR}_2)_2$ (**10b**), and the trinuclear complex $Cp_3Cr_3(CO)_2(S)(\mu\text{-PR}_2)$ (**10c**), together with $Cp_4Cr_4S_4$ (**4d**) as a minor product. These are demonstrated to arise from the thermal interaction of **10** and **2A**

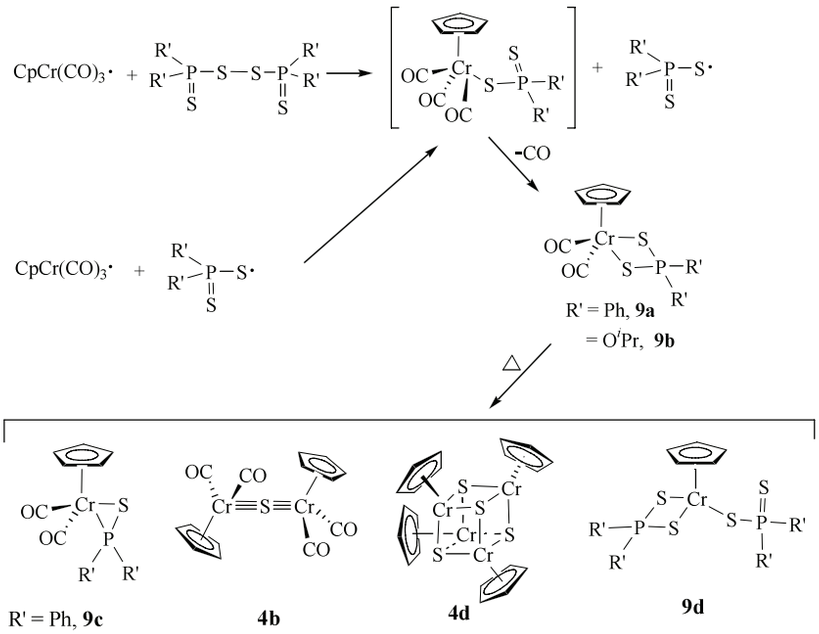
[16]. The structurally characterized complex **10c**, a phosphido-bridged tri-homometal cluster of a Group 6 element, adds to the family of such species of which the butoxide-bridged and nitrene-bridged analogues are known [17].

These results show that desulfurization of a thiophosphinito ligand at a CpCr center as in **10** provides a pathway to $\mu$-phosphido complexes, e. g. **10a – 10c**. These complexes have mainly been prepared from the reaction of metal carbonyls with diphosphanes $R_2PPR_2$ and phenyl phosphines $PPh_2H$ or $PPhH_2$.

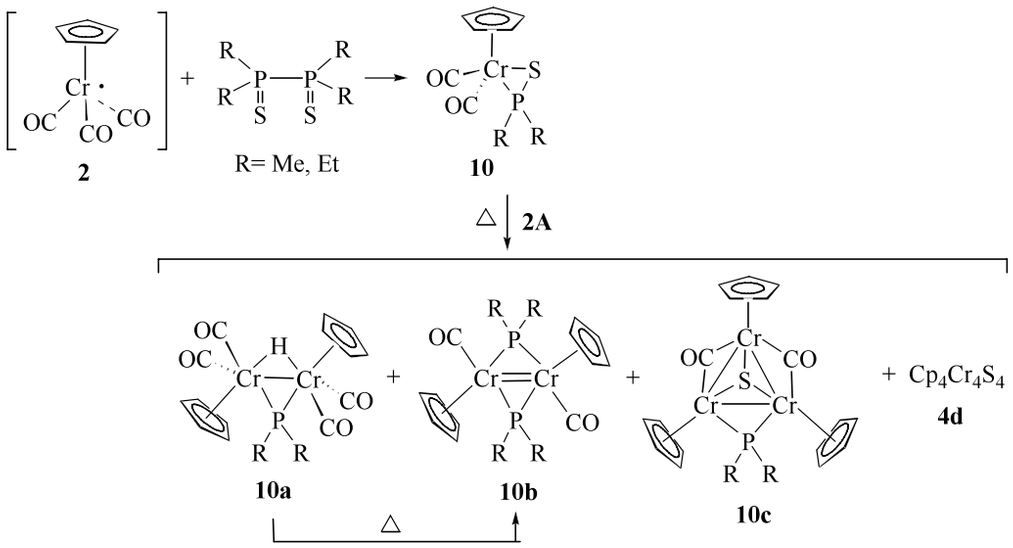
**Reactions with Lawesson's reagent $[CH_3OC_6H_4PS_2]_2$ (LR)**

The four-membered P–S bonded $P_2S_2$ ring with doubly-bonded S substituents on P in the molecule of **LR** presents a class of S- and P-containing substrate, very different from the S–S and P–P bonded systems discussed above. Though an effective thionation

**Scheme 4**




**Scheme 5**



agent towards organic substrates, **LR** has been little studied in transition metal chemistry.

The reaction of **LR** with an equimolar equivalent of **2A** gives products markedly dependent on reaction temperature, as shown in Scheme 6 [18]. Thus the ambient-temperature products are $Cp_2Cr_2(CO)_5(SPAr)$ (**11**), $Cp_2Cr_2(CO)_5(S_2PAr)$ (**12**), $Cp_2Cr_2(S_2P(O)Ar)_2$ (**13**), together with $Cp_2Cr_2(CO)_4S$ (**4b**), of which **11** and **12** are not detected at high temperatures, which result in additional new products, $CpCr(CO)_2(SP(H)Ar)$ (**15**), $[CpCr(CO)_2(SPAr)]_2$ (*cis*-**16**) and its isomer *trans*-**16**.

The molecular structures of **11** and **12** suggest that they both originate from a common intermediate, the radical species **I**, shown in Scheme 7, formed via interaction of **2** and the "monomer" of **LR**, route (i), or direct cleavage of the $P_2S_4$ central unit of **LR** by **2** (route (ii)). Subsequent reactions involving decarbonylation and desulphurization, with or without assistance from **2**, then generates the complexes **11** – **13**, described above.

The temperature-dependent distribution of product species and yields are consistent with bond homolysis 'a', leading to formation of **13** and **4b**, and homolytic dissociations 'b' and 'c', leading to **11**, **15** and **16** (Scheme 8)

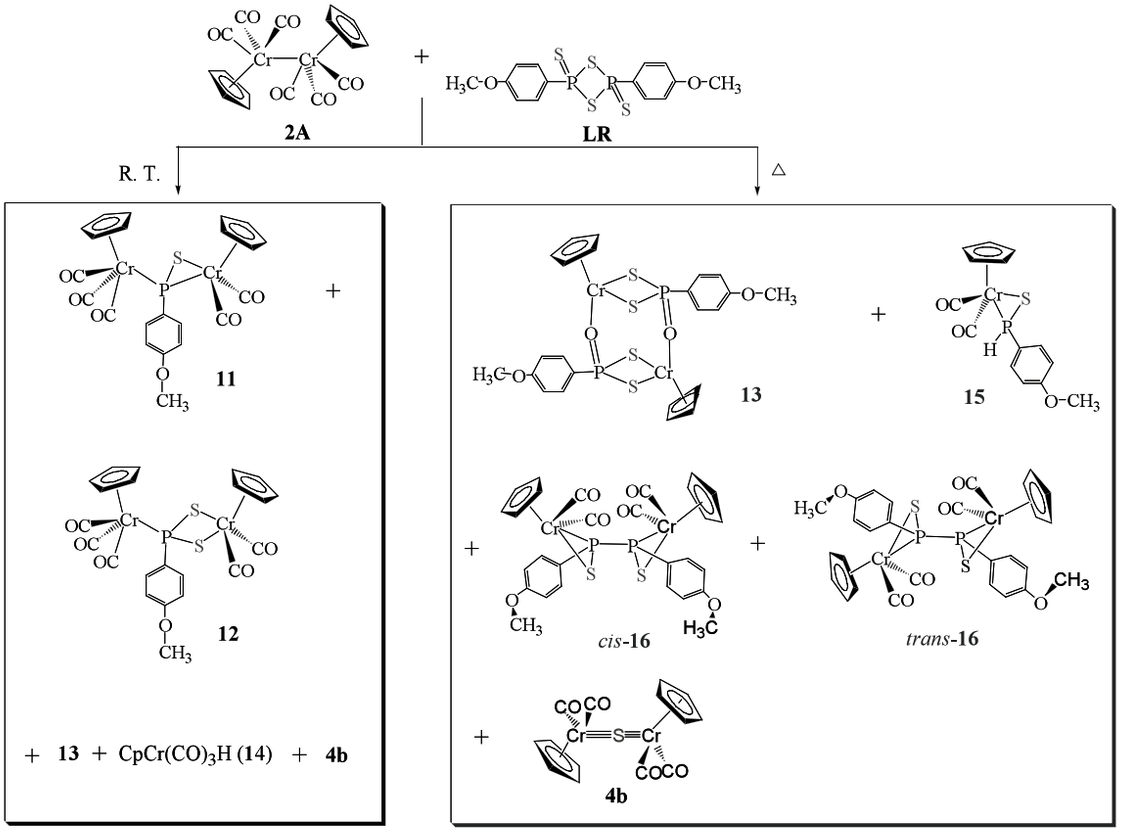
It was demonstrated that complex **16** also arises from Cr–P homolytic bond cleavage in **11**, followed by coupling of the P-centered radical species (**11a**) (Scheme 9).

This proposition is supported by (i) the reversal of the transformation by addition of **2A**, and (ii) the *ambient temperature* facilitation of the process by elemental sulfur or **LR**, which as effective scavengers for **2** drives the reaction towards formation of **16**.

## C–X (X = N, S) BOND CLEAVAGE AND C–C COUPLING

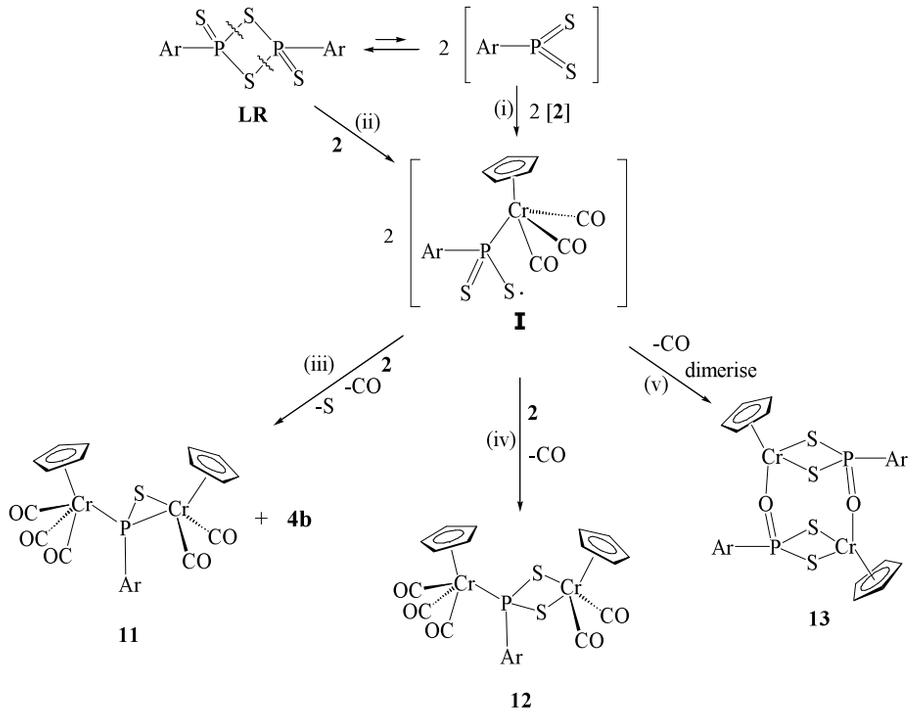
This article so far has shown the effectiveness of **2** in the cleavage of S–S, P–P and P–S bonds. The S- and N- containing organic substrates like thiuram disulfides and benzothiazoles provide situations for a study of the reactivity of **2** towards C–S and C–N bonds in the organic substrate in both its free and coordinated states.

**Scheme 6**



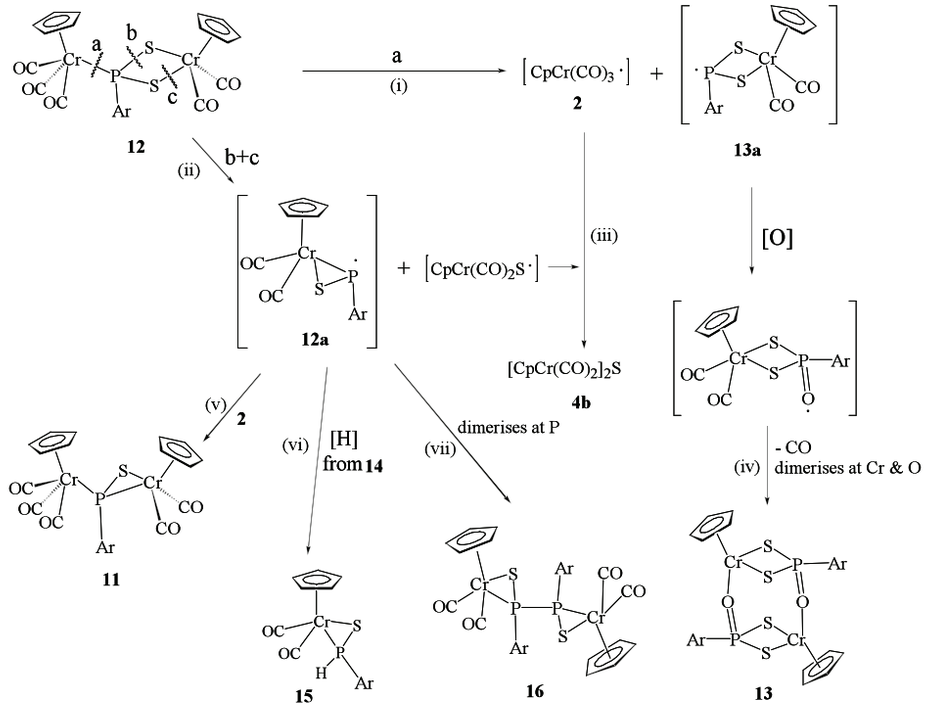
## Scheme 7

[Ar = $C_6H_4OCH_3$, ⌇ = bond cleavage]



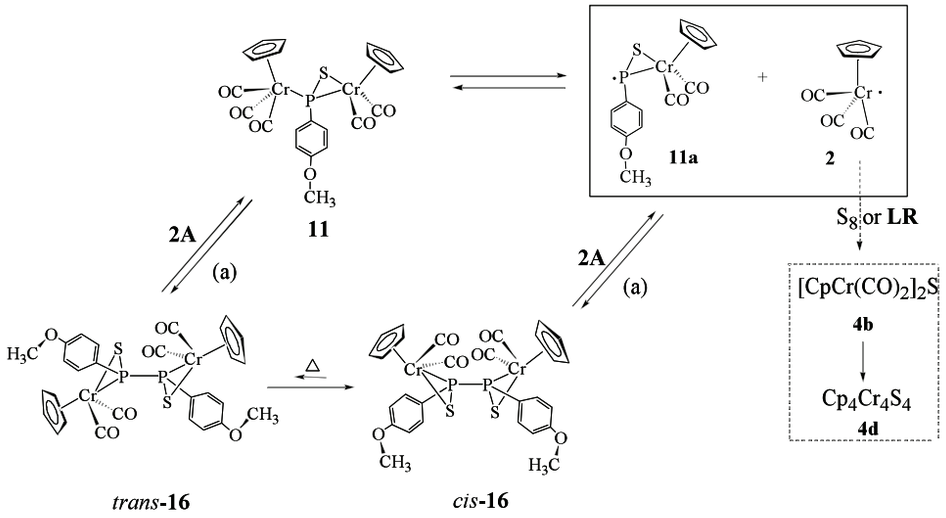

## Scheme 8

[ ⬭ = Cp or Cp*. a, b, c : bond homolysis]



**Scheme 9**

[(a) =△, $S_8$ or **LR**]




**Reaction with tetraalkylthiuram disulfides (D)**

Although the coordination chemistry of dithiocarbamate, $R_2NCS_2^-$, derived from tetraalkylthiuram disulfide (**D**), with both main group and transition metals, is well established, its organometallic chemistry is limited.

The facile reaction of **2** with **D** yields a product mixture, the composition of which is variant with temperature. *At temperatures below ambient*, the usual homolytic reaction of **2** produces the monodentate complex $CpCr(CO)_3(\eta^1\text{-}S_2CNR_2)$ (**17**), which readily decarbonylates at ambient temperature to give $CpCr(CO)_2(\eta^2\text{-}S_2CNR_2)$ (**18**) in high yield (Scheme 10). *At elevated temperatures*, the reaction leads to the isolation of **18** in reduced yield, together with a thiocarbenoid complex $CpCr(CO)_2(\eta^2\text{-}SCNR_2)$ (**19**), a thiocarboxamido dicubane-like cluster $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}SCNR_2)_2$ (**20**), a dithiocarbamate dicubane $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}S_2CNR_2)_2$ (**21**), the coordination compound $Cr(\eta^2\text{-}S_2CNR_2)_3$ (**22**), $Cp_2Cr_2(CO)_4S$ (**4b**) and $Cp_4Cr_4S_4$ (**4d**) [19 a,b]. A similar product composition is obtained from thermolytic degradation of **18** [19b].

In the presence of $[CpCr(CO)_3]_2$ (**2A**), the thermolysis of **18** gives additional products, viz. chromium carbyne complex $CpCr(CO)_2(CNR_2)$ (**23**) and an aminoacyl complex $CpCr(CO)_2(\eta^2\text{-}C,O\text{-}C(O)C(NR_2)CH(NR_2))$ (**24**). (Scheme 11) [19c]. An independent reaction shows that the carbyne complex **23** derives from thermal desulfurization of the thiocarbenoid complex **19** with **2A**.

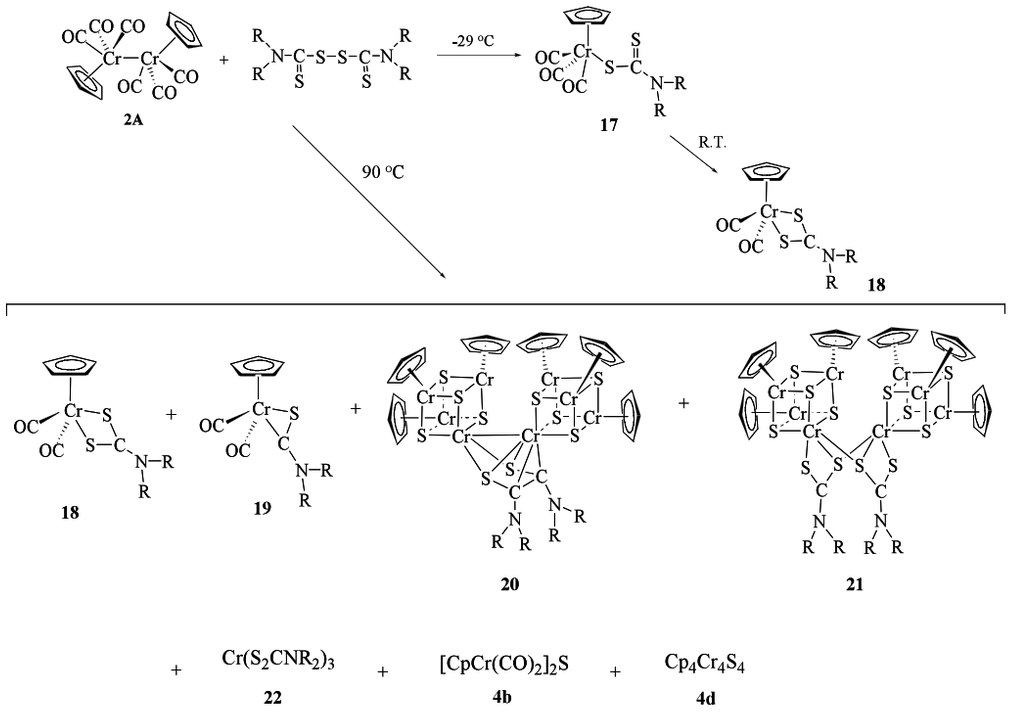
In an unprecedented reaction, complex **2A** "cleaves" the chelate rings in $Cr(S_2CNEt_2)_3$ (**22**) under thermolytic conditions, effecting a transfer of dithiocarbamate ligands to CpCr moieties to give a mixture of complexes **19**, **20**, **23** and **4d** (Scheme 12).

The products profile of these three reactions shows that while the cleavage of one sulfur atom from **18** is a thermally-achievable process, double desulfurization requires assistance from **2**. As in previous cases, the isolation of **4d** in substantial amounts provides evidence for the initial formation of the precursor complex $[CpCr(CO)_2]_2S$ (**4b**), a finding congruent with the observed thiophilicity of **2**. The thermal conversion of **18** to the double cubane-like complexes **20** and **21** containing $(\eta^5\text{-}CpCr)_3CrS_4$, is a new reactivity feature, not observed in the synthesis of the Mo or W analogues of **17** under thermal and photochemical conditions, respectively.

The significant feature of **20** is the presence of a dithiooxamide ligand (DTO = $Et_2NC(S)=C(S)NEt_2$), which links the two cubane-like cores with $\eta^2,\eta^4$ bonding mode, in addition to a weak M–M bond (3.101 Å) between the two 'cubanes'. The formation of the DTO ligand has involved a single C–S bond cleavage of each of two DTC ligands with C–C coupling of the resulting moieties. The cuboidal units in **21** are doubly bridged by two dithiocarbamate ligands, each bonding in a $\eta^1$-S,$\eta^2$-S,S' coordination mode, and do not involve any M–M bonding. In both these double

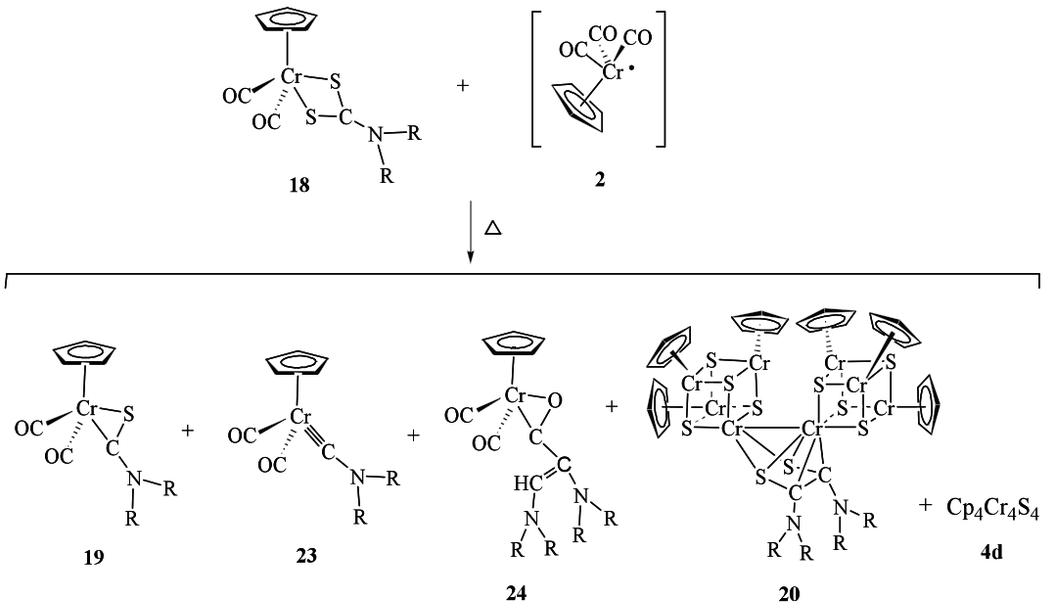
## Scheme 10

[ R= Me, Et or $^{i}$Pr.
the six Cr-Cr bonds in each of the cuboidal cores of **20** and **21**are omitted for clarity]



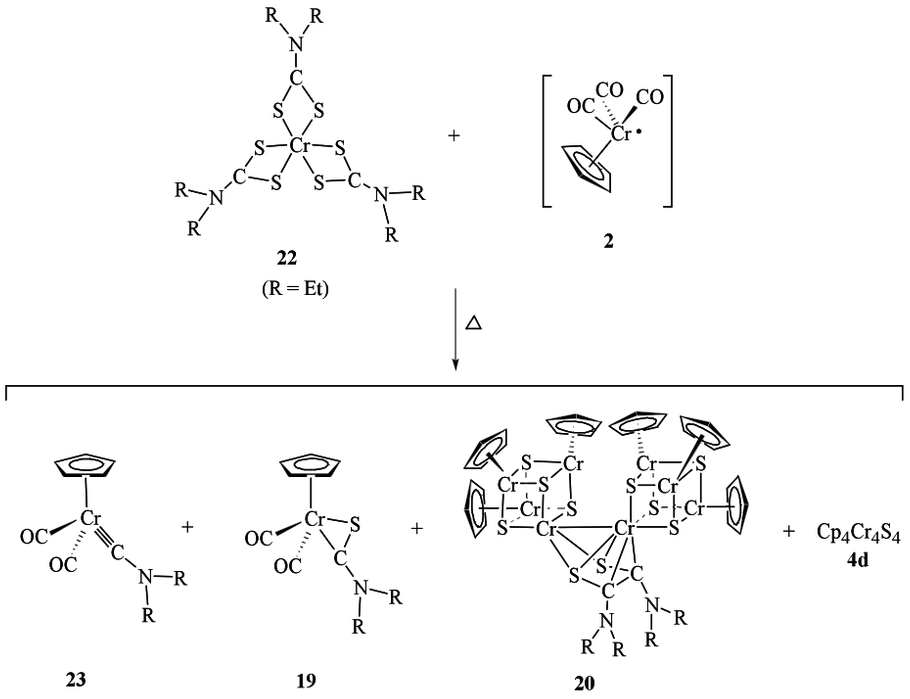

## Scheme 11

[R = Et.
The Cr-Cr bonds in the cuboidal core of **20** are omitted for clarity]



**Scheme 12**

[R = Et
The Cr-Cr bonds in the cuboidal core of **20** are omitted for clarity]




cubane-like molecules, dissociation of a $\eta^5$-Cp ligand has occurred at one Cr corner to accommodate the bridging ligands. Such Cp ligand dissociation seems to be facile in these CpCr systems, though rarely observed in Cp-metal chemistry.

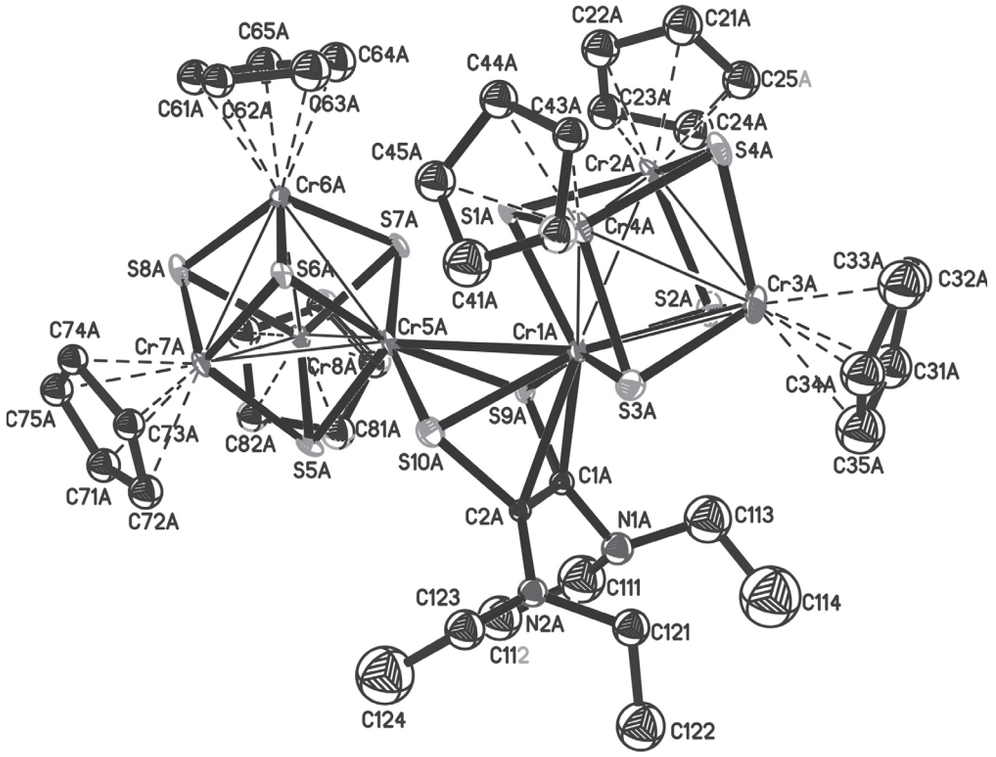
The complex **23** belongs to the rare group of aminocarbyne chromium complexes, the first example of which was isolated by Filippou and coworkers from a multiple-step synthesis from $Cr(CO)_6$ [20]. The intermediate formation of a carbenoid species $R_2NC$**:** is indicated by the presence of alkene and alkenyl acyl moieties in the structural composition of **20** and **24**, respectively, suggestive of carbene dimerisation as found in the formation of the DTO ligand discussed above. The carbyne moiety $R_2NC\equiv$ is evident in the structure of **23.**

The profile of the product composition shows that with respect to sulfur cleavage, the reaction pathways fall into three categories, in which (i) the dithiocarbamate (DTC) ligand remains intact, as in the DTC bridged cubane **21** and the coordination compound $Cr^{III}(DTC)_3$ **22**, (ii) the DTC ligand has undergone mono-sulfur cleavage, as found in the thiocarbenoid complex **19** and the dithiooxamide di-cuboidal compound **20**, and (iii) the DTC ligand has suffered double sulfur cleavage, as found in the Cr-aminocarbyne complex **23**, and the alkenylacyl compound **24,** respectively.

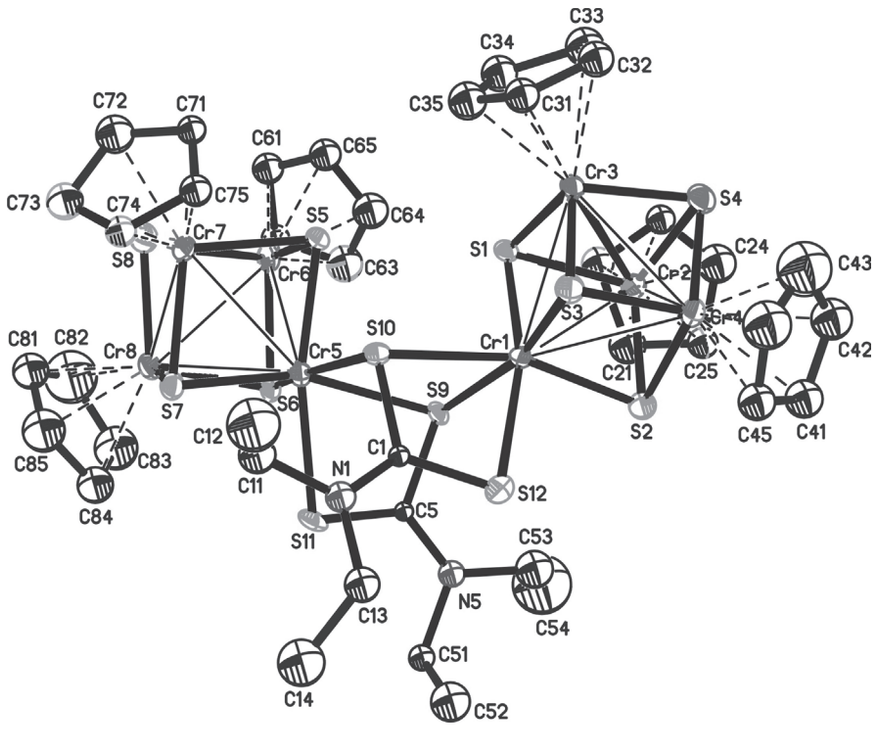
**Reaction with 2,2'-dibenzothiazolyl disulfide (E)**

The attractive features of thiazolyl disulfides for this study are the presence of a homolytically-cleavable S–S bond and a heterocyclic component often encountered in many bioactive molecules [21]. Since mechanisms of ring-opening and closure of heterocycles in biomolecules are of current active interest [21c], our intention was to examine the role of **2/2A** in probable ring-cleavage reactions in a thiazole ligated to CpCr.

The instantaneous reaction of **2A** with one mol equivalent of **E** at ambient temperature gives $CpCr(CO)_2(SCSN(C_6H_4))$ (**25**) in high yield. The further reaction of **25** with **2A** under thermolytic conditions produces $[Cp_2Cr_2(CO)_2(=CNS(C_6H_4))]_2$ (**26**), $Cp_5Cr_6S_4(SN(C_6H_4))(SNC_2(C_6H_4))$ (**27**), $Cp_6Cr_8S_4(OH)(SN(C_6H_4))_2(SNC_2(C_6H_4))_2$ (**28**), 2,2'-bibenzothiazole $(C_6H_4NSC)_2$ (**29**) and $Cp_4Cr_4S_4$ (**4d**) (Scheme 13) [22]. In contrast, in the absence of **2A**, **25** and **27** are thermolysed to non-characterisable compounds, while **26** remains unchanged.

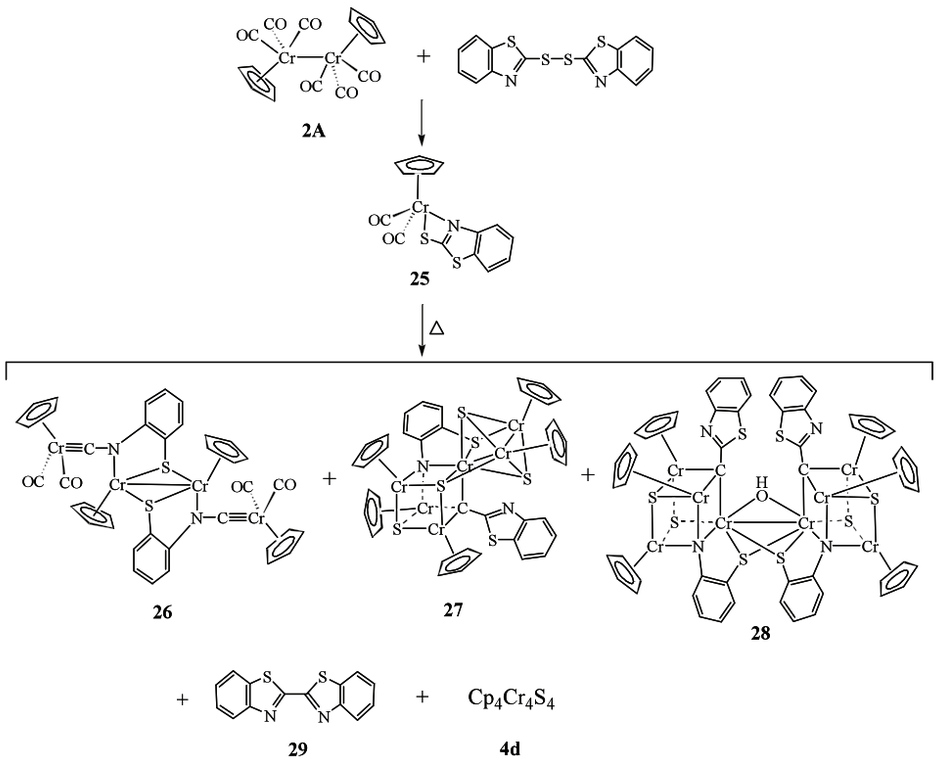



Molecular structure of $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}SCNEt_2)_2$ (**20**)
[Cr1A–Cr5A = 3.101 Å]

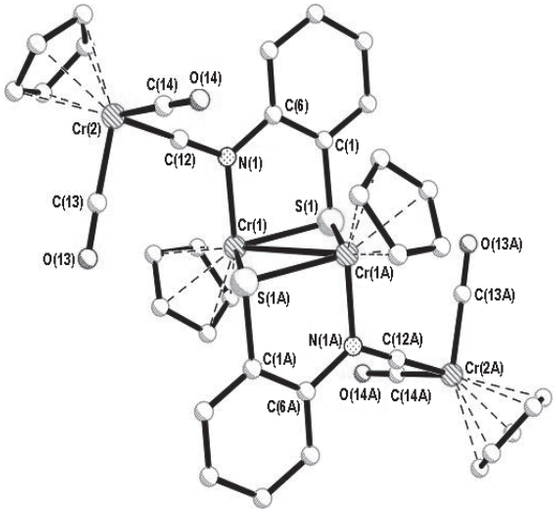



Molecular structure of $Cp_6Cr_8S_8(\eta^2,\eta^4\text{-}S_2CNEt_2)_2$ (**21**)
[Cr(1) … Cr(5) = 3.853(7) Å]

**Scheme 13**




The molecular structure of **26** possesses a crystallographic center of symmetry at the midpoint of the Cr(1)–Cr(1A) bond. A salient feature is the chair configuration in the central portion of the molecule with the planar four-membered $Cr_2S_2$ ring forming the "seat", wherein lies the Cr–Cr bond.



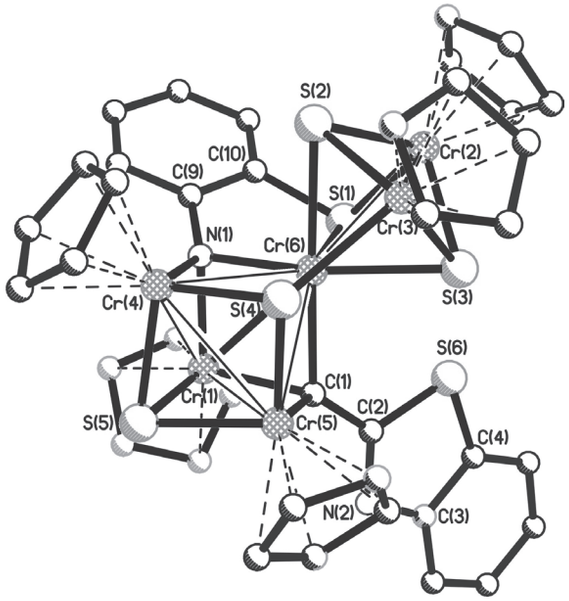

Molecular structure of $[Cp_2Cr_2(CO)_2(=CNS(C_6H_4))]_2$ (**26**) [Cr(1)–Cr(1A) = 3.070(1) Å]

A significant feature in **27** is the $Cr_4S_2CN$ cube, wherein three of the Cr corners are still attached to $\eta^5$-Cp rings, while the fourth corner (Cr(6)) is capped by a dichromium-trisulfur moiety, Cr(2) Cr(3)S(1)S(2)S(3) where S(1) is a component of the benzothiolatonitrido unit, which thus edge-bridges Cr(6) and the N(1) corner of the cube. The $\mu_4$-bonding S(4) is linked to Cr(3), Cr(4), Cr(5) and Cr(6). The carbido C(1) corner of the cube is singly-bonded to C(2), a component atom of a benzothiazole unit.

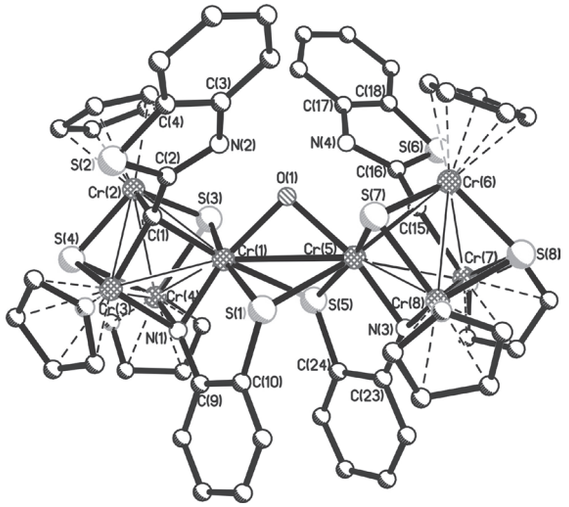
The molecular structure of **28** consists of double cubane moieties ($Cr_4S_2CN$), quadruply bridged by a weak Cr–Cr bond, a hydroxo ligand and the thiolato sulphur atoms of two benzothiolatonitrido units, the N atom of each of which constitutes one of the corners of each cube. The C atom in the cube is linked to a benzothiazole unit. The nature of the components of the cubes and of the bridge of this double 'cubane' has no precedent among the numerous cubane and double-cubane compounds, which have been extensively studied by Holm, Coucouvanis and Sykes [23].

The structural composition of **26**–**29** supports their formation from radical moieties, either discrete or quasi-associated, arising from the sequential

cleavage by **2** of C–S (steps (i) and (iv)), Cr–S (step (ii)), Cr–N (step (iii)) and C–N (step (v)) bonds in **25**, as proposed in Scheme 14.



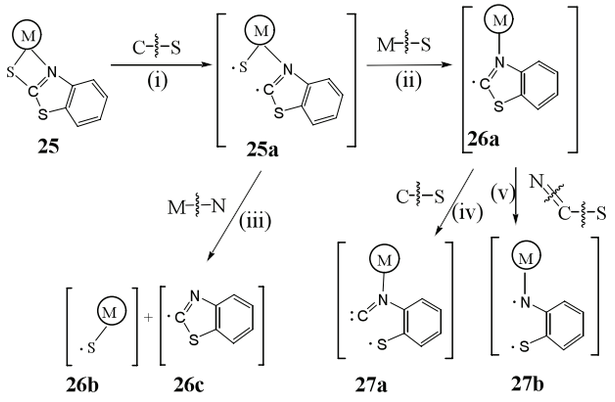

Molecular structure of $Cp_5Cr_6S_4(SN(C_6H_4\ (SNC_2–(C_6H_4))$ (**27**) [In the cube, Cr–Cr = 2.7065(6)–2.8972(7) Å. Others Cr(2)–Cr(3) = 2.9137(7), Cr(6)–Cr(2) = 2.8264(7) and Cr(6)–Cr(3) = 2.7933(7) Å]




Molecular structure of $Cp_6Cr_8S_4(OH)$-$(SN(C_6H_4))_2$ $(SNC_2(C_6H_4))_2$ (**28**) [Cr–Cr = 3.079(1), 3.087(1) Å in the two independent molecules in the unit cell In the cube, Cr–Cr = 2.6611(17) – 2.8211(18) Å]

**Scheme 14**

[ Ⓜ = $CpCr(CO)_2$. ⦚ = bond cleavage ]



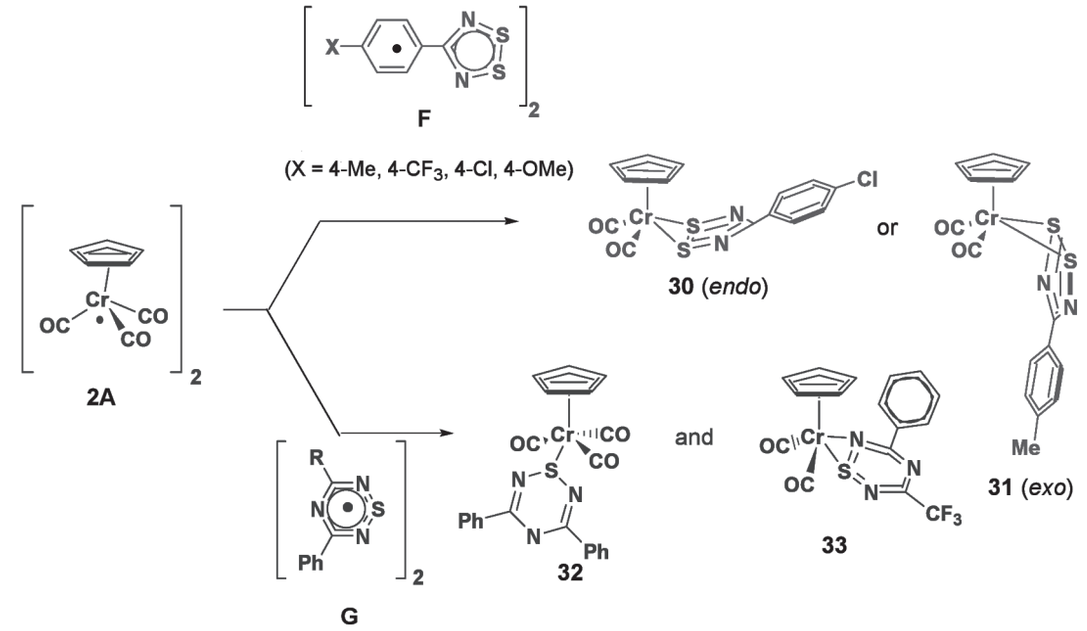

## REACTIONS WITH MAIN GROUP HETEROCYCLIC RADICALS (F and G)

The interaction between **2A** and dithiadiazolyl dimers $[S_2N_2CNR]_2$ (R = substituted aryl rings) has resulted in the isolation of a series of the first π organometallic complexes of C,N,S-heterocyclic compounds. Thus, the coupling of **2** and heterocyclic dithiadiazolyl radical **F** led to the formation of unique diamagnetic *endo* (**30**) and *exo* (**31**) isomers, in which the C,N,S rings are $\pi$:$\eta^2$-S,S'–coordinated to the metal. (Scheme 15). These isomers inter-convert in solution and remain redox-active through ligand-centered reductions.[24a,c] A similar reaction with **G** gave the complexes **32** and **33**, in which heterocyclic ring is S-bonded to Cr and $\eta^2$-N,S–coordinated, respectively.[24b] This study is particularly significant for providing the first examples of π-complexes of any thiazyl heterocycles and opens up a largely unexplored coordination chemistry of unsaturated C-N-S heterocyclic free radicals with paramagnetic organometallic species.

## SUMMARY

The 17-electron organometallic radical $[CpCr(CO)_3]$ (**2**) displays a remarkable capability in the scission of S–S, P–P and P–S bonds in organic substrates, forming radical-coupled products containing cyclopentadienyl chromium. By virtue of its high reactivity as a radical species and an avid thiophile, **2**

**Scheme 15**




further effects efficient cleavage of C–N, C–S, P–S, Cr–E (E = C, N, P and S) bonds in the CpCr complexes, generating radical species, which aggregate to yield a variety of new compounds, incorporating C–C and P–P bond formation in some cases. These findings suggest that fruitful results may be obtained from further investigations of the reactivity of **2**/**2A** towards radical or radical-like species from main group or transition metal compounds, particularly those containing sulfur ligands.

**Acknowledgements** – This work was supported by the University of Malaya and the National University of Singapore. The author acknowledges the intellectual and experimental contributions of her co-workers and students, whose names are cited in the references.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 105-108

# Microcantilever release process using micromachining technology

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*Received 14-02-2011; accepted 24-03-2011*

**Abstract** An isotropic deep reactive ion etching (DRIE) technique was used to release a suspended $SiO_2$ microcantilever from the substrate of SOI wafer using bulk micromachining technology. The dimension of the fabricated $SiO_2$microcantilever was 250 µm in length, 100 µm in width with thickness of 1 µm. Employing the plasma dry etching technique, the release of $SiO_2$ microcantilever from the frontside of the wafer was done using Plasmalab System 100.The etching parameters such as $SF_6$ flow, ICP power, RF power, temperature and $O_2$ flow were properly specified in order to obtain anisotropic condition with significant lateral etch rate for the microcantilever release. For optimum etching condition at maximum lateral etch rate, the chamber pressure was varied in the range of 10 to 30 mTorr. The optimum etching condition was realized at maximum chamber pressure of 30 mTorr which yielded lateral etch rate of 5 µm/min and vertical etch rate of 8 µm/min. In conclusion, by using an isotropic dry etching technique utilized from micromachining technology, a microcantilever release from the substrate of SOI wafer was successfully realized.

**Keywords** microcantilever release – MEMS – plasma isotropic etching – SOI wafer – DRIE system

## INTRODUCTION

The advances in MEMS devices development have been triggered by the improvement in fabrication techniques of micromachining technology. Among the MEMS devices that have been successfully fabricated are microneedles, microvalve, microtransformer and micropump [1-4]. The most critical process in the fabrication of microcantilever is releasing the suspended beam. Many fabrication techniques from the micromachining technology have been employed by researchers in obtaining the suspended microcantilever including dry and wet etching. Wet etching process such as KOH etching was commonly employed to release the cantilever structure from the substrate wafer by etching the backside of the wafer. However, this method always comes with additional preventive methods in avoiding the stiction problem encountered during the release and drying steps [5,6]. Because of these reasons, dry etching technique is preferable due to the fact that it offers simpler etching process sequences and excellent etching profile.

Deep reactive ion etching (DRIE) which falls under the dry etching technique is considered as an extension of RIE. It relies on the same etching mechanisms of ion bombardment and chemical etching as RIE. Compare to RIE, the DRIE enables the fabrication of deeper and narrower structures at higher etch rate. DRIE reactors are equipped with two power sources which are inductively coupled plasma (ICP) source and radio frequency (RF) source which employ sidewall passivation for process anisotropy. Recent microfabrication technology introduces anisotropic dry plasma etching technique from DRIE system for suspended microcantilever release from the backside of the wafer which offers higher etch rate, compatible with traditional IC processing and most importantly, higher Si: $SiO_2$ selectivity in releasing $SiO_2$ microcantilever beams from bulk silicon [7]. However, the removal of about 300-500 µm thick sacrificial silicon from the backside of the wafer in anisotropic dry etching can weaken the device structure. Apart from anisotropic etching of DRIE system, the isotropic etching process can also be conducted using the system. The isotropic profile of DRIE system has been utilized in the fabrication of thermal microbridge [8]. Similar studies of isotropic dry etching employing inductive coupled plasma (ICP) system have been reported [9]. However, the undercut rate and etch rate are difficult to control

because of the simplicity of the gas phase etching process in the ICP system [9].

In this paper, an isotropic dry plasma etching to release the suspended $SiO_2$microcantilever from the substrate of SOI wafer is developed. Employing the DRIE system, the frontside etching for the $SiO_2$ microcantilever release was done using Plasmalab System 100. The etching parameters such as $SF_6$ flow, ICP power, RF power, temperature and $O_2$ flow were properly specified in order to obtain anisotropic condition with significant lateral etch rate for the microcantilever release. For optimum etching in the microcantilever release, the chamber pressure was varied in the range of 10 to 30 mTorr. The effect of varying the chamber pressure on the lateral etch rate of the isotropic etching process was studied. This study also investigated the stability of photoresist and aluminum as etch masks in isotropic etching process.

## MATERIALS AND METHODS

SOI wafer was used in this study for its outstanding advantages in terms of process simplicity and uniform doping profile. The wafer consisted of about 300 µm thick susbstrate silicon, 1 µm thick buried oxide (BOX) layer and 2 µm thick silicon device layer. The BOX layer was utilized as the $SiO_2$ microcantilever beam while the silicon device layer was reserved as a piezoresistive layer that could be used for piezoresistive sensing in MEMS microcantilever sensor applications. The utilization of SOI wafer in microcantilever release process involved bulk micromachining technology where the suspended structure was realized by sequences of etching processes. The dimension of the fabricated $SiO_2$ microcantilever was 250 µm in length, 100 µm in width with thickness of 1 µm.

First, the investigation on the possibility of using photoresist as an etch mask in isotropic dry etching process was carried out. The etch mask was used to protect some parts of wafer while the unprotected parts were removed during etching process. The etch mask should be stable under the etching conditions. For this purpose, two types of etch masks, photoresist and aluminum were used for comparison. For photoresist mask, resist of AZ 4620 of about 7 µm thick was used. For aluminum mask, an aluminum layer of about 1.5 µm thick which was deposited using metal evaporation technique was used as the etch mask. The mask patterns were then transferred onto the wafer using optical lithography process. Using both mask types, isotropic dry etching was conducted on the patterned wafer for 20 min to observe the stability of both etch mask types.

The anisotropic DRIE process relies on inductively coupled $SF_6/O_2$ plasma at temperatures below -100ºC. The anisotropy of the etching process is enhanced by a thin passivation layer on sidewalls that prevents lateral etching. The thickness of the passivation layer can be adjusted by changing the process temperature and the $O_2$ flow. At higher temperatures and without $O_2$ flow, the passivation layer is not formed which results in isotropic etching profile. Therefore, for the isotropic etching using the DRIE system, the etching temperature was set at 20ºC with $SF_6$ flow only, no $O_2$ flow.The suitable etching parameters for the isotropic etching condition were specified as 70 sccm $SF_6$ flow, 2000 W ICP power, 3 W RF power, 20ºC etching temperature and 0 sccm $O_2$ flow. The chamber pressure was varied in the range of 10 to 30 mTorr to observe the effect of the chamber pressure on the lateral etch rate of the etching process.

## RESULTS AND DISCUSSION

The observation on the stability of photoresist as an etch mask showed that the resist could not sustain the plasma reactions in the isotropic etching condition. After about 20 min of isotropic etching under lateral etch rate of 5 µm/min, the photoresist etch mask had disappeared indicating that the photoresist was slowly consumed during the etching process (Fig. 1). After certain etch time, the photoresist had vanished and the silicon structure which was previously covered under the photoresist was exposed. The exposed structure was also etched away during the subsequent etching process (Fig. 1).

Consequently, the aluminum etch mask was found very stable under the isotropic etching condition (Fig. 2). The etch mask protected the microcantilever structure very well and could sustain the ion bombardment reaction throughout the etching process as it had better mechanical properties and selectivity than photoresist.

The observation on the effects of the chamber pressure showed that higher pressure resulted in higher etch rate (Table 1). For example, 10 mTorr chamber pressure yielded 2.46 µm/min lateral

etch rate while 30 mTorr pressure yielded higher lateral etch rate of 5.04 µm/min. The high process pressure contributed to an increment of the angular distribution of ions which indirectly increased the lateral etch rate of the etching process.

Employing the specified etching parameters of the isotropic etching, the $SiO_2$ suspended microcantilever release from SOI wafer was realized. Fig. 3 shows the lateral and vertical etching profile of the isotropic etching of partially released microcantilever while Fig. 4 shows an SEM image of the suspended microcantilever which had been completely released using the isotropic etching.

## CONCLUSION

By utilizing the isotropic profile of plasma dry etching from the DRIE system, the microcantilever release from the frontside of the SOI wafer has been successfully realized. The optimum etching condition of 0.63 isotropic ratio was obtained at 70 sccm $SF_6$ flow, 2000 W ICP power, 3 W RF power, 20ºC etching temperature and 0 sccm $O_2$ flow at maximum pressure chamber of 30 mTorr.

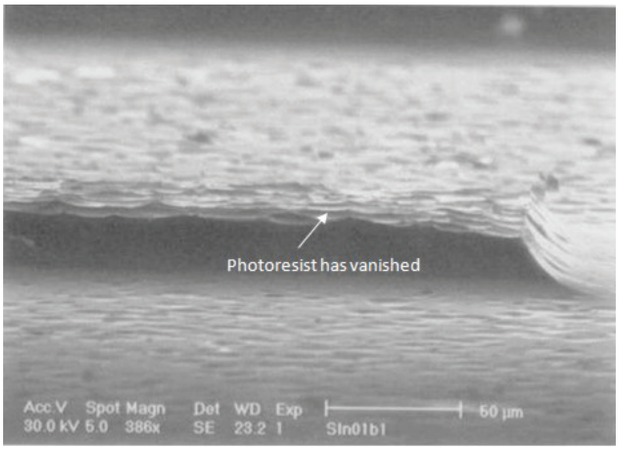


**Figure 1**. SEM image of microcantilever structure using photoresist etch mask after 20 min of isotropic dry etching.

**Table 1.** Etching parameters for microcantilever release.

| Parameter | Run 1 | Run 2 | Run 3 |
|---|---|---|---|
| $SF_6$ flow (sccm) | 70 | 70 | 70 |
| $O_2$ flow (sccm) | - | - | - |
| Pressure (mTorr) | 10 | 20 | 30 |
| ICP (W) | 2000 | 2000 | 2000 |
| RF (W) | 3 | 3 | 3 |
| Temperature (ºC) | 20 | 20 | 20 |
| Lateral etch rate (µm/min) | 2.46 | 2.84 | 5.04 |
| Vertical etch rate (µm/min) | 4.54 | 4.88 | 7.96 |
| Isotropic ratio | 0.54 | 0.58 | 0.63 |

**Acknowledgment** – The authors would like to thank the Malaysian Ministry of Science, Technology and Innovation under IRPA project (03-02-02-0015-SR003/07-01): MEMS Devices and Sensing Microstructure.

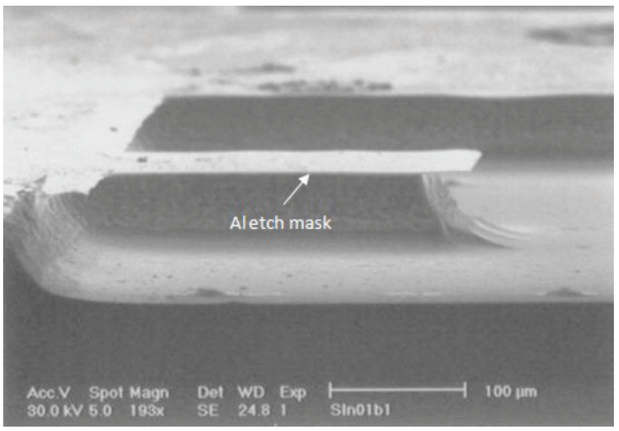


**Figure 2**. SEM image of microcantilever structure using aluminum etch mask after 20 min of isotropic dry etching.

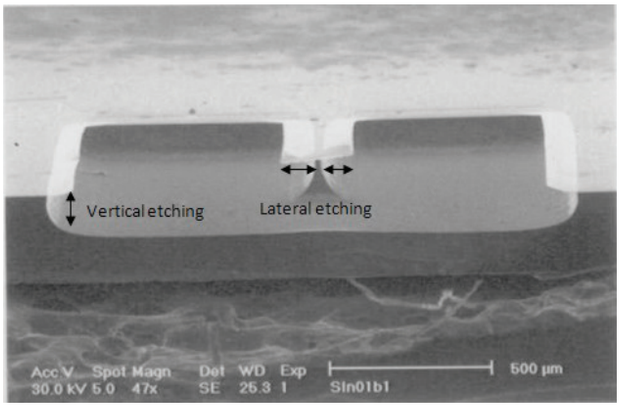


**Figure 3**. SEM image of partially released microcantilever showing lateral and vertical etching profiles.

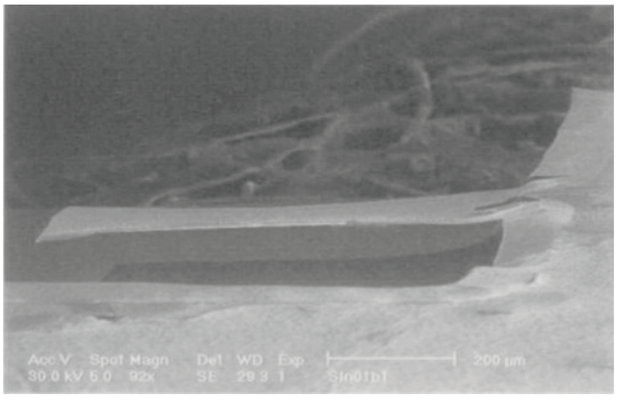


**Figure 4**. SEM image of 1 µm thick $SiO_2$microcantilever which was completely released.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 109-114

# Roto-dynamic faults investigation using acoustic emission technique

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**Abstract** This paper provides a method of acoustic emission (AE) technique to detect roto-dynamic faults of rotating machineries. An approach is to utilize dynamic envelope which was extracted from the raw time-domain signals and processed to its spectrum using Fourier transform. The transformed signals may contain unique characteristic features relating to the various types of bearing faults. The experiments on different operating conditions were investigated and they corresponded to (a) a balanced, and (b) an unbalanced with misaligned shaft. The diagnostic process will discriminate between different bearing conditions. The recognition rate achieved with the limited test sets was promising.

**Keywords** Acoustic emission – roto-dynamic – low speed – bearing

## INTRODUCTION

Bearings can be found on almost all machines. Their failure invariably has production consequences and sometimes even health and safety consequences. A reliable condition monitoring system is therefore highly desirable for it will alleviate the cost of these consequences and enhance the overall equipment effectiveness.

For bearing maintenance, two methods have been used, namely the statistical bearing life estimation and the bearing condition monitoring and diagnostics [1, 2]. The first method relies on a model of the bearing survival probability in terms of the dynamic load rating and the equivalent load to give a prediction of the fatigue life of a bearing [3-5]. However, since operating conditions can vary significantly from one machine to another, the prediction based on the assumption of normal duty on a bearing can be in serious error. The second method, in theory, is superior to the first if the signals monitored have useful features that can reliably indicate a potential failure well ahead of the occurrence of the corresponding functional failure [6-8]. Signals that have been studied for bearing condition monitoring include time and frequency domain of acoustic emission signal.

In this paper, a frequency-domain technique, namely the Fast Fourier Transform (FFT) was assessed in terms of its ability to discriminate between different types of signals that had transient features in them. These signals were collected from different bearing conditions: normal; unbalanced and misaligned shaft.

The objective of this research is to demonstrate that a condition-based monitoring using acoustic emission (AE) can provide not only timely detection of low speed bearing but also the fault identification so that maintenance or replacement can be performed prior to the loss of safety function. Therefore the use of acoustic emission method has been proposed for low speed machineries monitoring instead of the conventional method.

## THE PROPOSED APPROACH

Figure 1 shows a block diagram of the proposed bearing condition monitoring procedure. The acquired AE signals are first filtered and amplified to remove noise and then processed in order to obtain AE envelope signal. Then, the Fast Fourier Transform was used to produce its frequency domain pattern. The frequency domain has a horizontal axis representing frequency and a vertical axis representing the intensity of the frequency component.

The pre-processed parameter of the dynamic envelope of the AE signal can be obtained from AE Ultraspan (Holroyd Instruments, UK) (Fig. 2). The AE Ultraspan is the wireless sensor which provides

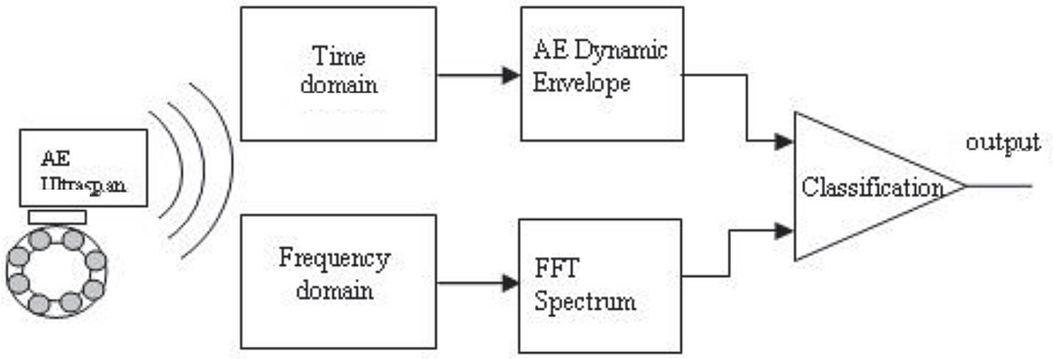


**Figure 1**. The proposed bearing monitoring block diagram.

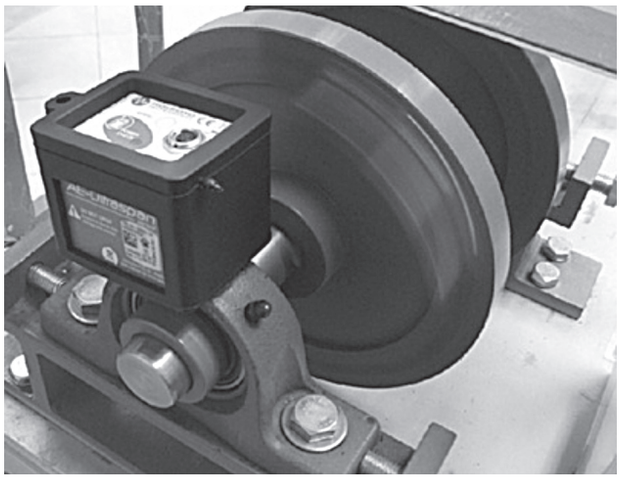

**Figure 2**. AE Ultraspan wireless sensor (Holroyd Instruments, UK).

many opportunities for diagnosing the nature of faults. The time dependence of the AE signal can reveal the occurrence and timing of subtle actions within machineries.

The AE dynamic envelope is suitable for both burst type and continuous AE signals. Effect of signal averaging is to improve statistical significance but distorts shape of activity giving time decays and reduced peak value. It is also represented in logarithmic scale of AE signals since it allows a greater signal range to be simultaneously observed with its unit in dB and is a ratio with respect to a reference voltage [9].

$$AE\ Log\ Magnitude in\ dB = 20\log_{10}\left(V_{sig}/V_{ref}\right)$$

To identify the roto-dynamic faults of low speed machineries, the Fast Fourier Transform is used in this study. The Fast Fourier Transform (FFT) does exactly that [10]. The Fourier transform of a signal x(t) is defined as:

$$F\left[x(t)\right] = X(f) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} x(t)e^{-j2\pi f t}dt$$

For Equation to be valid, the signal x(t) being transformed must be stationary, which means that its amplitude distribution does not depend on absolute time. In other words, the moments of the distribution – for example, mean, variance, and so on – are stationary.

## EXPERIMENTAL APPLICATION OF THE PROPOSED METHOD

The experimental setup consisted of roto-dynamic test rig which can produce multi fault operating conditions (Fig. 3a). Its schematic diagram of the test rig is shown in Figure 3b. The spindle was driven by a variable speed motor running at 1430 rpm. The two bearings were SKF 60062Z deep groove single row ball bearing. They were mounted in bearing housings which in turn were attached to a base plate. The test rig provided facilities to produce the different machine operating conditions characterized by: (1) the rotating shaft dynamically balanced (referred to as 'balanced shaft'); and (2) the rotating shaft dynamically unbalanced in one plane to the extent of 70 x $10^{-5}$ kg.m at roto-disc with misalignment achieved from moving one bearing laterally by 1 mm relative to the other (referred to as 'unbalanced and misaligned shaft').

Acoustic emission signal at the bearing was measured using AE Ultraspan sensor manufactured by Holroyd Instruments, UK (on top of both end and non-drive end housing). The AE sensor had resonance frequency at 100 kHz. The acquired AE signals, having been band-pass filtered at 20 kHz to

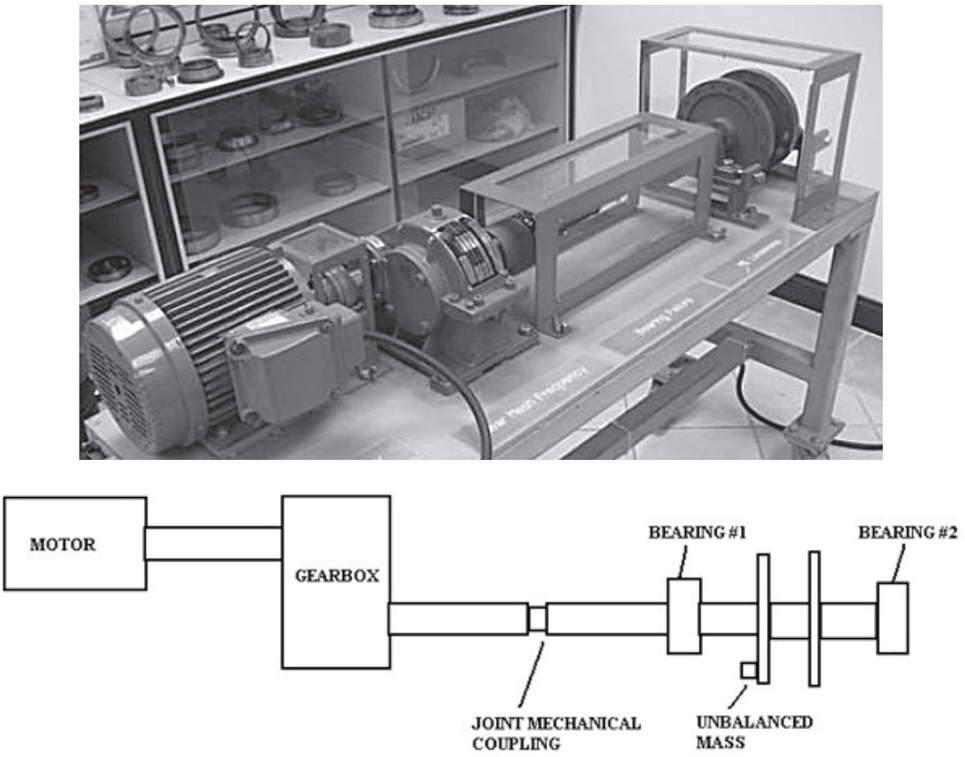


**Figure 3**. Test rig set (top) and test rig schematic diagram (bottom).

500 MHz for noise-removal and amplified to 60 dB, were sampled into a data acquisition card.

Measurements were obtained from different machine conditions: normal, unbalanced and misaligned shaft. For each condition, five signals were collected as for data processing.

## EXPERIMENTAL RESULTS AND DISCUSSION

Figure 4 shows the signal measured on bearing housing from different operating conditions – balanced, unbalanced shaft and misaligned shaft.

In the experiment, the AE dynamic envelope collected from a balanced shaft yielded mean of logarithm intensity about 13 dB whilst the intensity obtained from the unbalanced and misaligned shaft was about 20 dB. With machine fault condition resulting in greater energy release rates they produced higher continuous signal levels (such continuous signals result from the overlapping of many small transient signals).

For unbalanced shaft, the mass unbalanced ($70 \times 10^{-5}$ kg.mm.r product) occurred when the shaft center line and the mass center of the rotor did not coincide. Therefore the unbalanced is a once-per-revolution fault – then it occurs at the frequency of shaft speed (23.80 Hz) – and is sometimes difficult to distinguish from misaligned shaft. However, unbalanced shaft causes a rotating force; the force of misalignment is directional. Mass unbalance has a fixed phase angle with respect to a reference mark on the shaft. The spectrum has low-amplitude higher-order frequency components. In contrast to normal condition when motions are sinusoidal, nonlinear behavior of a bearing in the presence of excessive mass unbalance can cause truncated motions that introduce higher order (2x, 3x) (Fig. 5b).

For misalignment shaft, it can cause a rotating preload in the bearings, shaft and external couplings at the frequency of shaft speed. The magnitude of the resulting wave motion is dependent of the radial stiffness of the components in the system. Severe misalignment can cause nonlinear bearing behavior in one or both directions, depending on asymmetry in the bearing and the stiffness of the base-plate. The nonlinear behavior causes truncated waveforms and/or nonlinearly-generated second and higher orders of shaft frequency (Fig. 5b). The second order component of frequency in cases of severe misalignment can exceed the first order.

The experiments indicated that both AE dynamic envelope and its FFT spectrum of acoustic emission

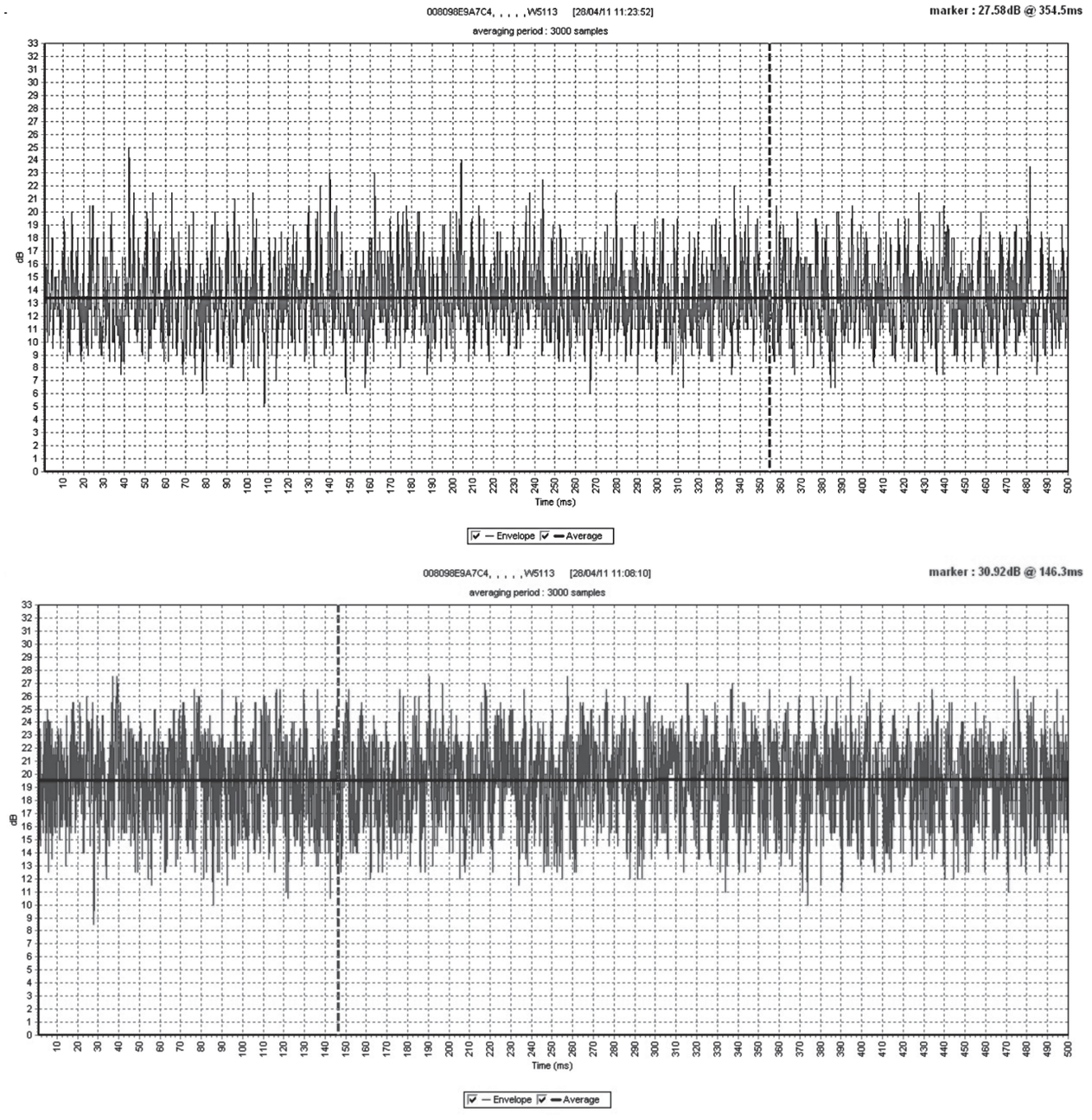


**Figure 4**. Results of AE dynamic envelope from balanced shaft (top), and unbalanced and misaligned shaft (bottom).

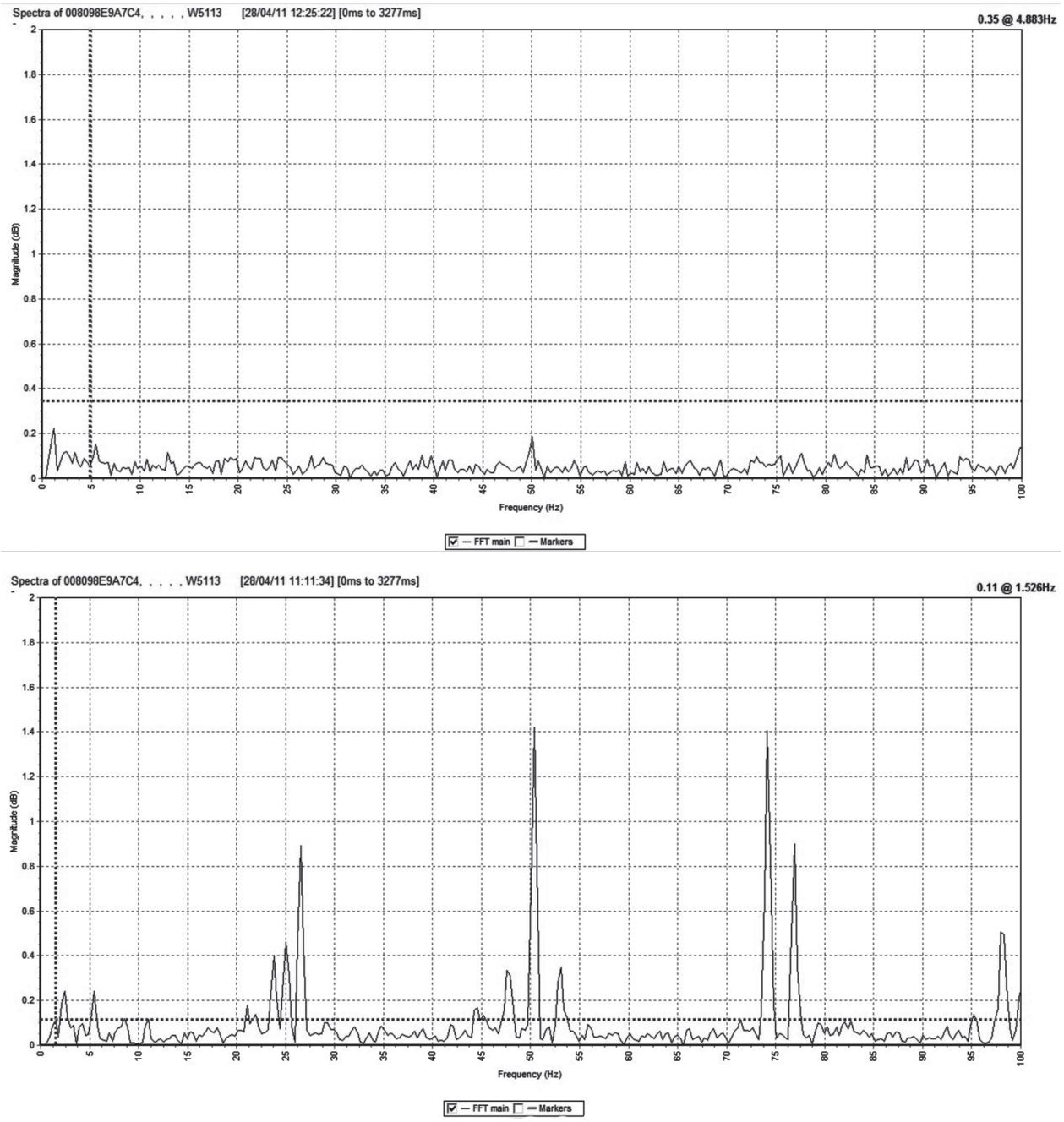


**Figure 5**. Results of spectrum from balanced shaft (top), and unbalanced and misaligned shaft (bottom).

signals from different roto-dynamic machine conditions – normal, unbalanced and misaligned bearing – produced distinct pattern of signals that could be discriminated by using its spectrum. The result was promising with 100 % recognition rate.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 115-119

# Effects of water treatment processes on basic water quality index (WQI) parameters, overall WQI and class of water

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**Abstract** This study, carried out in May and November 2007 at the Semenyih River Water Treatment plant, Precinct 19, Putrajaya, Peninsular Malaysia, examined the effects of five water treatment processes on the basic parameters of the water quality index (WQI), the overall WQI and the Class of Water. The basic WQI parameters include pH, biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), dissolved oxygen (DO) and ammoniacal-nitrogen ($NH_3$-N). The water treatment processes under study were coagulation, flocculation, sedimentation, filtration and disinfection. In this study, TSS and BOD are among the studied WQI parameters that underwent significant change during the treatment processes, resulting in more than 95% TSS and 25-67% BOD reduction. Results of this investigation show that the five studied treatment processes seem to affect most of the individual WQI parameters and the overall WQI but not necessarily the Class of Water.

**Keywords** water treatment – WQI parameters – Class of Water

## INTRODUCTION

Water is the most important inorganic liquid that exists naturally on earth. Oceans contain over 97% of the earth's water. However, salt water cannot be consumed directly by humans or used for many industrial processes. This means only less than 3% of water (fresh water) is readily available for use. Freshwater sources include rivers and lakes (surface water), groundwater and ice as well as glaciers [1].

In most countries, the major sources of water include surface water, groundwater and precipitation. In Malaysia, over 99% of drinking water comes from surface supplies, mainly rivers which are facing increasing levels of pollution, due to rapid industrialization and socio-economic development since the late 1980s [2].

Water Quality Index (WQI) relates a number of water quality parameters in a common scale and combines them in accordance with a chosen method or model of computation into a single number [3]. The main objective of the WQI system is to serve as a preliminary means of assessment of a water body for compliance with the standards adopted for five designated classes of beneficial uses [3].

Chemical oxygen demand (COD) measurements are used in both municipal and industrial wastewater treatment plants to indicate efficiency of the water treatment process [4]. According to Malaysia's Department of Environment (DOE), industrial activity is a major contributor to surface water biochemical oxygen demand (BOD) values in Selangor [5]. Numerous scientific studies suggest that 4 to 5 parts per million (ppm) of dissolved oxygen (DO) as the minimum amount needed to support a large, diverse fish population. Most fish die when dissolved oxygen falls below 3.0 ppm. DO is vital for maintaining aerobic conditions in natural waters that receive pollution matter and also in aerobic treatment processes intended to purify domestic wastewaters [4].

Ammoniacal-nitrogen ($NH_3$-N) is due to the presence of nitrate ($NO_3^-$), nitrite ($NO_2^-$), ammonia ($NH_3$) and organically-bonded nitrogen in water. Sewage and fertilizers are the main source of nitrates in lakes and rivers. The presence of excessive nitrates in water will encourage rapid growth of algae (eutrophication) and this later will increase the value of BOD when the algae start decaying [4].

Total suspended solids (TSS) include all particles suspended in the water which will not pass through a filter. Suspended solids can absorb heat from sunlight which increases the water temperature and decreases levels of dissolved oxygen (DO). Also since less

light penetrates the water, the rate of photosynthesis decreases and this also contributes to lower DO values.

The three major Malaysian river water pollutant indicators are ammoniacal-nitrogen, biochemical oxygen demand and suspended solids [3]. Ammoniacal-nitrogen originates from livestock farming and domestic sewage while suspended solids are due to earthworks and land-clearing activities. Biochemical oxygen demand is mainly due to discharges from agro-based and manufacturing industries. 80% of Malaysian rivers were polluted by $NH_3$-N in 1997, 43% in 1998 and 39% in 2006. Suspended solids polluted 31% of Malaysian rivers in 1997, 34% in 1998 and 40% in 2006 while BOD was responsible for 69% of Malaysian river water pollution in 1997, 21% in 1998 and 21% in 2006 [3]. Improvement in $NH_3$-N and BOD brought about reduction of polluted rivers from 25 in 1997, 16 in 1998 to 7 in 2006 [3].

Surface water from Malaysian rivers, lakes and dams undergo conventional water treatment processes such as coagulation, flocculation, sedimentation, filtration and disinfection before it is distributed to consumers as their domestic water supply or potable water.

This study examined the effects of five conventional water treatment processes on the individual parameters of the DOE water quality index. It also examined how the changes in the individual WQI parameter influenced the overall WQI and eventually the classes of beneficial uses of water.

## MATERIALS AND METHOD

Malaysia's Department of Environment (DOE) formula was used for this investigation and it included the following parameters: dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), ammoniacal-nitrogen ($NH_3$-N) and pH [3].

### Sampling site

This study was conducted at Sungai Semenyih Water Treatment plant in Precinct 19, Putrajaya, Peninsular Malaysia. This plant treats water from Semenyih dam via Semenyih river.

### Sampling frequency

Sampling was done twice, once in May 2007 and the other in November 2007.

### Sampling points

Samples were taken from 6 stages of the water treatment processes. These include raw water, coagulation, flocculation, sedimentation, filtration and disinfection.

### Sample analysis

In-situ parameters for pH, DO and temperature were obtained using the YSI environmental meter (Model 556). Ammoniacal-nitrogen concentrations were determined using Ion Chromatograph and BOD values were obtained using the BOD track apparatus. The DRB 200 digestion reactor and HACH DR 2010 spectrophotometer were utilized for analyzing COD values.

## RESULTS AND DISCUSSION

The various measured water quality parameters as well as calculated WQI and Class of Water values of all the treatment stages for May 2007 and November 2007 are tabulated in Table 1 and Table 2 respectively.

**Table 1**. Average water quality parameters, water quality index (WQI) and Class of Water for six stages of the treatment process at Precinct 19 Putrajaya Water Treatment Plant for May, 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| pH | 6.98 ± 0.05 | 6.13 ± 0.02 | 6.10 ± 0.02 | 5.92 ± 0.08 | 6.07 ± 0.02 | 7.32 ± 0.03 |
| DO (mg/L) | 6.96 ± 1.70 | 7.25 ± 1.10 | 7.01 ± 1.45 | 6.02 ± 1.45 | 6.90 ± 1.10 | 7.24 ± 0.80 |
| BOD (mg/L) | 2.8 ± 0.5 | 2.8 ± 0.7 | 1.1 ± 0.4 | 2.2 ± 0.4 | 2.7 ± 0.5 | 2.1 ± 0.5 |
| COD (mg/L) | 14.32 ± 1.24 | 13.60 ± 0.95 | 9.80 ± 1.04 | 6.00 ± 0.75 | 5.10 ± 0.90 | 4.73 ± 0.54 |
| TSS (mg/L) | 260.0 ± 5.4 | 200.0 ± 2.6 | 6.0 ± 1.2 | 10.0 ± 0.4 | 2.0 ± 0.2 | 0.2 ± 0.1 |
| $NH_3$-N (mg/L) | nd | nd | nd | nd | nd | nd |
| Temperature $^0$C | 25.9 ± 0.2 | 25.1 ± 0.1 | 25.3 ± 0.2 | 27.0 ± 0.1 | 25.9 ± 0.2 | 28.4 ± 0.3 |
| WQI (DOE) | 85.19 | 85.47 | 89.22 | 90.08 | 93.41 | 96.38 |
| Class of Water | II | II | II | II | I | I |

Table 3 and Table 4 show the changes in the status quality of the indexes for the individual major Malaysian river water pollutants ($NH_3$-N, BOD and SS) as these parameters undergo all the treatment stages.

There was no significant change in the pH during the treatment stages (Tables 1, 2). The average pH of raw water of 6.98 in May 2007 and 6.69 in November 2007 was within the recommended range of 5.5-9.0 for raw water quality criteria set by the Ministry of Health, Malaysia [6]. The final disinfection stage had an average pH of 7.32 in May 2007 and 7.18 in November 2007, both of which fell within the range of 6.5-9.0 for drinking water quality standards set by the Ministry of Health, Malaysia. In both studies, the highest average pH was obtained during disinfection stage which indicates the overall water treatment processes seem to improve the pH to alkaline range.

DO for both studies started decreasing during the flocculation stage, reaching the lowest at the sedimentation stage. During this stage, flocculants formed and agglomerated during the coagulation and flocculation stages were allowed to settle to the bottom using gravity force. After sedimentation, DO started increasing again and was the highest (7.24 and 7.50) during the disinfection stage for both studies.

BOD started decreasing during the coagulation stage (Tables 1 and 2). However, there seem to be some differences with BOD readings during flocculation stage between the two studies. The study done in May 2007 shows the lowest average BOD value for in contrast to the highest value for the November 2007. In both studies, it is evident that a significant change in the value of BOD occurred during coagulation-flocculation stage. A wastewater study [7] using 40 to 60 mg alum during coagulation in conventional water treatment processes indicated possibility of 95% BOD, turbidity and SS removal. Coagulation using alum coagulant is a typical process used in drinking water treatment to remove turbidity and natural organic matter [8, 9].

Disinfection processes also shows significant decrease in BOD values. The overall water treatment processes are able to convert the status quality of the individual BOD value (a major Malaysian river water pollutant) which is also one of the six parameters to calculate WQI, from slightly polluted to clean (Tables 3 and 4).

**Table 2**. Average water quality parameters, water quality index (WQI) and Class of Water for six stages of the treatment process at Precinct 19 Putrajaya Water Treatment Plant during November, 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| pH | 6.69 ± 0.02 | 6.68 ± 0.03 | 6.93 ± 0.03 | 6.99 ± 0.03 | 7.02 ± 0.06 | 7.18 ± 0.04 |
| DO (mg/L) | 7.19 ± 1.46 | 7.19 ± 1.20 | 6.50 ± 0.56 | 6.30 ± 0.85 | 7.20 ± 0.64 | 7.50 ± 0.94 |
| BOD (mg/L) | 3.90 ± 1.14 | 3.10 ± 0.94 | 3.70 ± 0.77 | 2.60 ± 1.01 | 2.20 ± 0.34 | 1.30 ± 0.28 |
| COD (mg/L) | 2.30 ± 0.45 | 37.75 ± 2.46 | 7.70 ± 1.15 | 2.40 ± 0.55 | 9.90 ± 1.33 | 4.90 ± 0.65 |
| TSS (mg/L) | 4.30 ± 0.88 | 4.70 ± 0.46 | 4.30 ± 0.22 | 4.00 ± 1.10 | 4.00 ± 0.94 | 4.00 ± 0.44 |
| $NH_3$-N (mg/L) | 0.65 ± 0.05 | 0.51 ± 0.07 | 0.46 ± 0.10 | 0.46 ± 0.08 | 0.43 ± 0.06 | 0.38 ± 0.03 |
| Temperature $^0$C | 25.2 ± 0.3 | 24.9 ± 0.2 | 24.8 ± 0.1 | 25.1 ± 0.1 | 24.1 ± 0.1 | 25.6 ± 0.1 |
| WQI (DOE) | 87.18 | 82.03 | 85.35 | 87.21 | 88.49 | 91.45 |
| Class of Water | II | II | II | II | II | II |

**Table 3**. Classification of major river pollutant indexes during conventional water treatment processes in terms of quality status for May 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| BOD | Slightly polluted | Slightly polluted | Clean | Clean | Slightly polluted | Clean |
| TSS | Polluted | Polluted | Polluted | Clean | Clean | Clean |
| $NH_3$-N | Clean | Clean | Clean | Clean | Clean | Clean |

**Table 4**. Classification of major river pollutant indexes during conventional water treatment processes in terms of quality status for November 2007.

| Water quality parameter | Raw water | Coagulation | Flocculation | Sedimentation | Filtration | Disinfection |
|---|---|---|---|---|---|---|
| BOD | Slightly polluted | Slightly polluted | Slightly polluted | Slightly polluted | Clean | Clean |
| TSS | Clean | Clean | Clean | Clean | Clean | Clean |
| $NH_3$-N | Polluted | Polluted | Polluted | Polluted | Polluted | Polluted |

COD values seem to be highest for coagulation where coagulants were added to produce flocculants (Tables 1 and 2). This value decreased further during flocculation and sedimentation. Disinfection shows the lowest average value of COD especially for May 2007 study. Disinfection in our study is the process of adding chlorine to public water supplies to make it safe from microbiological point of view as well as to leave a detectable residual of chlorine inside the distribution network [1].

During the overall water treatment process in May 2007, the value of TSS, a major water pollutant, decreased significantly from 260 mg/L to 0.06 mg/L (Table 1). Significant decrease in TSS occurred during coagulation. Coagulation appeared to be a very effective process to remove TSS if the initial concentration of TSS was high (260 mg/L).

Sedimentation and filtration also contributed significantly towards the decrease in TSS if the initial concentration of TSS in the raw water was high. In the May 2007 study, the combination of coagulation and flocculation processes managed to change the quality status of TSS index from polluted to clean (Table 3). Coagulation, sedimentation and filtration did not seem to work effectively if the initial TSS was very low (4.30 mg/L) as in the November 2007 study with a slight increase during coagulation and remained unchanged during sedimentation and filtration.

The overall treatment process was able to decrease ammoniacal-nitrogen from 0.6 mg/L (raw water) to 0.38 mg/L (disinfection) in the November 2007 study (Table 2). The greatest decrease seemed to occur during the coagulation process. However, it should be noted that both the coagulation and overall treatment processes were unable to remove even 50% of the ammoniacal-nitrogen. This was probably why the Sungai Semenyih plant operators shut down the treatment processes if ammoniacal-nitrogen was detected above the maximum acceptable value (1.5 mg/L) during routine monitoring [3]. The overall treatment processes was unable to improve the quality status of the $NH_3$-N index (Table 4).

In this study, coagulation with alum and disinfection with chlorine are the stages of the overall conventional water treatment processes that seem to have the most impact on the individual WQI parameters especially the major water pollutants like BOD, TSS and $NH_3$-N. Coagulation involves neutralizing negative water pollutants, for example clay and silt by using positive metal coagulants such as aluminium sulphate (alum) to form flocculants. Previous studies on alum coagulation which has been optimized for turbidity and organic matter removal indicated this process is extremely effective in removing *Cryptosporidium parvum* oocysts [9]. Alum has been shown to be a more effective coagulant to remove natural organic matter (NOM) than polyaluminium chloride [8]. A study had also reported that large molecules of NOM such as humic acid could be easily removed by coagulation [10]. Several studies also indicate possible alternative functions of coagulation. This can be achieved by manipulating alum dosage [7] which indicated efficient removal of phosphorus, bacteria and heavy metal with increased alum dosage. Increased alum dosage also improved removal efficiency of certain antibiotics in drinking water [11]. Other alternative coagulation functions can also be achieved by changing the type of coagulant used – for example, almost 100% asbestiform fibre removal was achieved from potable water using iron salts as the coagulant [12]. Similar study using other coagulant such as polyaluminium chloride (PACl) [11] indicated 50% removal efficiency for certain antibiotics.

Chlorine used in the disinfection process is usually added in the form of free chlorine or as hypochlorite [13]. It acts as a potent oxidizing agent in both forms and it often dissipates itself in side reactions so rapidly that little disinfection is achieved unless amounts in excess of the chlorine demand is added [13]. This, in turn creates a serious problem as chlorine also reacts with natural organic matter present in the water to form halogenated trihalomethanes (THMs) and haloacetic acids (HAAs) which are two major disinfection byproducts (DBPs) with harmful long-term effects [14]. However, with the increased use of chlorine for disinfection, there has been a corresponding decrease in the incidences of waterborne diseases such as cholera and typhoid. Disinfection with chlorine has also been shown to be an effective way to control nitrite levels in drinking water [15]. A study indicated 70% removal of certain common pesticides with chlorine disinfection [16].

In summary, the results of this study indicate that conventional water treatment processes especially coagulation and disinfection have the capacity to improve certain basic WQI parameters thus improving the overall WQI values. However, improvement of WQI values does not necessarily bring changes to the Class of Water.

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*Journal of Science and Technology in the Tropics* (2011) **7**: 121-125

# Structure activity relationship of flavonoid derivatives from two *Artocarpus* species as inhibitors of leukemia and breast cancer cells

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*Received 29-09-2011; accepted 3-10-2011*

**Abstract** Structure-activity relationship of a series of flavonoid compounds from Artocarpus kemando and Artocarpus odoratissimus were studied to clarify the structural requirement for inhibition of HL-60 and MCF-7 cancer cell lines by these compounds. The flavonoids are cycloartobiloxanthone (**1**), artomandin (**2**), artonol B (**3**) and artosimmin (**4**). Comparisons of these related flavonoids indicated that the prenyl and hydroxyl substituent groups, as well as the type of substitution pattern at A-ring and B-ring of tricyclic flavonoid skeleton were crucial for the inhibition of these cancer cells in MTT assays.

**Keywords** flavonoids – *Artocarpus kemando* – *Artocarpus odoratissimus* – cytotoxicity – HL-60 – MCF-7

## INTRODUCTION

Flavonoid constituents are one of the most abundant and diverse groups of natural products. They play an important role in the chemotaxonomy of the higher plant families (Coradin *et al*., 1985). The various classes of flavonoids differ in their level of substitution of the C ring of the basic benzopyrone structure [1]. Depending on the substitution of ring C, they are classified into six major subclasses: flavones, flavanols, flavanones, chalcones, isoflavones and anthocyanidins.

Previous studies on flavonoids from this plant and other species of *Artocarpus* have revealed them to exhibit a wide range of pharmacological activities such as anti-inflammatory [2], antioxidative [3], antiplatelet aggregation [4], 5α-reductase activities [5], inhibition of cathepsin K [6], and cytotoxicity [7]. Moraceae species have been reported to be rich in flavonoid derivatives [8]. Our research on *Artocarpus kemando* and *Artocarpus odoratissimus* has successfully led to the isolation of two furanocycloartobiloxanthone **(1-2)**, one xanthonolide **(3)** and a prenylated pyranoflavone **(4)**. These compounds were tested for their cytotoxic activities using HL-60 and MCF-7 cell lines. Until now, there is no information available on the pharmacological activities of flavonoids **1-4** on both types of cancer cells.

## MATERIALS AND METHOD

### Plant material

The stem bark of *A. kemando* and *A. odoratissimus* were collected from Sri Aman and Bintulu, Sarawak, Malaysia respectively.

### General

Infrared spectra were measured in KBr/NaCl pellet on a Perkin-Elmer FTIR Spectrum BX spectrometer. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500MHz NMR/JEOL 400MHz FT NMR spectrometer using tetramethylsilane

(TMS) as internal standard. Ultra violet spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-Visible Recording Spectrophotometer.

**Extraction and isolation**

The milled, air-dried stem bark (4.7 kg) of *A. kemando* was defatted with n-hexane and extracted exhaustively using ethanol, acetone and then methanol for more than 48 hours at room temperature. This gave 23.3 g, 50.2 g, 98.6 g and 198.5 g of hexane, ethanol, acetone, and methanol extracts respectively. The methanol extract was partitioned using chloroform to afford a 20.0 g extract. Solvent extractions on the dried powdered stem bark of *A. odoratissimus* (3.5 kg) gave hexane (6.0 g), chloroform (15.0 g), ethyl acetate (42.5 g) and ethanol (12.8 g) extracts. The ethanol extract of *A. kemando* was chromatographed using silica gel vacuum column chromatography using a stepwise gradient system (hexane/chloroform, chloroform / ethyl acetate, ethyl acetate /acetone and methanol) to give 20 fractions (250 mL). Fractions with similar profile on TLC were combined to give six major fractions. A portion of fraction 4 (2.57 g) was repeatedly chromatographed to give 19 subfractions. This resulted in cycloartobiloxanthone (**1**). Similarly, several fractionations of the acetone extract (45.0 g) of *A. kemando* by silica gel column and radial chromatography gave artomandin **(2)**. Similar column chromatographic separation as above on the chloroform extract (20.0 g) of *A. kemando* over silica gel yielded artonol B **(3).** The ethyl acetate extract (42.5 g) of *A. odoratissimus* gave artosimmin **(4).**

**Cycloartobiloxanthone (1)**

Dark yellow solid; mp 283-285 $^oC$ (Lit. 285-287 $^oC$ [9]; UV (MeOH) $\lambda_{max}$ (log ε) nm: 229 (4.31), 281 (4.31), 392 (4.02); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3434 (OH br), 2976, 2932 (C-H stretching), 1650 (C=O chelating), 1560, 1476 (C=C aromatic), 1358 ($CH_3$ alkane bending), 1272, 1160 (C-O); EIMS *m/z* (rel. int.): 434 [$M^+$, $C_{25}H_{22}O_7$]; FABMS *m/z* 435 [$(M+H)^+$, $C_{25}H_{23}O_7$]. $^1H$ and $^{13}C$ NMR spectral data are in agreement with published data [9].

**Artomandin (2)**

Yellow solid; mp 288-290 $^oC$; UV (MeOH) $\lambda_{max}$ (log ε) nm: 206 (3.89), 230 (3.91), 283 (3.93), 393 (3.64); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3351 (OH br), 2916, 2849 (C-H stretching), 1646 (C=O chelating), 1556, 1465 (C=C aromatic), 1348 ($CH_3$ alkane bending), 1272, 1159, 1017 (C-O); EIMS *m/z* (rel. int.): 434 [$M^+$, $C_{25}H_{22}O_7$]. $^1H$ NMR and $^{13}C$ NMR data are in agreement with published data [10].

**Artonol B (3)**

Fine yellow solid; mp 189-194 $^oC$ (Lit. 189-196 $^oC$ [11]; UV (MeOH) $\lambda_{max}$ (log ε) nm: 233 (2.39), 278 (2.35), 359 (2.24); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3423 (OH), 2969, 2921, 2851 (CH stretching), 1770 (C=O acetoxyl), 1717 (C=O cyclic), 1651 (conjugated C=O), 1606, 1581, 1479 (C=C aromatic), 1361, 1109 (C-O) ; EIMS *m/z* (rel. int.): 420 [$M^+$, $C_{24}H_{20}O_7$]. $^1H$ and $^{13}C$ NMR spectral data are in agreement with published data [11].

**Artosimmin (4)**

Yellow solid (95 % chloroform/ 5 % methanol); mp 213-215 $^oC$; UV (MeOH) $\lambda_{max}$ (log ε) nm: 213 (4.16), 271 (3.72), 340 (3.42); IR (KBr) $\nu_{max}$ $cm^{-1}$: 3527 (OH), 2853 (C-H stretching), 1734 (C=C unsaturated), 1653 (conjugated C=O), 1598, 1567, 1513, 1488, 1447 (C=C aromatic), 1306, 1239 (C-O); EIMS *m/z* (rel. int.): 436 [$M^+$, $C_{25}H_{24}O_7$]; $^1H$ NMR and $^{13}C$ NMR spectral data are in agreement with published data [12].

**Cancer cell-lines culture**

Two human cancer cell lines were used. They were HL-60 and the IRM-32 cell lines. Both of the cell lines were obtained from the National Cancer Institute, Maryland, USA. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5 % Fetal Bovine Serum (FBS), 100 IU $mL^{-1}$ penicillin and 100g/mL streptomycin by using 25 $cm^2$ flask in a 37 $^oC$ incubator with 5% $CO_2$.

**Cytotoxicity and MTT assay**

Cytotoxic assay was carried out using the microculture 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay as described previously [13]. The exponentially growing cells were performed by suspending the cells in 100 µL of stock culture ($1 \times 10^5$ cells $mL^{-1}$) per well which were plated in a 96-well plate and incubated for 24 hours at 37 $^oC$ under humidified 5% $CO_2$. The stock solution was prepared by dissolving the sample in absolute ethanol (EtOH) to a final concentration of 1 mg/mL. Serial dilutions of the stock solution in the growth medium to produce seven sample solutions at concentrations of 0.47, 0.94, 1.88, 3.75, 7.50, 15.00,

30.00 µg/mL. The growth medium was removed from the wells. The cells in each well were treated with 100 µL of varying concentrations of test specimens in their respective medium. The positive control was made containing only untreated cell population in 100 µL of growth medium. Each concentration of sample was tested in triplicate and allowed to proceed for 72 hours at 37 °C in 90% humidified 5% $CO_2$ atmosphere. Then, 100 µL of MTT stock solution (5 mg in 1 mL PBS) was added to each well to determine the fraction of adherent cells relative to the untreated cell population. The plates were further incubated for 4 hours at 37 °C. 100 µL of EtOH was added to each well to dissolve the water-insoluble purple formazan crystal. After 30 minutes, the absorbance (OD) of the samples and the reference was measured by using ELISA spectrophotometer microplate reader at wavelength 550 nm. The inhibition concentration of 50% reduction (killed cells) in cell number, $IC_{50}$ was appraised visually to determine the absorbance (OD) against concentration curve.

## RESULTS AND DISCUSSION

Flavonoids which are derived from the tricyclic flavones are a relatively homogeneous group of constituents in the *Artocarpus* species [8]. In this report, the flavonoids tested include modified flavonoid and rearranged flavonoid groups. They reveal an interesting trend of cytotoxic effects on HL-60 and MCF-7 cell lines with $IC_{50}$ values between 1.0 and 8.0 µg/mL and between 3.0 and 14.0 µg/mL respectively. However, these cytotoxic results show significant dissimilarity in inhibition effect probably due to the nature of the substituents and the substitution pattern at A-ring and B-ring of the tricyclic flavonoid skeleton. Structurally, these include 2',4'-dioxygenated and 3',4'-dioxygenated furanodihydroxanthone **(1-2)**, 3',4'-dioxygenated pyranoflavone **(4)**, as well as xanthonolide **(3)** flavonoids. The screening results of these compounds are summarized in Table 1.

Of the compounds tested, it is interesting to note that artosimmin **(4)**, cycloartobiloxanthone **(1)** and artomandin **(2)** are strongly active cytotoxic compounds towards HL-60 (1.1, 5.7 and 2.4 µg/mL) and MCF-7 (3.4, 13.4 and 3.1 µg/mL) cell lines, respectively. The activity might be due to the ortho or meta positions of the dihydroxyl moieties at the B-ring. The assays also indicated both artosimmin **(4)** and artomandin **(2)** which are 3',4'-dioxygenated at the B-ring of the structure exhibited a stronger cytotoxic activity when compared to that of **1** which has 2',4'-dioxygenated substitution. Hence, it is deduced that flavonoids with the presence of 2',4'- and 3',4'- positions of free hydroxyl moieties at B ring is inclined to exhibit a prominent inhibitory activity. Furthermore, the only example of a 5'-(3-methylbut-2-enyl) pyranoflavone type compound tested which is artosimmin **(4)** also demonstrates strong cytotoxic properties. From the structure-activity relationship study, compounds such as cycloartobiloxanthone **(1)**, artomandin **(2)** and artosimmin **(4)** consisting of C-3 isoprenyl unit substitution, gave a good degree of inhibition towards HL-60 cell line.

The C-3 isoprenyl unit substitution always tends to form a carbocyclic ring or an oxygen-bearing fused B-ring and C-ring, respectively [14]. Thus, it is suggested that the 3-prenyl flavone skeleton is a necessary requirement for the cytotoxic activity towards HL-60 and MCF-7 cell lines. In addition, it is observed that the modified and totally opened B-ring of flavonoids such as artonol B **(3)** tend to reduce the degree of inhibition effect towards the MCF-7

**Table 1**: Cytotoxic activities of **1-4** against HL-60 and MCF-7 cell lines.

| Compound | Type | HL-60 | MCF-7 |
|---|---|---|---|
| | | $IC_{50}$ ± SD (µg/mL) | $IC_{50}$ ± SD (µg/mL) |
| **1** | Furanodihydrobenzoxanthone | 5.7 ± 1.7 | 13.4 ± 2.4 |
| **2** | Furanodihydrobenzoxanthone | 2.4 ± 0.5 | 3.1 ± 0.4 |
| **3** | Xanthonolide | 7.2 ± 1.6 | > 30.0 |
| **4** | Pyranoflavone | 1.1 ± 0.1 | 3.4 ± 0.3 |
| [1]Goniothalamin | | 1.4 | - |
| [2]Tamoxifen | | - | 3.1 |

[1]positive control of HL-60 cell line; [2]positive control of MCF-7 cell line; - not tested.
Note:*$IC_{50}$ < 5.0 µg/mL = strong inhibition activity. *5.0 ≤ $IC_{50}$ ≤ 25.0 µg/mL = moderate inhibition activity. *$IC_{50}$ > 25.0 µg/mL = weak inhibition activity. [13]

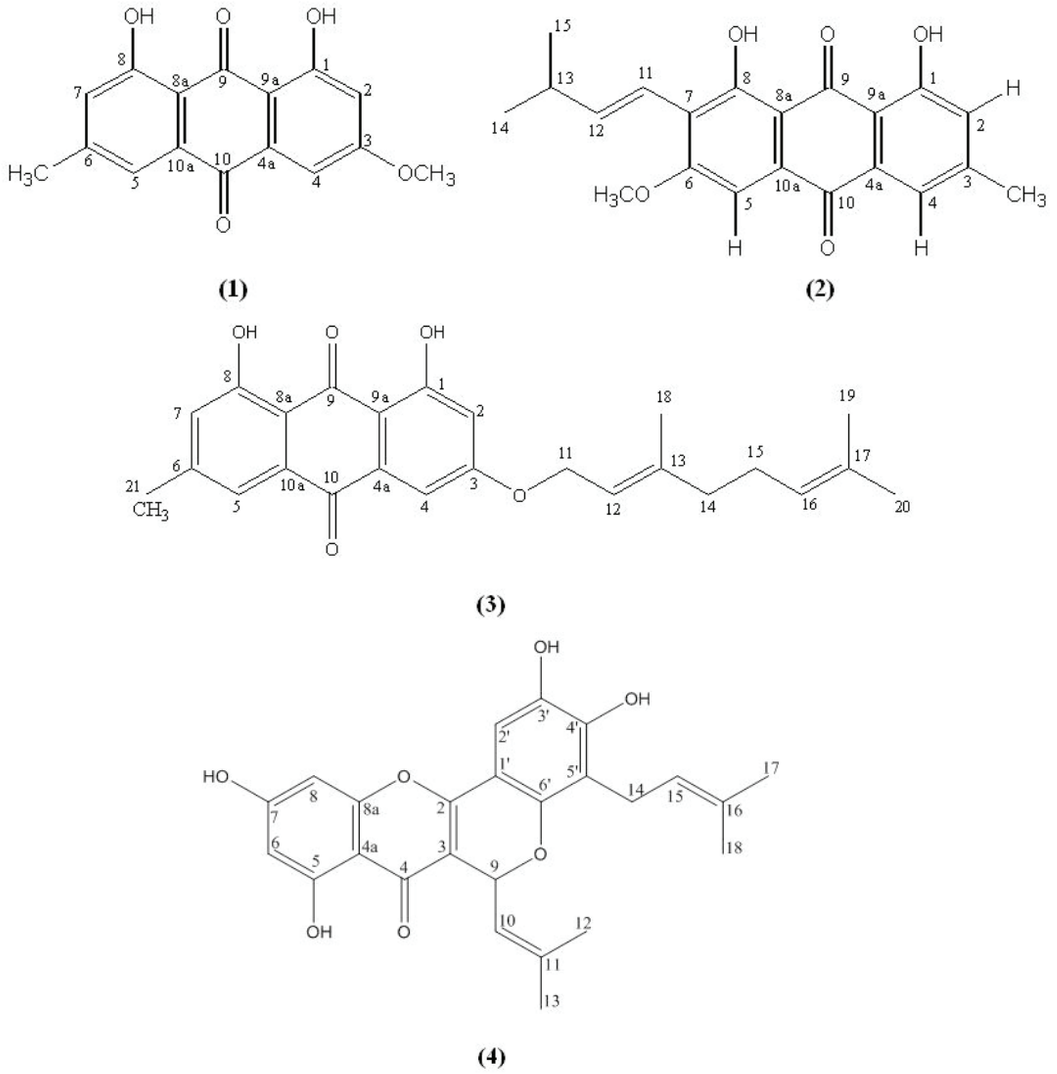


**Figure 1**. Structures of flavonoids.

cell line. However, **3** exhibited moderate cytotoxic activities towards HL-60 cell line with $IC_{50}$ values less than 10.0 µg/mL.

In summary, the unsaturated prenyl side chain (3-methylbut-2-enyl) at C-3/ C-5' and the presence of the 2',4'- and 3',4'- positions of free hydroxyl moieties at B-ring of flavonoid derivatives contribute to the prominent inhibitory activity. Hence, it can be concluded that flavonoids with 3, 5'-prenylated skeleton and are 2',4'- or 3',4'-dioxygenated might be lead compounds for HL-60 and MCF-7 cancer cell lines, respectively.

**Acknowledgements** – We thank the Malaysian FRGS grant for financial support, Assoc. Prof. Dr Jegak Uli for collection of plant samples and Mr Johadi Iskander for recording NMR spectra.

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# Think Malaysian Act Global: the Autobiography of Academician Dato' Ir. Lee Yee Cheong

ISBN 978-983-9445-47-3
Publisher: Academy of Sciences Malaysia, 2010

*Think Malaysian Act Global* is a testimony of the character and wisdom of Academician Dato' Ir. Lee Yee Cheong, a Foundation and Senior Fellow of the Academy of Sciences Malaysia. It portrays the life of this 'giant of a man' in the Academy who advocates and practises Think Malaysian Act Global.

Lee's remarkable insight deserves attention. It springs from his compassion, his humanity and his own rich life experiences in the pursuit of knowledge; professional engineering career; involvement in local and global academies, institutions in the sciences, technology, history, religion, economics, education, information and communication; as well as accounts of his tough, challenging human nature and character. This classic piece of lieterary prose is a truthful reflection of the rich fabric of his remarkable life.

This 378-page autobiography chronicles the details about the past; dreams for the future on the aspiration of an individual. It narrates vividly the successful science career-path of an engineer and his life's journey in pursuit of excellence. It would make an interesting read particularly for the young who want to learn, experience and experiment with life, and contribute to science, technology and innovation for the betterment of society; nationally and globally.

In the words of the then President of Academy of Sciences Malaysia, Tan Sri Dr Yusof Basiron: "This exposé of experiences (of Dato' Lee) will provide a window for students and stakeholders of the science industry on the importance of science and technology as well as create an excitement to pursue a career in science. This is precisely one of the ideals of the Academy and is consistent with our aims and objectives."

Lee was born in Ipoh, Perak, Malaya (now Malaysia) on 11 May 1937 – a double bull, being a Taurus and in the Year of the Ox. His family is of the Hakka stock. He attended Chinese schools from 1946-1950 (Min Tak Primary School in the Kayin Association building in Belfield Street, Yuk Choy Primary School in Gopeng Road), and St. Michael's Institution, Ipoh 1951-1956. He was a Colombo Plan scholar at the University of Adelaide (1956-1961) where he graduated with a First Class Honours in engineering and winning the Electricity Trust of South Australia prize for electrical power engineering.

For more information about this publication, contact: Academy of Sciences Malaysia, 902-4 Jalan Tun Ismail, 50480 Kuala Lumpur, Malaysia; www.akademisains.gov.my.

*Journal of Science and Technology in the Tropics* (2011) **7**: 128



## Portrait Of A Thousand Smiles: Academician Tan Sri Dato' Seri Dr. Salleh Mohd Nor

ISBN 978-983-9445-55-8
Publisher: Academy of Sciences Malaysia, 2010

Born in 1940 to a poor family in Ulu Inas in Negeri Sembilan, Peninsular Malaysia, Salleh received his early education at Tuanku Muhammad School, Kuala Pilah and the Royal Military College, Port Dickson. The military training at RMC with the motto "Serve to Lead" laid the foundation that served him throughout his career, that is service to the nation with integrity and honesty.

He had his initial tertiary education in Adelaide and Canberra, Australia where he studied forestry and enjoyed himself tremendously doing many other things besides study. His friendly disposition led him to be 'adopted' as the Malaysian son of an Australian Forest worker's family.

Salleh's stint in the Netherlands opened his eyes to the achievements of a small nation and what science and research can do for economic development. His final educational training was in Michigan State University, USA where despite working in a number of positions and raising a family, Salleh managed to complete a Masters and PhD with distinction in four years.

Salleh's international experience covered the four corners of the globe through his involvement with IUFRO, Forest Trends, NATMANCOM, Tropenbos, the CGIAR system, the MPTS Network and APAFRI, among others.

At home, Salleh shares his chanllenges in undertaking the first national forestry inventory, spending months camping in the 'jungles' of Peninsular Malaysia; the trials and tribulations and success in the formation of the Forest Research Institute of Malaysia; the challenges as the longest serving President of the Malaysian Nature Society; being Chief Executive Officer to the World Endurance 2008, the "Olympics" of endurance riding; his brief stint with Transparency International; his experiences with various universities in different capacities; and his involvement in the Academy of Sciences Malaysia and other NGOs, and his limited venture into business.

*Portrait Of A Thousand Smiles* is Salleh's foray into science as science permeates in all that the autobiography presents.

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