Sains Malaysiana 46(9)(2017): 1581–1586
http://dx.doi.org/10.17576/jsm-2017-4609-29

A New Cyclopentylidene and Other Chemical Constituents from Malaysian *Crotalaria pallida*

(Siklopentilidena Baru dan Juzuk Kimia Lain daripada *Crotalaria pallida* Malaysia)

SITI RABEAH FADZIL, ANN-CHEE YAP & YEUN-MUN CHOO*

ABSTRACT

Crotalaria pallida *Aiton is an herbaceous legume from the family Fabaceae. In the present study, one new cyclopentyliene, crotolidene (**1**) and seven known compounds, i.e. hydroxydihydrobovolide (**2**), octacosane (**3**),* trans*-phytyl palmitate (**4**), linoleic acid (**5**), methyl oleate (**6**), ethyl palmitate (**7**), and palmitic acid (**8**) were isolated from the* C. pallida *collected from Perak, Malaysia. These compounds were isolated and characterized using extensive chromatographic and spectroscopic methods.*

Keywords: Crotalaria pallida; *crotolidene; cyclopentylidene; spectroscopic analysis; (3E,5Z)-5-(4-hydroxy-2,2-dimethylcyclopentylidene)hex-3-en-2-one*

ABSTRAK

Crotalaria pallida *Aiton merupakan legum herba daripada famili Fabaceae. Satu siklopentilidena baru, crotolidine (**1**) dan tujuh sebatian lain, iaitu hidroksidihidrobovolide (**2**), octacosana (**3**),* trans*-phytil palmitat (**4**), asid linoleik (**5**), metil oleat (**6**), etil palmitat (**7**) dan asid palmitik (**8**) telah diasingkan daripada* C. pallida *dari Perak, Malaysia. Sebatian ini diasing dan dicirikan menggunakan kaedah kromatografi dan spektroskopi.*

Kata kunci: Analisis spektroskopi; Crotalaria pallida; *crotolidene; siklopentilidena; (3E,5Z)-5-(4-hidroksi-2,2-dimetilsiklopentilidene)heks-3-en-2-on*

INTRODUCTION

Crotalaria pallida Aiton is an herbaceous legume belonging to the family Fabaceae (sub-family Faboideae). It is grown in the temperate and tropical regions and synonyms with *C. mucronata* and *C. striata* (Chong et al. 2009). In Malaysia, *C. pallida* Aiton local name is *kiri-kiri, giring-giring* or *kacang kayu* (Abdullah 1990). *C. pallida* is use in traditional treatment for urinary problems, joints swelling, relieving fever and inflammation (Farida Hanum & Vander Maesen 1997). The *Crotalaria* plant yielded fibers similar to sunn hemp and used as cover crop to curb soil erosion and served as green manure. It has also been reported to produce the toxic pyrrolizidine alkaloids, monocrotaline and spectabiline, which causes poisoning in livestock and cattle (Williams & Molyneux 1987). In continuation of our natural product research programme to discover novel compounds, *C. pallida* collected from Perak, Malaysia was subjected to chemical constituent investigation. This is the first chemical constituents report of *C. pallida* from Malaysia.

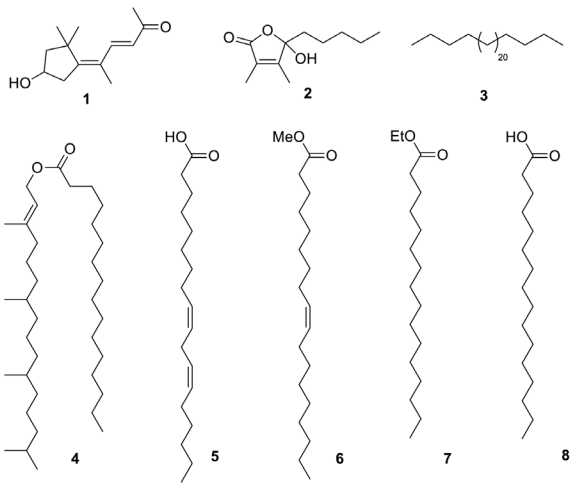

FIGURE 1. Compound **1** - **8**

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

NMR spectra data were obtained from 600 MHz Bruker AVANCE III (Bruker, Fallanden, Switzerland) NMR spectrometers with chemical shifts expressed in ppm and TMS as an internal standard in $CDCl_3$. HRESIMS data were obtained from the Agilent 6530 Q-TOF (Agilent Technologies, Santa Clara, CA, USA) mass-spectrometer equipped with the Agilent 1200 series Rapid Resolution LC system. The UV data were recorded using Agilent Cary 60 UV-Vis Spectrophotometer (Agilent Technologies, Santa

Clara, CA, USA) using quartz cell. IR was carried out on the Perkin-Elmer RX1 FT-IR (Perkin Elmer, Waltham, MA, USA) using NaCl cell. Optical rotation was measured on the Jasco P-1020 digital polarimeter (Jasco, Tokyo, Japan).

PLANT MATERIAL AND EXTRACTION

Crotalaria pallida was collected from Cameron Highland, Perak, Malaysia. A voucher of the specimen is available at the Herbarium of University of Malaya, Kuala Lumpur, Malaysia (voucher number KLU 47957). 1.8 kg of dried whole plant was extracted with 95% ethanol to give 195 g of crude ethanolic extract. The ethanolic extract was further partitioned into n-hexane, chloroform and ethanol extracts by sequential solvent-solvent extraction which yielded 23, 50 and 118 g of crude extracts, respectively. Preliminary TLC evaluation indicated that the n-hexane extract contains reasonable number of components, while the chloroform and ethanol extracts displayed similar undefined poor TLC results. The present study focused on the n-hexane extract (23 g) obtained from *C. pallida*.

ISOLATION

The n-hexane extract (23 g) was subjected to purification by column chromatography using silica gel 60 (0.040-0.063, Merck, Darmstadt, Germany) and solvent system of n-hexane:chloroform (1:1) which resulted in fractions A, B and C. Fraction A was further purified by column chromatograpy using silica gel 60 (0.040-0.063, Merck, Darmstadt, Germany) and gradient solvent system from n-hexane:ethyl acetate (9:1) to 100% ethyl acetate, yielded fractions A1, A2, A3 and A4. The centrifugal chromatography (Kieselgel 60 with gypsum silica gel; Merck, Darmstadt, Germany) was extensively used for the subsequent isolation and purification of pure compounds. Purification of fraction A1 using centrifugal chromatography with solvent system n-hexane:ethyl acetate (49:1) afforded compound **3**. Purification of fraction A2 using centrifugal chromatography with solvent system n-hexane:ethyl acetate (49:1) afforded compound **5**. Purification of fraction A3 using centrifugal chromatography with solvent system n-hexane:ethyl acetate (19:1) afforded compound **4** and **6**. Purification of fraction A4 using centrifugal chromatography with solvent system n-hexane:ethyl acetate (49:1) yielded compound **7**. Further purification of fraction C by column chromatograpy using silica gel 60 (0.040-0.063, Merck, Darmstadt, Germany) and solvent system of chloroform (100%) yielded fractions C1 and C2. Purification of fraction C1 using centrifugal chromatography with solvent system n-hexane:ethyl acetate (19:1) afforded compound **2** and **1**. Purification of fraction C2 using centrifugal chromatography with solvent system n-hexane:chloroform (1:1) yielded compound **8**. The yields of the compounds were as follows: **1** (0.9 mg), **2** (2.4 mg), **3** (10.5 mg), **4** (34.0 mg), **5** (14.5 mg), **6** (10.5 mg), **7** (540.0 mg) and **8** (3.5 mg).

CROTOLIDENE (1)

Colourless oil; molecular formula $C_{13}H_{20}O_2$; $[\alpha]_D$ -66.7 (*c* 0.237, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 205 (1.70), 276 (0.56) nm; IR (NaCl) ν_{max} 3250, 3010, 2959, 2937, 2870, 1674, 1653, 1608, 1457, 1363, 1259, 1175, 1049, 982, 803 and 771 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.19 (1H, d, *J* = 16 Hz, H-3), 6.09 (1H, d, *J* = 16 Hz, H-4), 3.99 (1H, m, H-8), 2.41 (1H, dd, *J* = 17 and 6 Hz, H-7a), 2.27 (3H, s, Me-1), 2.06 (1H, dd, *J* = 17 and 11 Hz, H-7b), 1.78 (1H, m, H-9a), 1.75 (3H, s, Me-11), 1.47 (1H, t, *J* = 12 Hz, H-9b), 1.10 (3H, s, Me-13), 1.09 (3H, s, Me-12). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 198.7 (C, C-2), 142.4 (CH, C-3), 135.9 (C, C-6), 132.6 (CH, C-4), 132.4 (C, C-5), 64.8 (CH, C-8), 48.6 (CH_2, C-9), 43.0 (CH_2, C-7), 37.1 (C, C-10), 30.3 (CH_3, C-12), 28.8 (CH_3, C-13), 27.5 (CH_3, C-1), 21.8 (CH_3, C-11). HMBC: ^2J C-2 to Me-1 and H-3; C-4 to H-3; C-5 to Me-11; C-8 to H-7b and H-9b; C-10 to H-9a and H-9b; C-12 to Me-13; C-13 to Me-12; ^3J C-3 to Me-1; C-4 to Me-11; C-5 to H-7a and H-7b; C-6 to H-4, Me-11, Me-12 and Me-13; C-7 to H-9a and H-9b; C-9 to Me-12 and Me-13; C-13 to H-9a and H-9b. HRESIMS *m/z* 223.1497 $[M+H]^+$ (calcd for $C_{14}H_{22}O_2$+H, 223.1698) and *m/z* 205.1316 $[M\text{-}OH]^-$ (calcd for $C_{14}H_{22}O_2$-OH, 205.1592).

HYDROXYDIHYDROBOVOLIDE (2)

Colorless oil; molecular formula $C_{11}H_{18}O_3$; $[\alpha]_D$ -13.3 (*c* 0.015, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 220 (3.49), 291 (0.23) nm; IR (NaCl) ν_{max} 3369, 2956, 2926, 2859, 1741, 1694, 1458, 1438, 1381, 1287, 1260, 1133, 1101, 1056, 974, 956, 903, 767 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.96 (1H, m, H-5a), 1.92 (3H, s, 3-Me), 1.80 (3H, s, 2-Me), 1.74 (1H, m, H-5b), 1.29 (2H, m, CH_2-8), 1.27 (2H, m, CH_2-7), 1.15 (2H, m, CH_2-6), 0.86 (3H, t, *J* = 7 Hz, CH_3-9). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 172.2 (C, C-1), 157.8 (C, C-2), 125.6 (C, C-3), 107.0 (C, C-4), 36.2 (CH_2, C-5), 31.7 (CH_2, C-7), 22.8 (CH_2, C-8), 22.6 (CH_2, C-6), 14.1 (CH_3, C-9), 10.9 (CH_3, 3-Me), 8.7 (CH_3, 2-Me); HRESIMS *m/z* 199.1335 $[M+H]^+$ (calcd for $C_{11}H_{18}O_3$+H, 199.1329) and *m/z* 181.1233 $[M+H - H_2O]^+$ (calcd for $C_{11}H_{18}O_3$-OH, 181.1223).

OCTACOSANE (3)

White amorphous solid; molecular formula $C_{28}H_{58}$; UV (Hex) λ_{max} (log ε) 207 (3.11) nm; IR (NaCl) ν_{max} 2926, 2854, 1459, 908, 734 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.26-1.24 (48H, m, CH_2-3 to CH_2-26), 1.11 (4H, m, CH_2-2 and CH_2-27), 0.88 (6H, t, *J* = 7 Hz, CH_3-1 and CH_3-28). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 32.3 (CH_2, C-3 and C-26), 30.0 (CH_2, C-5 to C-24), 29.6 (CH_2, C-4 and C-25), 23.0 (CH_2, C-2 and C-27), 14.4 (CH_3, C-1 and C-28).

TRANS-PHYTYL PALMITATE (4)

White amorphous solid; $[\alpha]_D$ -1.4 (*c* 0.237, $CHCl_3$); UV (Hex) λ_{max} (log ε) 210 (3.33) nm; IR (NaCl) ν_{max} 2916, 2849, 1738, 1732, 1641, 1462, 1456, 1372, 1236, 1165, 1114, 1046, 958, 756, 729, and 719 cm^{-1}; ^{1}H NMR ($CDCl_3$,

600 MHz) δ 5.30 (1H, t, *J* = 7 Hz, H2'), 4.56 (2H, d, *J* = 7 Hz, CH_2-1'), 2.28 (2H, t, *J* = 8 Hz, CH_2-2), 1.97 (2H, t, *J* = 7 Hz, CH_2-4'), 1.68 (3H, s, 3'-Me), 1.58 (2H, m, CH_2-3), 1.50 (1H, m, H-15'), 1.38-1.25 (36H, m, CH_2-4 to CH_2-15, CH_2-5', CH_2-7', CH_2-9' and CH_2-11' to CH_2-13'), 1.13 (2H, m, CH_2-14'), 1.05 (6H, m, CH_2-6', CH_2-8' and CH_2-10'), 0.88 (3H, m, CH_3-16), 0.86 (6H, m, 15'-CH_3 and CH_3-16'), 0.84 (6H, m, 7'-CH_3 and 11'-CH_3). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.1 (C, C-1), 142.7 (C, C-3'), 118.5 (CH, C-2'), 61.4 (CH_2, C-1'), 40.1 (CH_2, C-4'), 39.6 (CH_2, C-14'), 37.5-37.3 (CH_2, C-6', C-8' and C-10'), 36.8 (CH_2, C-12'), 34.6 (CH_2, C-2), 33.0 (CH, C7' and C-11'), 32.2 (CH_2, C-14), 29.9-29.4 (CH_2, C-4 to C-13), 28.2 (CH, C-15'), 25.2-24.7 (CH_2, C-3, C-5', C-9' and C-13'), 22.9 (CH_2, C-15), 22.8 (CH_3, 15'-CH_3 and C-16'), 19.9 (CH_3, 7'-CH_3 and 9'-CH_3), 16.6 (CH_3, 3'-CH_3), 14.3 (CH_3, C-16).

LINOLEIC ACID (5)

Light yellowish solid; UV (EtOH) λ_{max} (log ε) 224 (1.28), 272 (0.36) nm; IR (NaCl) ν_{max} 3436, 3024, 2984, 2918, 2850, 1741, 1710, 1648, 1465, 1374, 1243, 1047 and 758 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.32-5.36 (4H, m, H-9, H-10, H-12, and H-13), 2.79 (1H, t, *J* = 7, H-11a), 2.75 (1H, t, *J* = 7, H-11b), 2.32 (2H, t, *J* = 8 Hz, CH_2-2), 2.03 (4H, m, CH_2-8 and CH_2-14), 1.61 (2H, m, CH_2-3), 1.26 (14H, m, CH_2-4 to CH_2-7 and CH_2-15 to CH_2-17), 0.87 (3H, t, *J* = 7 Hz, CH_3-18). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 179.8 (C, C-1), 130.5 (CH, C-13), 130.3 (CH, C-9), 128.3 (CH, C-12), 128.1 (CH, C-10), 34.2 (CH_2, C-2), 31.8 (CH_2, C-16), 29.9-29.3 (CH_2, C-4 to C-7 and C-15), 27.4 (CH_2, C-8 and C-14), 25.9 (CH_2, C-11), 24.9 (CH_2, C-3), 22.8 (CH_2, C-17), 14.3 (CH_3, C-18); HRESIMS with *m/z* 279.2371 $[M-H]^-$ (calcd for $C_{18}H_{32}O_2$-H, *m/z* 279.2330).

METHYL OLEATE (6)

Yellowish oil; UV (Hex) λ_{max} (log ε) 202 (2.80), 222 (0.68), 277 (0.11) nm; IR (NaCl) ν_{max} 2926, 2855, 1744, 1458, 1437, 1376, 1364, 1245, 1196, 1171, 1018, 882 and 722 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.32 (2H, m, H-9 and H-10), 3.64 (3H, s, 1-OCH_3), 2.28 (2H, t, *J* = 8 Hz, CH_2-2), 1.98 (4H, m, CH_2-8 and CH_2-11), 1.59 (2H, m, CH_2-3), 1.28 (20H, m, CH_2-4 to CH_2-7 and CH_2-12 to CH_2-17), 0.86 (3H, t, *J* = 7 Hz, CH_3-18). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 174.6 (C, C-1), 130.2 (CH, C-10), 130.0 (CH, C-9), 52.7 (CH_3, 1-OCH_3), 34.3 (CH_2, C-2), 32.1 (CH_2, C-16), 30.0-29.3 (CH_2, C-4 to C-7 and C-12 to C-15), 27.4 (CH_2, C-8 and C-11), 25.2 (CH_2, C-3), 22.9 (CH_2, C-17), 14.3 (CH_3, C-18); GCMS 296 (3) $[M]^+$, 264 (14), 222 (10), 180 (8), 137 (9), 123 (17), 110 (27), 97 (52), 83 (61), 73 (60), 69 (74), 55 (100).

ETHYL PALMITATE (7)

Yellowish oil; UV (Hex) λ_{max} (log ε) 206 (3.10), 274 (0.11) nm; IR (NaCl) ν_{max} 2924, 2853, 1740, 1465, 1420, 1373, 1349, 1302, 1244, 1177, 1116, 1098, 1036, 859, 804, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 4.08 (2H, q, *J* = 7 Hz, 1-O<u>CH_2</u>CH_3), 2.38 (2H, t, *J* = 8 Hz, CH_2-2), 1.57 (2H, m, CH_2-3), 1.24 (24H, m, CH_2-4 to CH_2-15), 1.21 (3H, t, *J* = 7, 1-OCH_2<u>CH_3</u>), 0.84 (3H, t, *J* = 7 Hz, CH_3-16). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.8 (C, C-1), 60.4 (CH_2, 1-O<u>CH_2</u>CH_3), 34.6 (CH_2, C-2), 32.2 (CH_2, C-14), 30.0-29.4 (CH_2, C-4 to C-13), 25.3 (CH_2, C-3), 23.0 (CH_2, C-15), 14.5 (CH_3, 1-OCH_2<u>CH_3</u>), 14.4 (CH_3, C-16); GCMS *m/z* 284 (4) $[M]^+$, 255 (2), 241 (6), 157 (11), 101 (53), 88 (100), 70 (26), 55 (27).

PALMITIC ACID (8)

White amorphous solid; molecular formula $C_{16}H_{32}O_2$; UV (EtOH) λ_{max} (log ε) 208 (0.73), 268 (0.18) nm; IR (NaCl) ν_{max} 3409, 2917, 2850, 1707, 1464, 1411, 1297, 908, 735 and 650 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.33 (2H, t, *J* = 8 Hz, CH_2-2), 1.61 (2H, m, CH_2-3), 1.28 (24H, m, CH_2-4 to CH_2-15), 0.86 (3H, t, *J* = 7 Hz, CH_3-16). ^{13}C NMR ($CDCl_3$, 150 MHz) δ 178.7 (C, C-1), 34.0 (CH_2, C-2), 32.1 (CH_2, C-14), 29.9-29.3 (CH_2, C-4 to C-13), 24.9 (CH_2, C-3), 22.9 (CH_2, C-15), 14.2 (CH_3, C-16); HRESIMS with *m/z* 255.2369 $[M-H]^-$ (calcd for $C_{16}H_{32}O_2$-H, *m/z* 255.2330).

RESULTS AND DISCUSSION

The n-hexane extract of *C. pallida* from Perak, Malaysia yielded one new cyclopentylidene, crotolidine (**1**) and seven known compounds, i.e. hydroxydihydrobovolide (**2**), octacosane (**3**), *trans*-phytyl palmitate (**4**), linoleic acid (**5**), methyl oleate (**6**), ethyl palmitate (**7**) and palmitic acid (**8**) through extensive chromatographic purifications of the crude extracts (Figure 1). The structures of the compounds **1**-**8** were characterized using NMR, HRESIMS, IR and UV spectroscopy and the NMR data are available in the supplementary document.

Crotolidene (**1**) is a new cyclopentylidene isolated as colourless oil from the n-hexane extract of *C. pallida*. The HRESIMS showed *m/z* 223.1497 $[M + H]^+$ and 205.1316 $[M - OH]^-$ consistent with molecular formula $C_{14}H_{22}O_2$ and four degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl group with the observation of IR band at 3250 cm^{-1}. The IR bands at 3010 and 1674 cm^{-1} suggested the presence of double bond and conjugated ketone in the structure of **1**. The ^{13}C NMR spectrum of **1** showed a total of 13 carbon signals attributed to four methyls, two methylenes, three methines and four quaternary carbons. The observation of the methine signals at δ_C 64.8 (δ_H 3.99) suggested oxymethine functionality consistent with the IR results. The quaternary carbon signals at δ_C 198.7 suggested the presence of carbonyl functionality in agreement with the IR results. The results from the ^{1}H NMR spectrum indicated the presence of four methyl singlets (δ_H 1.09, 1.10, 1.75, and 2.27), one sp^3 oxymethine (δ_H 3.99), two sp^2 methines (δ_H 6.09 and 7.19), and two set of sp^3 methylenes (δ_H 2.14, 2.06 and δ_H 1.47, 1.78). The methyl signals at δ_H 2.27 (δ_C 27.5) is typical of the methyl ketone functionality. The COSY spectrum gave two partial structures consisted of CH_2-CH(OH)-CH_2 and CH=CH, consistent with the IR,

^{13}C and ^{1}H NMR data as discussed above. The first partial structure CH_2-CH(OH)-CH_2 containing two methylenes and an oxymethine corresponds to C(7)-C(8)-C(9), while the second alkene-methine partial structure CH=CH is assigned to C(3)-C(4). The observed coupling constant of 16 Hz for the *sp*^{2} methines suggested H(3) and H(4) are arranged in *trans*-configuration (3*E*). The HMBC spectrum (Figure S1) showed J^2 correlations from carbonyl C(2) to Me-1 and H(3) and J^3 correlation from C(3) to Me-1 connecting the methyl ketone functionality to the CH=CH (i.e. C(3)-C(4)) partial structure providing the Me-CO-CH=CH- fragment. The J^2 HMBC correlations from C(5) to Me-11 and J^3 HMCB correlations from C(4) to Me-11 and C(6) to H(4) and Me-11, extended the conjugation of the fragment to Me-CO-CH=CH-C(Me)=C- (methyl-hexadienone) corresponding to C(1)-C(2)-C(3)-C(4)-C(5) C(11)-C(6)-. Hence, three of the four degrees of unsaturation indicated by the molecular formula of **1** can be attributed to the C(2) ketone and C(3)-C(4) and C(5)-C(6) double bonds.

The C(5) of the methyl-hexadienone fragment showed J^3 HMCB correlation to H(7), connecting the Me-CO-CH=CH-C(Me)=C- (methyl-hexadienone) to the CH_2-CH(OH)-CH_2 partial structures together. The *vice versa* J^3 HMCB correlation between Me-12 and M-13 suggested both the methyls group are connected to the same carbon center (C(10)). The observation of J^3 HMBC correlation from C(6) to Me-12 and Me-13 suggested C(6) is α positioned to the C(10) carbon center which is connecting to both Me-12 and Me-13. The final key HMBC J^2 correlation from C(10) to H(9) and J^3 correlations from C(9) to Me-12 and M-13 formed the cyclopentyl ring of structure **1**. The cyclopentyl ring accounted for the last degree of unsaturation in the structure of **1**.

The configuration of the C(5) and C(6) alkene is assigned as *Z*. In the 5*Z* configuration, the Me-11 is located in further distance from other protons such as CH_2(7) and H(3). However, in the case of 5*E* configuration, the Me-11 is located in near proximity with Me-12 and Me-13. The NOESY experiments of compound **1** show no correlations between Me-11 and other protons suggested no proximate protons in the Me-11 vicinity supporting the 5*Z* configuration. Due to the minute amount of compound **1**, we were not able to assign the configuration of OH-8. The enantiomeric relationship of α-OH-8 and β-OH-8 structures was not resolvable in the NMR experiments. Hence, the structure of crotolidene (**1**) is (3*E*,5*Z*)-5-(4-hydroxy-2,2-dimethylcyclopentylidene) hex-3-en-2-one (Figure 2).

Hydroxydihydrobovolide (**2**) was isolated as colourless oil with optical activity $[\alpha]_D$ -13.3 (*c* 0.02, $CHCl_3$). The molecular formula $C_{11}H_{18}O_3$ was determined by HREIMS with observation of *m/z* 199.1335 $[M+H]^+$ (calculated for $C_{11}H_{18}O_3$ + H 199.1329) and *m/z* 181.1233 $[M+H-H_2O]^+$ (calculated for $C_{11}H_{18}O_3$ - OH 181.1223). The IR spectrum shows bands at 3369, 1741 and 1694 cm^{-1}, suggesting the presence of hydroxyl, conjugated

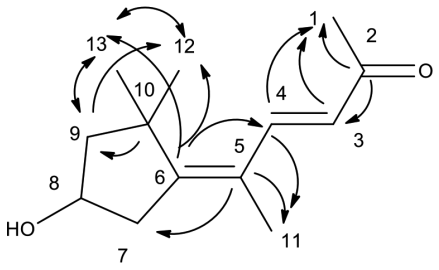

FIGURE 2. HMBC correlations of compound **1**

ester and conjugated alkene functionality. The ^{13}C NMR spectrum showed 11 carbon signals consist of three methyls, four methylenes and four quaternary carbons. The carbon signals at δ_C 172.2 is typical of the carbonyl carbon (C(1)) of the 2-furanone moiety, while the carbon signal at δ_C 107.0 (C(4)) suggested that this carbon is flanked between two oxygen atoms. The ^{1}H NMR spectrum showed the presence of three methyl signals at δ_H 1.92 (3-Me), 1.80 (2-Me) and 0.86 (CH_3-9), and three set of methylene signals.

The COSY spectrum showed only one partial structure, such as $CH_3CH_2CH_2CH_2$ corresponding to C(5)-C(6)-C(7)-C(8)-C(9) pentyl side chain. The HMBC spectrum shows ^3J correlations from C(1) and C(3) to 2-Me and C(2) and C(4) to 3-Me confirming the presence of the dimethyl-2-furanone moiety. The observation ^{13}C NMR chemical shift of C(4) at δ_C 107.0 suggested that the hydroxyl group is attached to this carbon. The pentyl side chain is connected to the hydroxyl-dimethyl-2-furanone moiety at C(4), a deduction from the HMBC results with the observation of ^2J correlation from C(4) to H(5), hence completing the structure of **2** as 5-hydroxy-3,4-dimethyl-5-pentylfuran-2(5H)-one. Compound **2** has been previously isolated from microorganism (Koshino et al. 1989; Wu et al. 2011; Yuan et al. 2016) and named as hydroxydihydrobovolide and was reported to show mild antimicrobial activity. The experiment data were similar with those reported and this is the first isolation report of hydroxydihydrobovolide from a plant source (Figure 3).

Octacosane (**3**) was isolated as white solid from the most non-polar fractions. The IR spectrum showed absorption bands attributing to the aliphatic functionality

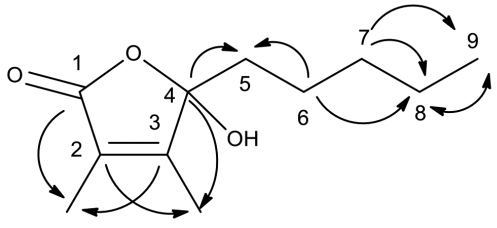

FIGURE 3. HMBC correlations of compound **2**

at 2926, 2854, 908, 734, 668 and 651 cm^{-1}. The ^{1}H NMR spectrum showed presence of two methyl signal at δ_H 0.88 (Me-1 and Me-28); and a methylene cluster signal at δ_H 1.26 integrated for 56 protons or twenty-six set of methylenes which were assigned to C(2) to C(27). The ^{13}C NMR spectrum displayed five carbon signals attributed to twenty-eight carbons, with signal at δ_C 14.4 assigned to the terminal methyl C(1) and C(28) and four other signals at δ_C 23.0 (C-2 and C-27), 32.2 (C-3 and C-26), 29.6 (C-4 and C-25) and 30.0 (C-5 to C-24) assigned to the methylene carbons. Octacosane (**3**) is a known hydrocarbon and was previously isolated from other plants such as *Euphorbia esula*, *Heracleum sphondylium* and *Moschosma polystachyum* (Farnsworth et al. 1968; Lawrie et al. 1968; Rajkumar & Jebanesan 2004) and was reported to display mosquitocidal activity. The experimental data were similar with those of the reported data (Sigma-Aldrich 2017; Speight et al. 2011).

trans-Phytyl palmitate (**4**) (Asand et al. 1991), linoleic acid (**5**) (Gunstone 2007; Gunstone & Jacobsberg 1972), methyl oleate (**6**) (Gunstone 2007; Rakoff et al. 1979), ethyl palmitate (**7**) (Gunstone 2007; Joshi et al. 2009) and palmitate acid (**8**) (Gunstone 2007; Miranda-Vilela & Grisolia 2009) are common known fatty-acids or fatty-esters. The structures of the compounds were established by comparing the experiment data with the reported data. Both set of data were found to be similar.

Conclusion

In the present studies, we have successfully isolated and characterized a new cyclopentylidene, crotolidene (**1**) which possessed the (3*E*,5*Z*)-5-(4-hydroxy-2,2-dimethylcyclopentylidene)hex-3-en-2-one structure and seven known compounds, i.e. hydroxydihydrobovolide (**2**), octacosane (**3**), *trans*-phytyl palmitate (**4**), linoleic acid (**5**), methyl oleate (**6**), ethyl palmitate (**7**) and palmitate acid (**8**), from Malaysia *C. pallida*. This is the first isolation report from the Malaysia *C. pallida* to best of our knowledge. Compound **1** and **2** were not subjected to further bioassays characterization due to the minute amount of samples isolated in this study. The NMR spectrum **1**-**8** are available in the supplementary document.

Acknowledgements

This work was supported by the University of Malaya UMRG under Grant RP002A-13Bio; Government of Malaysia MOSTI E-science fund under Grant SF004-2014 (06-01-03-SF0905); and Postgraduate Research Fund of University Malaya under Grant PG039-2015A.

References

Abdullah, A.S. 1990. *Poisonous Plants of Malaysia*. Kuala Lumpur: Tropical Press.

Asand, M., Urixm, A.H. & Ahmad, V.U. 1991. Diterpene fatty acid ester from *Leucas nutans*. *J. Nat Prod*. 54(5): 1444-1446.

Chong, K.Y., Tan, H.T.W. & Corlett, R.T. 2009. *A Checklist of the Total Vascular Plant Flora of Singapore: Native, Naturalised and Cultivated Species*. Singapore: Raffles Museum of Biodiversity Research, National University of Singapore.

Farida Hanum, I. & Vander Maesen, L.J.G. 1997. *Plant Resources of South-East Asia* No.11. Auxiliary Plants. Leiden: Backhuys Publisher.

Farnsworth, N.R., Wagner, H., Hoerhammer, L., Hoerhammer, H.P. & Fong, H.H.S. 1968. *Euphorbia esula*. I. Preliminary phytochemical and biological evaluation. *J. Pharm. Sc*. 57(6): 933-939.

Gunstone, F.D. 2007. *^{13}C-NMR Spectroscopy of Fatty Acids and Derivatives*. The AOCS Lipid Library. http://lipidlibrary.aocs.org/nmr/nmrmono/index.html.

Gunstone, F.D. & Jacobsberg, F.R. 1972. Fatty acids, part 36 the synthesis, silver ion chromatographic, and NMR spectroscopic properties of the nine 9,12-diunsaturated n-C18 acids. *Chem. Phy. Lipids*. 9(2): 112-122.

Joshi, H., Joshi, A.B., Sati, H., Gururaja, M.P., Shetty, P.R., Subrahmanyam, E.V.S. & Satyanaryana, D. 2009. Fatty acids from *Memecylon umbellatum* (Burm.). *Asian J. Research Chem*. 2(2): 178-180.

Koshino, H., Yoshihara, T., Sakamura, S., Shimanuki, T., Sato, T. & Tajimi, A. 1989. Novel C-ll epoxy fatty acid from stromata of epichloe typhina on phleum pretense. *Agric. Biol. Chem*. 53(9): 2527-2528.

Lawrie, W., McLean, J. & Younes, M.E.G. 1968. Constituents of the seeds of *Heracleum sphondylium*. *Phytochemistry* 7(11): 2065-2066.

Miranda-Vilela, A.L. & Grisolia, C.K. 2009. Characterization of the major nutritional components of *Caryocar brasiliense* fruit pulp by NMR spectroscopy. *Quim. Nova*. 32(9): 2310-2313.

Rajkumar, S. & Jebanesan, A. 2004. Mosquitocidal activities of octacosane from *Moschosma polystachyum* Linn. (lamiaceae). *J. Ethnopharmacology* 90: 87-89.

Rakoff, H., Weisleder, D. & Emken, E.A. 1979. ^{13}C-Nuclear magnetic resonance of mono- and dihydroxy saturated and unsaturated fatty methyl esters. *Lipids* 14(1): 81-83.

Sigma-Aldrich. *1H NMR of Octacosane*. 17 March 2017. Retrived from http://www.sigmaaldrich.com/catalog/product/aldrich/o504?lang=en&region=MY

Speight, R.J., Rourke, J.P., Wong, A., Barrow, N.S., Ellis, P.R., Bishop, P.T. & Smith, M.E. 2011. ^{1}H and ^{13}C solution- and solid-state NMR investigation into wax products from the Fischer-Tropsch process. *Solid State Nuclear Magnetic Resonance* 39: 58-64.

Williams, M.C. & Molyneux, R.J. 1987. Occurrence, concentration, and toxicity of pyrrolizidine alkaloids in crotalaria seeds. *Weed Science* 35(4): 476-481.

Wu, J., Tsujimori, M., Hirai, H. & Kawagishi, H. 2011. Novel compounds from the mycelia and fruiting bodies of *Climacodon septentrionalis*. *Biosci. Biotechnol., Biochem*. 75(4): 783-785.

Yuan, W., Cheng, A., Fu, W., Zhao, M., Li, X., Cai, Y., Dong, J., Huang, K., Gustafson, K.R. & Yan, P. 2016. Structurally diverse metabolites from the soft coral *Sinularia verruca* collected in the South China Sea. *J. Nat Prod*. 79(4): 1124-1131.

Yeun-Mun Choo
Department of Chemistry
University of Malaya
50603 Kuala Lumpur, Federal Territory
Malaysia

*Corresponding author; email: ymchoo@um.edu.my

Received: 9 January 2017
Accepted: 18 March 2017