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A polyisoprenylated ketone from *Calophyllum enervosum*

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Abstract

A polyisoprenylated ketone named enervosanone has been isolated from the stem bark of *Calophyllum enervosum* together with three known compounds, cambogin, osajaxanthone and epicatechin. Their structures were determined by spectroscopic analysis. The antimicrobial evaluations of the isolated compounds were also reported.

Keywords: *Calophyllum enervosum*; Guttiferae; Stem bark; Isolation; Polyisoprenylated ketone; Enervosanone; Antimicrobial evaluation

1. Introduction

The genus *Calophyllum* is a member of Guttiferae (Clusiaceae) family. The Guttiferae family is well known to contain xanthones, coumarins, flavonoids and benzophenones (Sultanbawa, 1980). *Calophyllum enervosum* Henderson and Wyatt-Smith is a big tree reaching a height of 33 m and 250 cm girth. The Malay local name of *Calophyllum* genus is called as "bintangur" (Burkill, 1966; Whitemore, 1973). The most popular species from this genus is the *C. lanigerum* that found in Sarawak, Malaysia. Its chemical constituents have been shown to inhibit the cytophatic effects of in vitro HIV infection (Kashman et al., 1992).

A series of polyisoprenylated bicyclo[3.3.1]nonane natural products have been reported from this genus by many researchers (Olivera et al., 1999; Henry et al., 1999; Lokvam et al., 2000; and Sahu et al., 1989).

Herein, we wish to report the isolation and structural elucidation of a novel polyisoprenylated ketone, enervosanone and three other compounds **2**–**4** from the stem bark of *C. enervosum* as well as their antimicrobial evaluations.

2. Results and discussion

Extraction of the air dried and powdered stem bark of *C. enervosum* by soxhlet apparatus with *n*-hexane, EtOAc and acetone successively gave the *n*-hexane, EtOAc and acetone extracts. The *n*-hexane extract was subjected to column chromatography on silica gel afforded enervosanone (**1**) and cambogin (**2**) (Rama Rao et al., 1980). The ethyl acetate extract yielded osajaxanthone (**3**) (Devon and Scott, 1975) and the acetone extract afforded (−)-epicatechin (**4**) (Khoon and Das, 1989).

Compound **1** was obtained as white needles. The ultraviolet spectrum of **1** showed the absorbance maxima at 212 and 268 nm. The infrared spectrum showed absorption bands for carbonyl groups at 1730 cm^{-1}. A

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band at 1607 cm^{-1} was attributed to C=C stretching. The bands at 2967, 2912 cm^{-1} were assigned to C–H aliphatic stretching (Silverstein et al., 1991; Williams and Fleming, 1995).

The ^1H NMR of **1** showed the presence of two tertiary methyl groups appearing at δ 0.94 and 1.24, seven olefinic methyl groups at δ 1.64 (3H, *s*), 1.62 (3H, *s*), 1.61 (3H, *s*), 1.58 (3H, *s*), 1.54 (3H, *s*), 1.49 (3H, *s*) and 1.24 (3H, *s*). The C-3 *gem* methylene protons appeared at δ 2.93 (1H, *d*, $J = 17.0$ Hz) and 3.53 (1H, *d*, $J = 17.0$ Hz). Four triplet signals at δ 5.13 (1H, *t*, $J = 7.5$ Hz), 4.98 (1H, *t*, $J = 6.2$ Hz), 4.90 (1H, *t*, $J = 8.0$ Hz), and 4.84 (1H, *t*, $J = 6.6$ Hz) corresponding to olefinic protons indicated the presence of both isopent-2-enyl and geranyl groups in the structure. The COSY spectrum of **1** showed the connectivity of a triplet signal at δ 5.13 and a doublet signal at δ 2.47 ($J = 7.5$ Hz), other connectivity could be shown between a triplet signal at δ 4.98 and the multiplet signals at δ 1.99 and 1.95. These findings confirmed the presence of geranyl side chain, which was attached to C-5. Two isoprenoid units were readily identified from ^1H, COSY and ^{13}C NMR spectra as 2-methylbut-2-enyl units that were joined to C-1 and C-7.

The ^{13}C NMR of **1** showed the presence of 31 carbons. The DEPT experiment confirmed that **1** contains five methine carbons (at δ 124.1, 122.6, 118.0, 117.4 and 46,4), seven methylene carbons at (δ 62.8, 40.6, 39.9, 31.1, 29.2, 27.1 and 26.6), nine methyl carbons at (δ 26.3, 26.0, 25.7, 25.6, 22.9, 17.8, 17.8, 17.6 and 16.2) and seven quaternary carbons at (δ 139.1, 136.3, 133.5, 131.4, 70.2, 64.7 and 51.2). Three signals for carbonyl groups were seen at δ 210.3 and 202.7 (there were two overlapping).

Compound **1** contains a bicyclo[3.3.1]nonane moiety, which is commonly observed in the Guttiferae family and the location of functionalities and ligands were deduced from the NMR data (Table 1).

In the EIMS spectrum, the molecular ion peak was observed at *m/z* 466 corresponding to the molecular formula of $C_{31}H_{46}O_3$ (calculated for 466.34488). The presence of nine degrees of unsaturation which consisted of four double bonds, three C=O groups and two cyclic systems (Silverstein et al., 1991; Williams and Fleming, 1995) confirmed that the compound has a molecule as shown in structure **1**. Cleavage of **1** to fragment *m/z* 329 indicated the elimination of geranyl side chain. A fragmentation ion at *m/z* 205 showed the cleavage of the non-ketone cyclic fragment and the base peak at *m/z* corresponded to an isopent-2-enyl group. When the ^1H and ^{13}C NMR spectral data of **1** were compared with those of Guttiferone B (**5**) as previously isolated by Gustafson et al. (1992), it showed that they have a similarity in structure but lacked a benzoyl group.

Table 1
NMR spectral data of **1** (in $CDCl_3$)

Position	δ_C[a]	δ_H (int. mult. *J*)	^1H–^1H COSY
1	70.2	–	–
2	202.7	–	–
3	62.8	2.93 (1H, *d*, $J_{ax} = 17.0$ Hz) 3.53 (1H, *d*, $J_{eq} = 17.0$ Hz)	H-3
4	202.7	–	–
5	64.7	–	–
6	40.6	1.30 (1H, *m*) 1.43 (1H, *m*)	H-6, H-7
7	46.4	2.04 (1H, *m*)	H-17, H-6
8	51.2		–
9	210.3	–	–
10	26.6	2.55 (2H, *d*, $J = 8.0$ Hz)	H-11
11	118.0	4.90 (1H, *t*, $J = 8.0$ Hz)	H-10, H-13, H-14
12	133.5	–	–
13	22.9	1.58 (3H, *s*)	H-11, H-14
14	26.0	1.58 (3H, *s*)	H-11, H-13
15	25.6	1.24 (3H, *s*)	H-16
16	17.6	0.94 (3H, *s*)	H-15
17	29.2	2.19 (2H, *b dt*)	H-8, H-18
18	122.6	4.84 (1H, *t*, $J = 6.6$ Hz)	H-17, H-20, H-21
19	136.3	–	–
20	26.3	1.49 (3H, *s*)	H-21, H-18
21	16.2	1.64 (3H, *s*)	H-20, H-18
22	31.1	2.47 (2H, *d*, $J = 7.5$ Hz)	H-23
23	117.4	5.13 (1H, *t*, $J = 7.5$ Hz)	H-22, H-26
24	139.1	–	–
25	39.9	1.95 (2H, *m*)	H-27
26	17.8	1.62 (3H, *s*)	H-23
27	27.1	1.99 (2H, *m*)	H-25, H-28
28	124.1	4.98 (1H, *t*, $J = 6.2$ Hz)	H-27, H-30, H-31
29	131.4	–	–
30	25.7	1.61 (3H, *s*)	H-31, H-28
31	17.8	1.54 (3H, *s*)	H-30, H-28

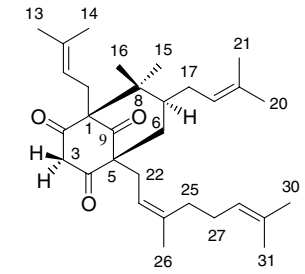
[a] All carbon signals were assigned by the aid of DEPT experiments.

Table 2
Antibacterial activity of compounds **1**, **2** and **3**

Micro-organisms	Inhibition zone in mm (MIC value in μg/μL)				
	1	**2**	**3**	**4**	Standard
B. subtilis	12 (0.0125)	8 (0.05)	–		
E. coli	9.5 (0.0125)	–	–	n.a.	21 (3.9×10^{-4})
P. aeruginosa	11 (0.0125)	8 (0.05)	–		
S. aureus	11.5 (0.0125)	8 (0.05)	–		

'–': No activity against the tested bacteria; n.a.: not available; MIC: minimum inhibition concentration; standard: streptomycin sulfate.

Antimicrobial assay was tested against two strain bacteria, Gram-positive (*Bacillus subtilis* and *Escherecia coli*) and Gram-negative (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) using the disc diffusion method. As shown in Table 2, compound **1** was found to have the strongest activity against four tested bacteria although it is much less active than the positive control streptomycin sulfate (MIC of 3.9×10^{-4} μg/μL). Compound **2** was found to be inactive against *S. aureus* and compound **4** was found to be inactive against all tested bacteria.



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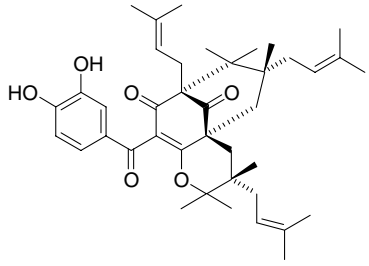

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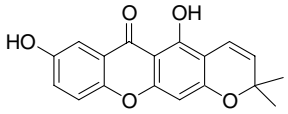

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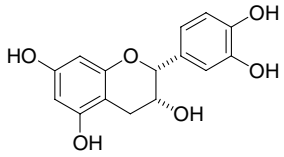

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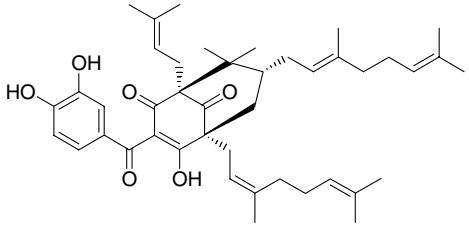

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3. Experimental

3.1. General experimental procedures

Melting points were determined by using a Leica Gallen III apparatus and were uncorrected. UV spectra were measured on UV-100PC Shimadzu using methanol solution. IR spectra were measured on a FT-IR Perkin–Elmer 1600 as KBr discs. Optical rotations were measured by a Polarimeter Type AA-5. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Varian Unity INOVA spectrophotometer, using TMS as internal standard. EIMS were recorded on a Varian mass spectrometer at 70 eV. CC: silica gel (Merck 70–230 mesh and 230–400 mesh). Spots were visualized by UV (254 and 365 nm), $FeCl_3$ and *p*-anisaldehyde spraying reagent. Streptomycin sulfate standard was purchased from Oxoid (Hampshire, UK).

3.2. Plant material

Stem bark of *C. enervosum* was collected from Tilatang Kamang, 6 km East of Bukittinggi, West Sumatra, Indonesia in September 1998. The voucher specimen (MT-03) is deposited at the Herbarium of Universitas Andalas (AND), Padang, Indonesia.

3.3. Extraction and isolation

The dried and powdered of the stem bark of *C. enervosum* (950 g) was extracted by soxhlet extractor for 18 h with *n*-hexane (2.5 L), EtOAc (2.5 L) and acetone (2.5 L) successively. Each solvent was removed under reduced pressure by rotary evaporator. The *n*-hexane extract (31.7 g, 3.33%), EtOAc extract (12.7 g, 1.33%) and acetone extract (16 g, 1.68%) all gave a sticky brown liquid.

The *n*-hexane extract (30 g) was separated by column chromatography (60 cm × 6 cm i.d.) on silica gel 70–230 mesh (Merck) (150 g) and eluted with petrol, petrol–EtOAc (9:1), (8:2), (7:3), (6:4), (5:5), (2:8) and EtOAc to give fractions A, B, C, D, E, F and G. Then, fraction C (5.2 g) was repeatedly purified by column chromatography (42 cm length, 3.5 cm diameter) on silica gel 70–230 mesh (80 g) using the same solvent system giving fractions C1, C2, C3 and C4.

The combined fraction C2 was subjected to column chromatography (35 cm length, 2.5 cm diameter) on silica gel 230–400 mesh (60 g) eluting with petrol,

petrol–EtOAc (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (2:8), (1:9) and EtOAc. Fraction with the same R_f values were combined and washed with petrol to yield **1** as white needles (341.1 mg).

Fraction D (1.7 g) was purified by column chromatography (35 cm × 2.5 i.d.) on silica gel 70–230 mesh (80 g), eluted with *n*-hexane–EtOAc (9:1) (8:2) (7:3), (6:4) (5:5), (4:6), EtOAc, and EtOAc–MeOH (9:1) afforded fractions D1, D2 and D3. Fraction D3 was repeatedly washed with Et_2O to afford **2** as pale yellow cubes (249.1 mg).

Separation of the EtOAc extract (10 g) by column chromatography (42 cm × 3.5 cm i.d.) over silica gel 70–230 mesh (100 g) and elution with petrol–EtOAc (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), EtOAc, and EtOAc–MeOH (9:1, 8:2) afforded fractions H, I, J, K and L. Purification of fraction I by preparative thin layer chromatography (1 mm layer thickness) eluted with petrol–EtOAc (7:3) yielded **3** as yellow needles (7.4 mg).

The acetone extract (10 g) was purified by vacuum liquid chromatography (7.5 cm × 7.5 cm i.d.). Silica gel 230–400 mesh (100 g) was used as stationary phase. It was eluted with Et_2O, EtOAc and acetone successively giving three fractions. The EtOAc soluble fraction was separated by column chromatography (35 cm × 2.5 cm i.d.) on silica gel 70–230 mesh (60 g), eluting with petrol–EtOAc (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), EtOAc, and EtAOc–MeOH (9:1), (8:2), and (7:3) to give fractions M, N and O. Fraction N was repeatedly washed with acetone to give **4** as white plate crystals (850 mg).

3.4. Antimicrobial assay

The isolated compounds from the stem bark of *C. enervosum* were tested against micro-organisms, *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus*. The antimicrobial assay was carried out according to Zavala et al. (1997), the paper discs were impregnated with 10 μL of methanol solution of each sample (1 mg/mL) and allowed to evaporate at room temperature. Streptomycin sulfate (30 μg/disc) was used as standard positive control. The discs were then incubated on the plate containing micro-organism at 37 °C for 18 h. The diameter of inhibition zone around each disc was measured and recorded at the end of the incubation period.

3.5. 8,8-Dimethyl-5-geranyl-1,7-bis(3-methylbut-2-enyl)bicyclo[3.3.1]nona-2,4,9-trione (*1*)

White needles, m.p. 119–120 °C. TLC (silica gel): R_f 0.67 in EtOAc: $CHCl_3$ (1:1). $[\alpha]_D^{24}$ +10° (MeOH; *c* 0.2000). UV λ_{max}^{MeOH} nm (log ε): 212 (2.18) and 268 (1.86). IR υ_{max}^{KBr} cm^{-1}: 2967, 2912 (C–H aliphatic), 1730 (C=O), 1607 (C=O), and 1531 (C=C). ^1H NMR (400 MHz, $CDCl_3$) see Table 1. ^{13}C NMR (100 MHz, $CDCl_3$) see Table 1. EIMS: *m/z* $[M]^+$ 466 for $C_{31}H_{46}O_3$ (calculated 466.34488), 329 (30), 261 (22), 205 (50), 135(18), 109 (38), 81(50), 69 (100), and 41 (76).

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