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Hopane and Lupane Triterpenes from Leaves and Stem Bark of *Aegle marmelos* (Rutaceae)

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Two hopane and two lupane triterpenes have been isolated from leaves and stem bark of *Aegle marmelos* originated from Yogyakarta, Indonesia. Hopane triterpenes identified as 6α,22-dihydroxyhopane (**1**) and 15α,22-dihydroxyhopane (**2**) were isolated from petroleum ether extract of the leaves, while lupane triterpenes; 20(29)-lupene-3α-ol (**3**) and 20(29)-lupene-3-on (**4**) were isolated from the stem bark. The structure of the compounds were elucidated using spectroscopic methods and by comparison with previous data.

Key Words: *Aegle marmelos*, Hopane triterpenes, Lupane triterpenes.

INTRODUCTION

Aegle marmelos Correa; synonym of *Crataeva marmelos* Linn. and *Crataeva religiosa* Ainslie belongs to Rutaceae family[1] and locally known as bel (Malaysia) and maja (Indonesia). The plant grows easily in Indian Subcontinent and Southeast Asia. The plant is known in traditional medicine uses *e.g.*, the leaves are utilized for treatment of fertility control, the bark is used for diabetes, while the root and aerial part were used in Ayurvedic system in Sri Lanka as medicine for some ailments such as to treat intermittent fever and for fish poison[2,3]. In India the leaves are mostly used in religious offering in temples. The older folks used its half-ripe fruit to relieve dysentery, cholera and constipation[4]. The ripe fruit can be used as a laxative and the extract as for rectum inflammation, whereas the fresh ripe-fruit is made into sherbet or syrup. The fixed oil of seed is used for purgative and claimed to have antimicrobial and anthelminthic activity[5]. Previous phytochemical work on *Aegle marmelos* have afforded several classes of constituents such as aegeline[6] and coumarin derivatives[6,7], cinnamide alkaloids[8], anthraquinone[7] and some lignin glycoside[9]. In continuation of our work on Rutaceous and local medicinal plants, here we report phytochemical investigation of leaves and stem barks of *Aegle marmelos*.

EXPERIMENTAL

The Infrared spectra were recorded using KBr mini disc on Perkin Elmer FTIR spectrophotometer model 1725X, while ultraviolet spectra were recorded on Shimadzu UV-VIS 160 Spectrometer in absolute ethanol or methanol. Mass spectra were recorded by AE1-MS 12 spectrometer with ionisation induced by electron impact at 70 eV. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded by JEOL FTNMR or Bruker DRX-500 with $CDCl_3$ used as solvent. Melting points were determined by Kohfler melting points apparatus XSP-12 Model 500X equipped with microscope and were uncorrected.

The plant sample of *Aegle marmelos* was collected from Yogyakarta, Indonesia. The plant was identified by Dr. Suwijio Pramono from Gadjah Mada University, Yogyakarta, Indonesia. The voucher specimen was deposited in the herbarium of Faculty of Pharmacy, Gadjah Mada University.

Isolation of constituents: The ground air-dried leaves (2.5 kg) and stem bark (3.5 kg) of the *A. marmelos* were extracted three times successively with petroleum ether, chloroform and methanol. The leaves extracts were concentrated under reduced pressure using rotary evaporator to give dark gummy solid of petroleum ether (21 g), chloroform (67.7 g) and methanol (133 g) extracts, respectively while stem bark yielded petroleum ether (14.1 g), chloroform (22.5 g) and methanol (130 g) extracts, respectively. Each of these extracts was subjected to column chromatography over silica gel using mixtures of petroleum ether, chloroform and methanol as eluents. Column chromatography separation of petroleum ether extract of the leaves (17.8 g) yielded 6α,22-dihydroxyhopane (**1**, 249 mg) and 15α,22-dihydroxyhopane (**2**, 95 mg), while separation

TABLE-1
NMR DATA OF COMPOUNDS (**1**) AND (**2**)

	^{1}H NMR (500 MHz) $CDCl_3$		^{13}C NMR (125 MHz) $CDCl_3$		COSY		HMBC	
	(1)	**(2)**	**(1)**	**(2)**	**(1)**	**(2)**	**(1)**	**(2)**
1	0.88, m	0.81, m	41.4	41.1	H-2	-	C-25	-
2	1.64, m	1.40, m	19.4	19.5	H-1	H-3	-	-
3	1.31, m	1.17, m	44.8	42.6	-	H-2	-	-
4	-	-	34.6	34.3	-	-	-	C-23, C-24
5	1.12, m	0.86, m	61.6	56.7	H-6	-	-	C-7
6	4.23, dt (J=10.0, 4.5Hz)	1.59, m	68.3	21.8	H-5, H-7	-	-	-
7	1.83, m	2.12, m	46.2	37.7	-	-	-	C-5
8	-	-	43.4	44.2	-	-	-	-
9	1.36, m	1.35, m	50.7	51.5	-	-	C-26	C-7
10	-	-	42.6	38.3	-	-	-	-
11	1.56, m	1.58, m	21.1	19.8	-	-	-	-
12	1.53, m	1.51, m	24.8	25.1	-	-	-	-
13	1.38, m	1.42, m	50.2	50	-	-	-	-
14	-	-	39.8	48.1	-	-	-	-
15	1.29, m	4.11, dd (J=9.7, 5.1 Hz)	35.1	74.9	-	H-16	-	C-8,C-14,C-16,C-27
16	2.18, m	2.80, ddd (J=9.7, 5.1, 2.0 Hz)	22.7	33.7	H-17	H-15, H-17	-	C-15, C-17, C-18
17	1.47, m	1.62, m	55.1	51.6	H-16, H-21	H-16, H-21	C-16, C-21	-
18	-	-	44.7	45.1	-	-	C-16, C-21	-
19	1.00, m	1.61, m	42.1	41.9	-	H-20	C-16	-
20	1.87, m	1.81, m	27.4	27.7	H-21	H-19, H-21	C-21	-
21	2.41, dd (J=20.0, 9.2 Hz)	2.41, dd (J=20.0, 9.1 Hz)	52	51.8	H-17, H-20	H-17, H-20	C-17, C-20, C-22, C-29, C-30	C-15, C-17, C-20, C-22, C-29, C-30
22	-	-	72.9	72.8	-	-	-	-
23	1.60, s	0.88, s	37.7	33.9	-	-	C-3, C-4, C-24	C-4, C-24
24	1.27, s	0.84, s	23	22.2	-	-	C-4, C-23	C-4, C-23
25	0.96, s	0.89, s	17.9	16.7	-	-	C-10	C-9, C-10
26	1.11, s	1.20, s	18.9	16.6	-	-	C-8, C-14	C-8
27	1.03, s	1.33, s	17.7	12.9	-	-	C-14, C-15	C-14
28	0.95, s	1.03, s	16.8	18	-	-	C-18	C-18
29	1.38, s	1.38, s	30.2	30	-	-	C-21	C-22
30	1.43, s	1.42, s	31.7	31.9	-	-	C-21	C-22

of petroleum ether extract of the stem bark (12.1 g) using vacuum column chromatography yielded 20(29)-lupene-3α-ol (**3**, 536 mg) and 20(29)-lupene-3-on (**4**, 63.5 mg). Compound (**3**) (125 mg) was also obtained from crude chloroform extract of the stem bark (20 g).

6α,22-Dihydroxyhopane (zeorin) (**1**) colourless prisms (249 mg), $C_{30}H_{52}O_2$, m.p. 225-227 ºC (lit. m.p[10] 221-223 ºC). UV (λ_{max} EtOH) 206 nm (log = 4.63). IR (KBr, ν_{max}, cm^{-1}): 3321, 3278, 2938, 1632, 1467, 1385, 1219, 1159, 1027, 944, 762. MS *m/z* (% intensity): 444 (M^+, 0.9), 207 (52), 189 (56), 161 (10), 149 (39), 109 (18), 95 (30), 69 (31), 59 (100). ^{1}H NMR and ^{13}C NMR data are tabulated in Table-1.

15α,22-Dihydroxyhopane (dustanin) (**2**) colourless prisms (95 mg), $C_{30}H_{52}O_2$, m.p. 248-250 ºC (lit. m.p.[11] 249-250 ºC). UV (λ_{max} EtOH) 206 nm, (log = 4.63). IR (KBr, ν_{max}, cm^{-1}): 3419, 3375, 2944, 1463, 1382, 1158, 1054, 1035, 936. MS *m/z* (% intensity): 444 (M^+, 5), 411 (5), 223 (10), 205 (30), 191 (100), 163 (10), 147 (16), 135 (18), 95 (40), 69 (38), 59 (85). ^{1}H NMR and ^{13}C NMR data are tabulated in Table-1.

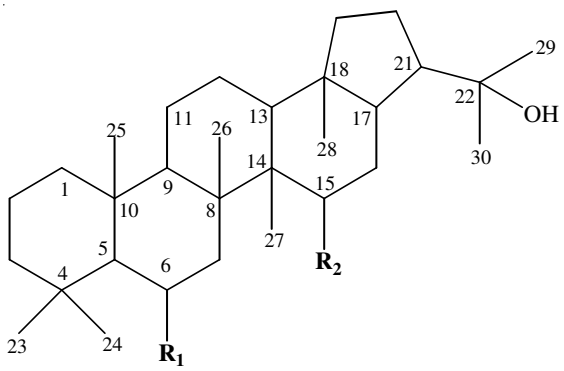
20(29)-Lupene-3α-ol (epi-lupeol) (**3**) white needles (536 mg), $C_{30}H_{50}O$, m.p. 202-204 ºC (lit. m.p.[12] 202.5 ºC). UV (λ_{max}, MeOH) 203 nm (log = 3.8). IR (KBr, ν_{max}, cm^{-1}): 3412, 3071, 2938, 1642, 1456, 1382, 1042. MS *m/z* (% intensity): 424 (M^+, 10), 383 (5), 272 (5), 257 (19), 234 (12), 207 (52), 189 (60), 161 (23), 147 (28), 135 (51), 121 (52), 107 (60), 95 (65), 81 (67), 68 (100). ^{1}H and ^{13}C NMR data were in good agreement with published data[12].

20(29)-Lupene-3-on (lupenone) (**4**) colourless needles (63.5 mg), $C_{30}H_{48}O$, m.p. 172-174 ºC (lit. m.p.[13] 170 ºC). UV (λ_{max}, MeOH) 203 nm (log = 3.8). IR (KBr, ν_{max}, cm^{-1}): 3072, 2944, 1706, 1643, 1457, 1384. MS *m/z* (% intensity): 424 (M^+, 10), 409 (9), 369 (8), 257 (9), 245 (18), 218 (24), 205 (64), 189 (24), 161 (20), 149 (22), 135 (34), 121 (38), 109 (60), 95 (57), 68 (100), 55 (60). ^{1}H and ^{13}C NMR data were in good agreement with published data[13].

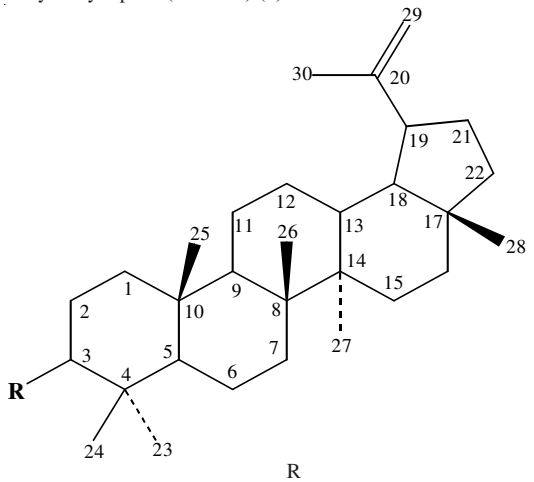
RESULTS AND DISCUSSION

Phytochemical investigation of leaves and stem bark of *Aegle marmelos* have led to the isolation of several classes of constituents including alkaloids, coumarins andtriterpenes. Our previous study reported the isolation of alkaloids and coumarins from various parts of the plant[14,15]. Here we wish to describe the isolation and characterization of hopane and lupane triterpenes from *Aegle marmelos*. Hopane triterpenes; 6α,22-dihydroxyhopane (**1**) and 15α,22-dihydroxyhopane (**2**) (Fig. 1) were obtained from petroleum ether crude extract of

the leaves. The isolation of these compounds never been reported from *Aegle marmelos* previously while lupane triterpenes; 20(29)-lupene-3α-ol (**3**) and 20(29)-lupene-3-on (**4**) were isolated from petroleum ether and chloroform crude extracts of the stem bark.



	R1	R2
6α,22-dihydroxyhopane (zeorin) (**1**) :	OH	H
15α,22-dihydroxyhopane (dustanin) (**2**):	H	OH



	R
20(29)-lupene-3a-ol (epi-lupeol) (**3**):	OH
20(29)-lupene-3-on (lupenone) (**4**) :	=O

Fig. 1. Structures of hopane and lupane triterpenes

Compound (**1**) was isolated as colourless crystals (249 mg), m.p. 225-227 ºC and was recrystallized from petroleum ether-acetone. The molecular ion peak at *m/z* 444 corresponds to molecular formula $C_{30}H_{52}O_2$. Its IR spectrum exhibited the presence of broad peak at 3321 cm^{-1} which corresponded to hydroxyl, aliphatic C-H stretching (2938 cm^{-1}), methyl and methylene groups (1632 and 1385 cm^{-1}), whereas the peak at 1159 cm^{-1} corresponded to C-O group. The integration of ^1H NMR showed the presence of fifty two protons. A doublet of triplet signal appeared at downfield region at δ 4.23 (*J* = 10.0, 4.5 Hz) was due to H-6 indicating the presence of hydroxyl group. H-21 peak appeared as doublet of doublet at δ 2.41 (*J* = 20.0, 9.2 Hz), while multiplet signal at δ 2.18 was correspond to H-16. Eight three-proton singlets at δ 1.60, 1.43, 1.38, 1.27, 1.11, 1.03, 0.96 and 0.95 were due to methyl groups which attached to C-4 (2 × CH_3), C-10, C-8, C-14, C-22 (2 × CH_3) and C-18. The other assignments of methylene and methine protons are summarized in Table-1.

^{13}C NMR spectrum revealed the presence of 30 peaks corresponds to 30 carbon atoms in the molecule. Resonances for methine carbons appeared at δ 61.6 (C-5), 68.3 (C-6), 50.7 (C-9), 50.2 (C-13), 55.1 (C-17) and 52.0 (C-21). Signals at δ 34.6, 43.4, 42.6, 39.8, 44.7 and 72.9 were due to quaternary carbons of C-4, C-8, C-10, C-14, C-18 and C-22 respectively. The remaining peaks correspond to other carbons of steroidal skeleton. ^{13}C NMR chemical shifts of the compound were in good agreement with the data of 6α,22-dihydroxyhopane reported earlier from lichen species *Physcia aipolia*[10]. ^1H-^1H COSY spectrum further confirmed the structure of compound (**1**) which shows the correlation between H-6, H-5 and H-7, while H-21 correlated with H-17 and H-20. Its HMBC spectrum showed correlations between H-21 with carbon C-17, C-20, C-22, C-29 and C-30. The details of COSY and HMBC correlation are tabulated in Table-1. 6α,22-Dihydroxyhopane (zeorin) (**1**) has mostly been isolated in cryptogams such as lichen and fern. The only report of its isolation from higher plant was from Tripterygium regelii[16].

The other hopane triterpenes was identified as 15α,22-dihydroxyhopane (dustanin) (**2**) (95 mg), m.p. 248-250 ºC and was recrystallized from acetone-methanol. The molecular ion peak appeared at *m/z* 444 correspond to the molecular formula $C_{30}H_{52}O_2$. Its IR spectrum showed the presence of free hydroxyl group at broad peaks around 3375 and 3419 cm^{-1}. A sharp band at 2944 cm^{-1} indicated the presence of aliphatic C-H bond, while peak at 1158 cm^{-1} was due to C-O stretching bond. The ^1H NMR data summarized in Table-1 showed almost similar with that of compound (**1**). The appearance of doublet of doublet signal at downfield region at δ 4.11 (*J* = 9.7, 5.1 Hz) suggested the presence of hydroxyl group at position H-15. Similar to compound (**1**), H-21 appeared as doublet of doublet signal at δ 2.41 (*J* = 20.0, 9.1 Hz), while doublet of doublet of doublet at δ 2.80 (*J* = 9.7, 5.1, 2.0 Hz) was attributed to H-16.

The ^1H and ^{13}C NMR spectra also showed the presence of eight methyl groups at δ 33.9 (C-23), 22.2 (C-24), 16.7 (C-25), 16.6 (C-26), 12.9 (C-27), 18.0 (C-28), 30.0 (C-29) and 31.9 (C-30) corresponded to δ 0.88, 0.84, 0.89, 1.20, 1.33, 1.03, 1.38 and 1.42, respectively. The signals at δ 74.9 (C-15), 56.7 (C-5), 51.8 (C-21), 51.6 (C-17), 51.5 (C-9) and 50.0 (C-13) represent the methine carbons. Signal for C-22 appeared at lower field at δ 72.8 which due to deshielding effect of hydroxyl group. The quaternary carbons appeared at δ 48.1 (C-14), 45.1 (C-18), 44.2 (C-8), 38.3 (C-10) and 34.3 (C-4). The ^{13}C NMR data were also compared with the data reported previously[17]. COSY spectrum showed correlations between H-15 and H-16; between H-21 with H-17 and H-20. HMBC spectrum shows long range coupling of proton H-15 with carbon C-8, C-14, C-16 and C-27, while proton H-21 correlates with carbon C-15, C-17, C-20, C-22, C-29 and C-30. Full COSY and HMBC correlation data can be referred in Table-1. To our knowledge, this is the first isolation of 15α,22-dihydroxyhopane (dustanin) (**2**) from higher plant.

Meanwhile, two lupane triterpenes; epi-lupeol (**3**) and lupenone (**4**) were isolated from the stem bark of *Aegle marmelos*. 20(29)-lupene-3α-ol or epi-lupeol, (**3**) (536 mg) was obtained from petroleum ether and chloroform extracts. The compound appears as white needles with melting point 202-204 ºC. The appearance of molecular ion peak at *m/z* 426 corresponds to molecular formula $C_{30}H_{50}O$. Its IR spectrum

revealed the presence of hydroxyl group (3412 cm^{-1}), aromatic and aliphatic C-H bonding (3071 and 2938 cm^{-1}), while absorption peak for C=C, CH_3 and C-O groups were at 1642, 1382 and 1042 cm^{-1}, respectively. The compound has been identified as 20(29)-lupene-3α-ol (**3**) and its NMR data were in good agreement with the reported data[12]. Compound (**4**) was identified as 20(29)-lupene-3-on or lupenone (63.5 mg) and was obtained from petroleum ether extract. The compound appeared as colourless needle crystals with melting point at 172-174 ºC and recrystallized in petroleum ether-acetone. The mass spectrum showed molecular ion peak at m/z 424 which corresponds to molecular formula $C_{30}H_{48}O$. Its IR spectrum exhibited the similar absorption peak to compound (**3**) but with an additional peak at 1706 cm^{-1} due to the presence of C=O group. The compound was identified as 20(29)-lupene-3-on (**4**) based on similarity of its physical and spectral data with literature values[4].

Conclusion

Phytochemical investigation on *Aegle marmelos* originated from Yogjakarta, Indonesia has afforded two hopane triterpenes from the petroleum ether crude extract of the leaves and two lupane triterpenes isolated from the stem bark. To our best of knowledge the hopane triterpenes were found to be the first time isolated from *Aegle marmelos* collected from Yogyakarta, Indonesia.

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