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DAMMARANE TRITERPENOIDS FROM *AMOORA YUNNANENSIS*

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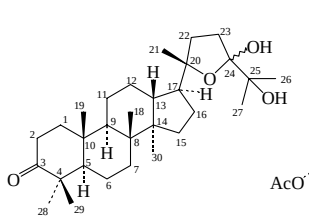
Abstract - Four dammarane triterpenoids, 20*S*,24-epoxy-24,25-dihydroxydammar-3-one, 20*S*,23*R*,24*R*-23-chloro-20,24-epoxy-dammarane-3α,24,25-triol 3-acetate, 20*S*,23*R*,24*S*-23-chloro-20,24-epoxy-dammarane-3α,24,25-triol 3-acetate, and 20*S*,24-epoxy-24,25-dihydroxy-3,4-secodammar-4(28)-en-3-oic acid, in addition to eight known dammarane triterpenoids, 3-acetylcabraleadiol, cabraleadiol, cabralea-hydroxylactone, ocotillone, cabraleone, 24,25-dihydroxy-dammar-20-en-3-one, shoreic acid, and 20*S*,24-epoxy-25,26,27-trisnor-24-oxo-3,4-secodammar-4(28)-en-3-oic acid, were isolated from the bark of *Amoora yunnanensis*. Their structures were elucidated by a combination of 1D and 2D NMR spectral analysis and comparison with closely related compounds.

The genus *Amoora*, which consists of about 25-30 species, is mainly distributed in India and the Malay Peninsula, and six species are found in the Yunnan province, P. R. China. *A. yunnanensis* (H. L. Li) C. Y. Wu is mainly distributed in southern Yunnan.[1] According to Pennington and Styles,[2] *Amoora* cannot be considered as a valid genus. In our continuing chemical studies on the Meliaceae, *A. yunnanensis* was investigated since there has been no report regarding its chemical constituents. However, no tetranortriterpenoids or protolimonoids were isolated from this species, in spite of these being considered as chemotaxonomic markers for the Meliaceae. On the other hand, 12 dammarane triterpenoids were obtained from the bark of this plant. These consisted of four new compounds, 20*S*,24-epoxy-24,25-dihydroxydammar-3-one (**1**), 20*S*,23*R*,24*R*-23-chloro-20,24-epoxy-dammarane-3α,24,25-triol 3-acetate (**2**), 20*S*,23*R*,24*S*-23-chloro-20,24-epoxy-dammarane-3α,24,25-triol 3-acetate (**3**), and 20*S*,24-epoxy-24,25-dihydroxy-3,4-secodammar-4(28)-en-3-oic acid (**4**), and eight known dammaranes, 3-acetylcabraleadiol (**5**),[3] cabraleadiol (**6**),[3] cabrelea-hydroxylactone (**7**),[4] ocotillone (**8**),[5] cabraleone (**9**),[5]

24,25-dihydroxy-dammar-20-en-3-one (**10**),[6] shoreic acid (**11**),[5] and 20*S*,24-epoxy-25,26,27-trisnor-24-oxo-3,4-seco-dammar-4(28)-en-3-oic acid (**12**).[7]



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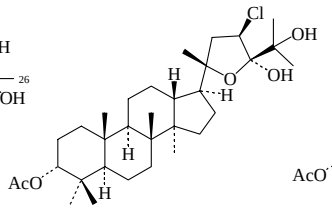

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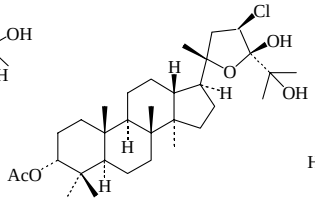

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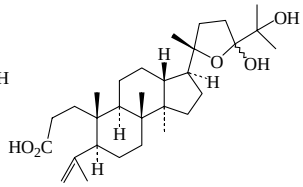

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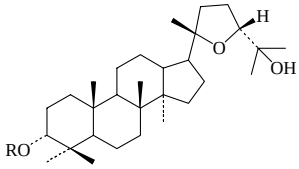
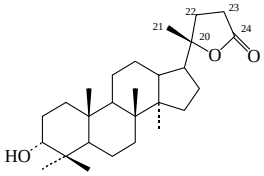


5 **R** = Ac
6 **R** = H



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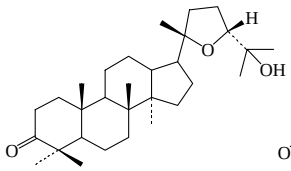

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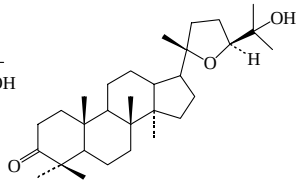

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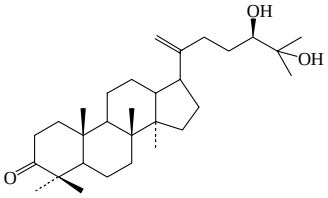

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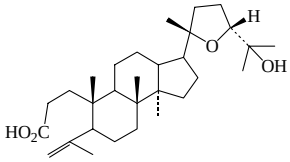

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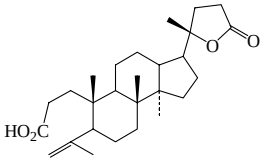


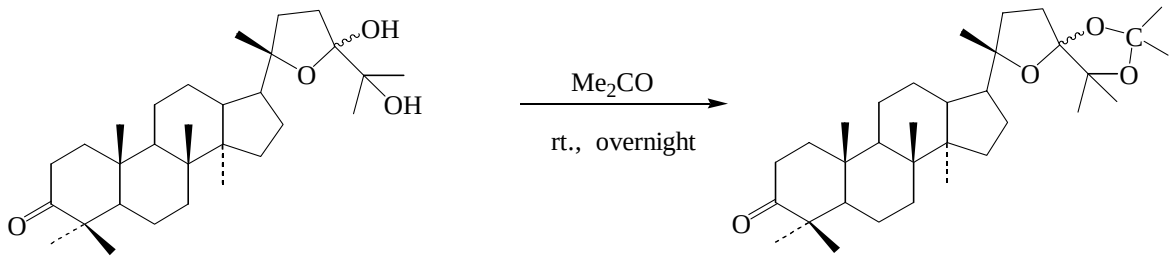
12

The concentrated residue of EtOH extract of the bark was suspended in H_2O and extracted with EtOAc. The EtOAc extract was then subjected to CC on silica gel to give 12 compounds. All 12 compounds had very similar ^1H and ^{13}C NMR spectra, which suggests that they constituted a series of structurally similar ocotillone-type triterpenoids, and possessed a 20*S* configuration as determined by comparing the chemical shift of C-21 with those of related compounds. C-21 resonated at about *ca.* δ_C 24 in 20*S*, and at about *ca.* δ_C 20 in 20*R*.[5, 8, 9]

The molecular formula of compound (**1**) was assigned to be $C_{30}H_{50}O_4$ based on its negative-ion HRFABMS. Its IR spectrum showed hydroxyl (3450 cm^{-1}) and carbonyl group (1705 cm^{-1}) absorption bands. Inspection of the ^1H and ^{13}C NMR spectra revealed the presence of eight tertiary methyl groups (δ_H 0.85, 0.91, 0.98, 1.00, 1.05, 1.09, 1.21, 1.23), 10 methylene groups, four characteristic methine groups [δ_C 55.3 (C-5), 50.4 (C-9), 50.2 (C-17) and 43.4 (C-13)], six quaternary carbons (δ_C 88.7, 74.2, 50.2, 47.2, 40.2, 36.8) and a ketonic carbonyl (δ_C 217.7). These data suggested the presence of a damaran-3-one skeleton.[5] The ^{13}C NMR spectrum of compound (**1**) was very similar to that of ocotillone.[5] Further comparison of the ^1H and ^{13}C NMR spectra of these two compounds revealed that **1** did not show δ_C 86.4 (*d*, C-24) or the corresponding proton δ_H 3.64 (1H, m) observed for ocotillone. Instead, a hemiacetal

carbon δ_C 108.5 (s) was evident in the ^{13}C NMR spectrum of **1**, which was consistent with **1** having another oxygen atom. This observation suggested that δ_C 108.5 (s) was a hemiacetal carbon that could be attributed to C-24. This assumption was supported by an HMBC experiment, with correlations between δ_H 1.23 and 1.21 (each 3H, s, H-26, H-27) and the carbons at δ_C 108.5 (C-24), and 74.2 (C-25).

Compound (**1**) was not stable in acetone solution. Condensation occurred overnight even at room temperature (Scheme 1). Isolation of the condensation product 24,25-isopropylidene derivative of compound (**1**) further supported the structure for **1**.



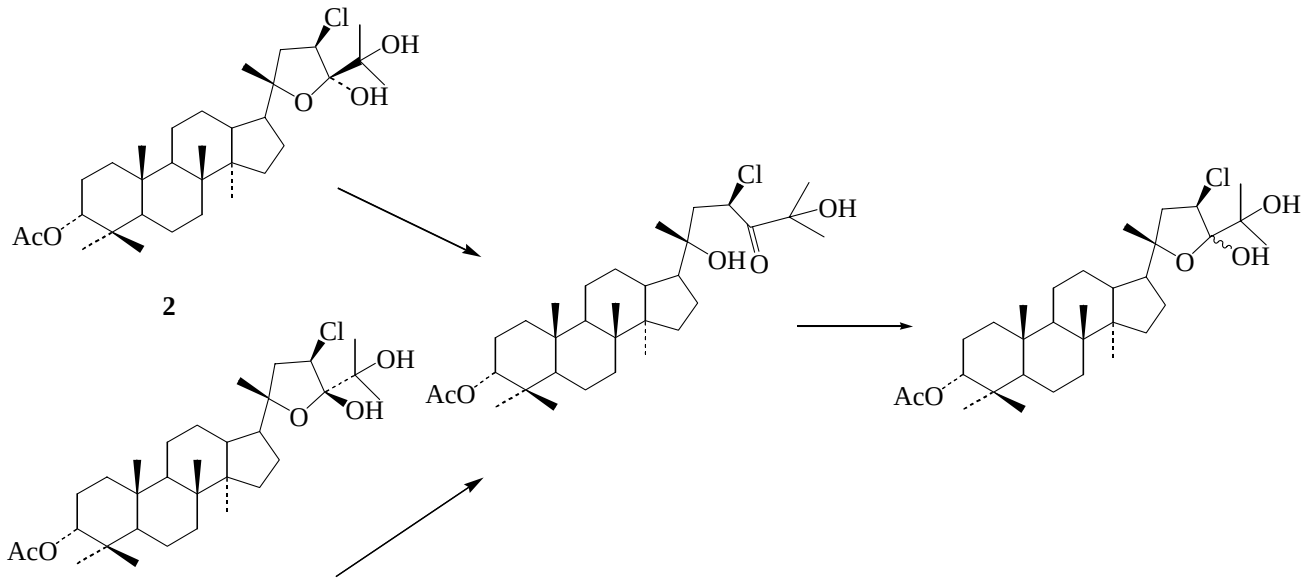
1 **1a**

Scheme 1

Compound (**2**) had a molecular formula of $C_{32}H_{53}O_5Cl$, as indicated by negative-ion HRFABMS. It contained a chlorine atom based on δ_C 56.2 (*d*) and the corresponding proton δ_H 4.44 in the ^1H and ^{13}C NMR spectra.[10] In the EIMS spectrum, the fragment ion peak at *m/z* 516 also suggested the loss of HCl from the molecular ion peak at *m/z* 552. The IR spectrum showed absorption bands for hydroxyl (3522 cm^{-1}) and carbonyl groups (1732 cm^{-1}). The ^1H and ^{13}C NMR spectra also exhibited signals due to eight tertiary methyl groups, nine methylene groups, six methine groups, one of which was oxygenated, four quaternary carbons, two hydroxylated tertiary carbons, a hemiacetal carbon and an acetate group. These data suggested that **2** was a triterpenoid with a skeleton similar to **1**. The signal δ_H 4.59 was assigned to the proton adjacent to the carbon (C-3) bearing an acetate based on its HMBC spectrum, in which δ_H 0.85 and 0.80 (each 3H, s, H-28, H-29) showed cross peaks to δ_C 78.4 (*d*, C-3), whereas δ_H 4.59 (H-3) displayed a cross peak to δ_C 170.7. Small coupling constants (*t*, *J* = 2.6 Hz) for H-3 due to *ee* and *ea* couplings suggested an α substituted acetoxyl group at C-3. The side chain was elucidated from an analysis of the HMBC and NOESY spectra of **2**. δ_H 1.31 and 1.29 (each 3H, s, H-27, H-26), which showed cross peaks to the hemiacetal carbon δ_C 104.8 (C-24) and to the hydroxylated tertiary carbon δ_C 73.9 (C-25), respectively, indicated a 2-hydroxyisopropyl group attached to the hemiacetal carbon (C-24), as in compound (**1**). The correlations from δ_H 4.44 (1H, *t*, *J* = 9.5 Hz, H-23) to δ_C 104.8 (C-24), H-23 to δ_C 73.9 (C-25), H-23 to δ_C 45.2 (C-22), δ_H 2.27 (2H, *d*, *J* = 9.5 Hz, H-22) to δ_C 85.0 (C-20), and H-22 to

δ_C 51.1 (C-17) placed the chlorine atom at C-23. The stereochemistry at C-24 was determined to be 24*R* by the ROESY spectrum, in which NOE interaction was observed between H-21 and both H-26 and 27. The absence of NOE interaction between H-23 and H-21, 26 and 27 strongly supported a 23*R* configuration. Thus, compound (**2**) was elucidated to be 20*S*,23*R*,24*R*-23-chloro-20,24-epoxy-dammarane-3α,24,25-triol 3-acetate.

Compound (**3**) was also shown to have a molecular formula of $C_{32}H_{53}O_5Cl$ by negative-ion HRFABMS. The ^1H and ^{13}C NMR spectra of compounds (**2**) and (**3**) were almost identical. An HMBC experiment again indicated that the chlorine atom was attached at C-23. The ROESY spectrum suggested that compound (**3**) was a 24-epimer of **2**. In addition, compounds (**2**) and (**3**) were not stable, and both were liable to lose stereospecificity at C-24, which was attributed to the unstable hemiacetal carbon at the side chain and the formation of a pair of 24-epimers (Scheme 2).



Scheme 2

Compound (**4**), based on its negative-ion HRFABMS, together with its ^{13}C NMR and DEPT spectra, was assigned a molecular formula of $C_{30}H_{50}O_5$. The ^1H and ^{13}C NMR showed six shielded tertiary methyls and an olefinic methyl (δ_H 1.78), four methines, three quaternary carbons, two hydroxylated tertiary carbons, two olefinic carbons, one of which was disubstituted, and one carboxyl group. In an HMBC experiment, olefinic protons (δ_H 4.95, 4.87) showing cross peaks to δ_C 23.7 (*q*, C-29), revealed cleavage of the A ring. These data were very similar to those of shoreic acid and eichlerianic acid.[5] Compound (**4**) was suggested to be a 3,4-secodammarane with a double bond between C-4 and C-28. The molecular formula of **4** has one more oxygen atom than that of shoreic acid. A detailed comparison of the ^{13}C NMR spectral data of

the two compounds revealed a hemiacetal carbon δ_C 109.5 in **4**, in contrast to δ_C 86.1 (*d*) in shoreic acid. These finding supported a hydroxyl attached to C-24, causing the hemiacetal carbon in **4**. This assumption was supported by cross signals between δ_H 1.61 and 1.56 (each 3H, s, H-26, 27) and δ_C 109.5 (C-24), and between δ_H 1.61 and δ_C 73.6 (C-25) in the HMBC spectrum. Thus, compound (**4**) was elucidated to be 20*S*, 24-epoxy-24,25-dihydroxy-3,4-secodammar-4(28)-en-3-oic acid. Like compound (**1**), **4** also condensed in acetone solution at room temperature overnight, which supported a 24,25-dihydroxyl structure moiety.

Table 1. ^{13}C NMR Spectral Data for Compounds (**1-4**)*

C	1	2	3	4	C	1	2	3	4
1	39.9 t	35.1 t	35.0 t	25.1 t	17	50.2 d	51.1 d	49.8 d	51.6 d
2	34.6 t	21.2 t	21.1 t	35.6 t	18	16.1 q	16.5 q	16.0 q	16.5 q
3	217.7 s	78.4 d	78.3 d	176.5 s	19	15.2 q	15.5 q	15.5 q	20.6 q
4	47.2 s	37.2 s	37.2 s	148.1 s	20	88.7 s	85.0 s	85.3 s	87.8 s
5	55.3 d	50.5 d	50.3 d	41.3 d	21	24.6 q	24.5 q	24.9 q	23.8 q
6	19.7 t	18.1 t	18.0 t	31.7 t	22	36.8 t	45.2 t	46.6 t	36.0 t
7	34.1 t	34.3 t	34.2 t	34.3 t	23	34.6 t	56.2 d	57.5 d	32.8 t
8	40.2 s	40.6 s	40.6 s	40.3 s	24	108.5 s	104.8 s	104.9 s	109.5 s
9	50.4 d	50.8 d	50.7 d	50.6 d	25	74.2 s	73.9 s	74.3 s	73.6 s
10	36.8 s	36.8 s	36.8 s	40.3 s	26	24.5 q	24.5 q	24.6 q	25.6 q
11	22.0 t	22.9 t	22.9 t	22.4 t	27	24.1 q	24.1 q	24.0 q	25.6 q
12	25.9 t	25.5 t	26.8 t	26.0 t	28	27.3 q	27.9 q	27.9 q	113.7 t
13	43.4 d	43.3 d	42.8 d	43.7 d	29	21.0 q	21.7 q	21.1 q	23.7 q
14	50.2 s	50.1 s	50.3 s	50.6 s	30	16.1 q	16.0 q	16.0 q	15.6 q
15	31.5 t	31.4 t	30.9 t	29.6 t	OAc		170.7 s	170.9 s	
16	26.7 t	26.9 t	26.8 t	27.3 t			21.3 q	21.3 q	

* Compounds (**1**) and (**3**) were measured on a Bruker AM-400, and **2**, **4** on a DRX-500 spectrometer with TMS as internal standard; **1**-**3** were measured in $CDCl_3$, while **4** in pyridine-d_5; chemical shifts are in ppm.

Eight known dammaranes, 3-acetylcabraleadiol (**5**),[3] cabraleadiol (**6**),[3] cabrelea-hydroxylactone (**7**),[4] ocotillone (**8**),[5] cabraleone (**9**),[5] 24,25-dihydroxy-dammar-20-en-3-one (**10**),[6] shoreic acid (**11**),[5] and 20*S*,24-epoxy-25,26,27-trisnor-24-oxo-3,4-seco-dammar-4(28)-en-3-oic acid (**12**),[7] were identified by direct comparison of their spectral data with those of authentic samples.

EXPERIMENTAL

General Experimental Product -- Melting points were obtained on an XRC-1 micromelting apparatus and are uncorrected. Optical rotations were taken with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR spectra (KBr) were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H, ^{13}C NMR and 2D-NMR

spectra were recorded on a Bruker AM-400 and a DRX-500 NMR spectrometer with TMS as internal standard. MS data were obtained on a VG Autospec-3000 spectrometer, at 70 eV for EI. Si gel (200-300 mesh) for column chromatography and GF_{254} for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China.

Plant material -- The bark of *A. yunnanensis* was obtained from Xishuangbanna, Yunnan Province, P. R. China, in December 1996. It was identified by Prof. Tao, G-D., Xishuangbanna Botanical Garden, The Chinese Academy of Sciences. A voucher specimen (No. 39795) was deposited in the herbarium of the Department of Taxonomy. Kunming Institute of Botany, The Chinese Academy of Sciences, Kunming, P. R. China.

Extraction and isolation -- The air-dried and powdered bark (4.1 kg) of *A. yunnanensis* was extracted with EtOH (10 L) three times under reflux (each process lasting three hours), and the solvent was removed *in vacuo*. The residue (580 g) was suspended in H_2O and extracted with EtOAc. The EtOAc part was concentrated *in vacuo* to obtain 56 g of residue. The residue was fractionated on silica gel with $CHCl_3$-Me_2CO (9:1-2:1) as an eluent to give 10 fractions (A-J) under TLC monitoring. Fraction B (5.3 g) was further purified on silica gel CC using petroleum ether-acetone (8:1) as an eluent to yield **5** (3.25 g) and **8** (180 mg). Fraction C (3.5 g) was subjected to column chromatography (CC) on silica gel eluted with petroleum ether-EtOAc (3:1) as an eluent to give **9** (170 mg), **7** (168 mg) and **6** (232 mg). Fraction D (1.9 g) was subjected to CC on silica gel with petroleum ether-EtOAc (2:1) as an eluent under TLC monitoring to give two main parts, which were purified on silica gel with $CHCl_3$-EtOAc (5:1), to give **1** (132 mg), **2** (10 mg) and **3** (19 mg). Fraction E (0.7 g) was purified on silica gel with $CHCl_3$-EtOAc (3:1) as an eluent to give **10** (8 mg). Fraction G (2.8 g) was subjected to CC on silica gel and eluted repeatedly with $CHCl_3$-acetone (5:1) under TLC monitoring to give three main subfractions, which were purified on silica gel using petroleum ether-EtOAc (2:3) as an eluent. Finally, recrystallization from acetone gave **4** (62 mg), **11** (1.62 g) and **12** (28 mg).

20*S*,24-Epoxy-24,25-dihydroxydammar-3-one (**1**) Colorless needles (Me_2CO); mp 80-82 °C; $[\alpha]_D^{29}$ + 47.1° ($CHCl_3$; *c* 0.35); EIMS *m/z* (70eV): 474 $[M]^+$ (1), 456 $[M\text{-}H_2O]^+$ (20), 441 (3), 429 (73), 391 (10), 370 (8), 313 (15), 237 (8), 205 (18), 173 (73), 155 (25), 135 (30), 123 (41), 109 (35), 81 (67), 59 (100); negative ion HRFABMS *m/z* found: 473.3562 $[M\text{-}H]^-$ (requires: $C_{30}H_{49}O_4$, 473.3631). IR (KBr) ν_{max} cm^{-1}: 3450, 2970, 2950, 1705, 1461, 1424, 1384, 1313, 1247, 1222, 1160, 1098, 1075, 1047, 1010, 989, 960, 931, 902, 882; ^1H NMR (500 MHz, $CDCl_3$): δ 1.23 (3H, s, H-27), 1.21 (3H, s, H-26), 1.09 (3H, s, H-21), 1.05 (3H, s, H-19), 1.00 (3H, s, H-30), 0.98 (3H, s, H-18), 0.91 (3H, s, H-29), 0.85 (3H, s, H-28); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

20*S*,23*R*,24*R*-23-Chloro-20,24-epoxy-dammarane-3α,24,25-triol 3-acetate (**2**) Colorless needles (Me_2CO);

mp 170-172 °C; $[\alpha]_D^{28}$ + 23.1° ($CHCl_3$; *c* 0.26); EIMS *m/z* (70eV): 552 $[M]^+$ (1), 534 (8), 516 (2), 498 (5), 458 (10), 433 (28), 300 (22), 285 (8), 193 (50), 175 (37), 159 (25), 149 (32), 135 (37), 119 (98), 101 (55), 83 (66), 69 (50), 59 (100); negative ion HRFABMS *m/z* found 551.3431 $[M-H]^-$ (requires: $C_{32}H_{53}O_5Cl$, 551.3503). IR (KBr) ν_{max} cm^{-1}: 3522, 2947, 2874, 1732, 1464, 1376, 1313, 1248, 1182, 1149, 1059, 1037, 1020, 987, 967, 874, 824, 732; ^1H NMR (400 MHz, $CDCl_3$): δ 4.59 (1H, t, *J* = 2.6 Hz, H-3), 4.44 (1H, t, *J* = 9.5 Hz, H-23), 2.27 (2H, d, *J* = 9.5 Hz, H-22), 2.06 (3H, s, OAc), 1.31 (3H, s, H-26), 1.29 (3H, s, H-27), 1.13 (3H, s, H-21), 0.94 (3H, s, H-19), 0.88 (3H, s, H-18), 0.85 (3H, s, H-28), 0.82 (3H, s, H-30), 0.80 (3H, s, H-29); ^{13}C NMR (100 MHz, $CDCl_3$): Table 1.

20*S*,23*R*,24*S*-23-Chloro-20,24-epoxy-dammarane-3α,24,25-triol 3-acetate (**3**) Colorless needles (Me_2CO); mp 242-244 °C; $[\alpha]_D^{29}$ + 36.9° ($CHCl_3$; *c* 0.33); EIMS *m/z* (70eV): 552 $[M]^+$ (1), 537 (3), 534 (3), 498 (4), 458 (20), 433 (68), 398 (13), 300 (52), 285 (15), 229 (15), 206 (35), 189 (70), 175 (98), 159 (43), 139 (47), 121 (66), 107 (72), 95 (80), 81 (84), 69 (71), 59 (100); negative ion HRFABMS *m/z* found 551.3442 $[M-H]^-$ (requires: $C_{32}H_{53}O_5Cl$, 551.3503); IR (KBr) ν_{max} cm^{-1}: 3522, 2947, 2874, 1729, 1465, 1376, 1313, 1249, 1182, 1149, 1059, 1037, 1021, 987, 968, 874, 825, 732; ^1H NMR (500 MHz, $CDCl_3$) δ 4.60 (1H, t, *J* = 2.6 Hz, H-3), 4.48 (1H, t, *J* = 9.3 Hz, H-23), 2.45 (1H, dd, *J* = 13.0, 9.3 Hz, H-22a), 2.15(1H, dd, *J* = 13.0, 9.3 Hz, H-22b), 2.07 (3H, s, OAc), 1.34 (3H, s, H-21), 1.31 (3H, s, H-26), 1.27(3H, s, H-27), 0.93 (3H, s, H-19), 0.87 (3H, s, H-18), 0.86 (3H, s, H-28), 0.83 (3H, s, H-30), 0.81 (3H, s, H-29); ^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

20*S*,24-Epoxy-24,25-dihydroxy-3,4-secodammar-4(28)-en-3-oic acid (**4**) Crystalline solids (Me_2CO); mp 148-150 °C; $[\alpha]_D^{28}$ + 25.6° (CH_3OH; *c* 0.23); EIMS *m/z* (70eV): 472 $[M-H_2O]^+$ (46), 454 (5), 431 (12), 414 (18), 397 (15), 329 (40), 315 (15), 235 (22), 189 (15), 175 (26), 161 (37), 141 (81), 123 (60), 107 (11), 95 (78), 81 (88), 69 (71), 59 (100); negative ion HRFABMS *m/z* found: 489.3600 $[M-H]^-$ (requires: $C_{30}H_{49}O_4$, 489.3580). IR (KBr) ν_{max} cm^{-1}: 3387, 3079, 2971, 2873, 1711, 1638, 1458, 1421, 1384, 1311, 1284, 1227, 1209, 1181, 1162, 1053, 959, 930, 778; ^1H NMR (400 MHz, pyridine-d_5) δ 4.95, 4.87 (each 1H, s, H-28), 1.78 (3H, s, H-29), 1.61 (3H, s, H-27), 1.56 (3H, s, H-26), 1.22 (3H, s, H-21), 0.96 (3H, s, H-30), 0.84 (6H, s, H-18, H-19); ^{13}C NMR (100 MHz, pyridine-d_5): Table 1.

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