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Chemical Evaluation of *Betula* Species in Japan. III. Constituents of *Betula maximowicziana*

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The constituents of *Betula maximowicziana* REGAL in Japan were identified as follows. Fresh leaves: 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane, 20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane, 3-*epi*-ocotillol II, 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,25-dihydroxydammarane, 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane 3-*O*-β-D-glucopyranoside (betulamaximoside A)*, 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane 3-*O*-β-D-(6-*O*-acetyl)-glucopyranoside (betulamaximoside B)*,12-*O*-acetylbetulafolienetetraol, betulafolienetetraol, 6-methoxykaempferol, 6-methoxy-3-*O*-methylkaempferol and naringenin. Outer bark: betulin, betulin 3-*O*-caffeate, lupeol, lupane-3β,20,28-triol, lupane-3β,20,28-triol 3-*O*-caffeate, acetyloleanolic acid. Inner bark: acerogenin E, 16-hydroxy-17-*O*-methylacerogenin E*, alnusdiol β-D-glucopyranoside*, lyoniresinol 3α-*O*-α-L-rhamnopyranoside, 3,4,5-trimethoxyphenol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, benzyl alcohol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, (+)-catechin 7-*O*-β-D-xylopyranoside and monogynol A. Root bark: dammarenediol II 3-*O*-caffeate, dammar-24-ene-3β,20(*S*),26-triol 3-*O*-caffeate*, dammar-24-ene-3β,20(*S*),26-triol 3-*O*-*p*-coumarate*, oleanolic acid 3-*O*-caffeate. The six compounds with asterisks are new.

Key words *Betula maximowicziana*; dammarane glucoside; triterpene; caffeoyl ester; diarylheptanoid; lignan

The genus *Betula* (*B.*) in Japan contains 11 species, of which *B. platyphylla* var. *japonica*,[1] *B. ermanii*[2] and *B. maximowicziana* have white bark. The constituents of the former two have been investigated in our earlier studies. As the third object of the series, *B. maximowicziana* REGAL was selected, though the constituents of the outer bark of the same species in Russia had been reported.[3] In this paper, we describe the detailed chemistry of *B. maximowicziana*.

Constituents of Fresh Leaves From the MeOH extract of fresh leaves collected in June, two new dammarane-type glycosides, **5** and **6**, were isolated, together with 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane (**1**),[4] 20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane (**2**),[4] 3-*epi*-ocotillol II (**3**),[4] 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,25-dihydroxydammarane (**4**),[1] 12-*O*-acetylbetulafolienetetraol (**7**),[5] betulafolienetetraol (**8**),[5] 6-methoxykaempferol (**9**),[6] 6-methoxy-3-*O*-methylkaempferol (**10**)[7] and naringenin (**11**).[8]

Compound **5** was given the formula $C_{38}H_{64}O_{11}$ from the high resolution fast atom bombardment MS (HR-FAB-MS). The ^{1}H- and ^{13}C-NMR data for **5** were similar to those of **1**, except for the presence of additional signals of a hexosyl and differences in the chemical shifts around C-3 (Table 1).[9] On alkaline methanolysis, **5** gave a deacetyl compound (**5a**) which was then hydrolyzed with a glycosidase mixture of turbo to get **1a** and D-glucose. Compound **1a** was identified as 20(*S*),24(*R*)-epoxy-3α,12β,17α,25-tetrahydroxydammarane by direct comparison with an authentic sample derived from **1**. Thus, the structure of **5** was determined to be 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane 3-*O*-β-D-glucopyranoside, and it was named betulamaximoside A.

Compound **6** was given the formula $C_{40}H_{66}O_{12}$ from the HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data were in good agreement with those of **5** except for the presence of additional signals of an acetyl group and differences

Table 1. ^{13}C-NMR Data in C_5D_5N

C	1	1a	5	5a	6
1	34.1	34.3	34.1	34.3	34.1
2	26.4	26.5	21.9	21.6	22.3
3	75.3	75.3	82.6	81.9	83.2
4	38.1	38.2	37.5	37.5	37.5
5	49.8	49.9	50.7	50.6	50.8
6	18.6	18.7	18.4	18.4	18.4
7	34.5	34.7	34.2	34.4	34.2
8	40.5	40.9	40.4	40.8	40.4
9	50.3	50.9	50.0	50.9	50.0
10	37.7	37.7	37.4	37.6	37.4
11	29.1	32.2	29.0	32.3	29.0
12	72.1	68.5	72.1	68.3	72.1
13	49.1	52.5	49.0	52.4	49.0
14	52.4	51.5	52.2	51.4	52.2
15	33.4	34.2	33.4	34.1	33.4
16	39.5	39.4	39.5	39.3	39.5
17	84.8	85.2	84.7	85.2	84.7
18	15.8	16.0	15.8	16.0	15.8
19	16.4	16.5	16.4	16.6	16.4
20	91.1	90.3	91.1	90.2	91.1
21	24.0	22.9	23.8	22.8	23.8
22	34.0	34.1	33.9	34.1	33.9
23	27.5	25.9	27.4	25.9	27.4
24	84.3	85.8	84.2	85.7	84.3
25	72.2	70.0	72.1	70.1	72.1
26	27.9	28.5	27.8	28.4	27.8
27	26.8	26.8	26.8	26.8	26.8
28	29.3	29.4	29.5	29.5	29.4
29	22.4	22.5	22.6	22.6	22.7
30	18.3	18.7	18.3	18.8	18.3
G-1			102.8	102.2	103.1
G-2			75.0	75.1	74.8
G-3			78.9	78.8	78.6
G-4			71.7	72.1	71.6
G-5			78.2	78.3	75.0
G-6			63.2	63.2	64.7
Ac	170.1		170.2		170.8
	21.8		21.8		170.2
					21.8
					20.8

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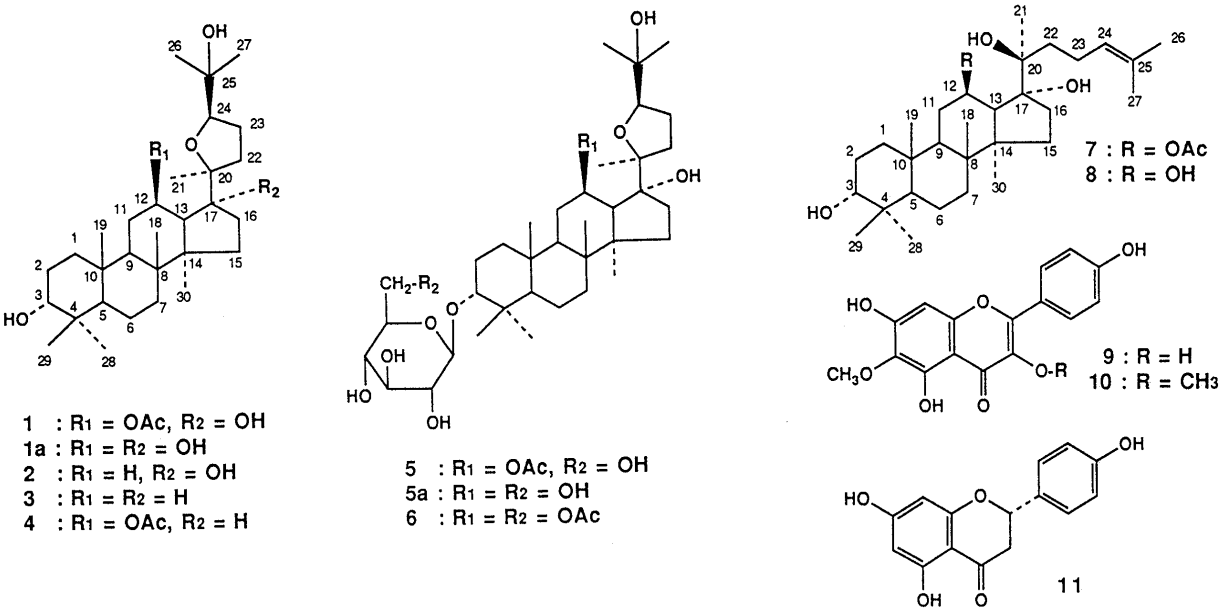


Chart 1. Constituents of Leaves

in the chemical shifts around C-6′ (Table 1). On alkaline methanolysis, **6** gave **5a**. Thus, the structure of **6** was determined to be 12β-acetoxy-20(*S*),24(*R*)-epoxy-3α,17α,25-trihydroxydammarane 3-*O*-β-D-(6-*O*-acetyl)-glucopyranoside, and it was named betulamaximoside B.

Constituents of Outer Bark From the air-dried outer bark collected in June, betulin (**12**),[2] betulin 3-*O*-caffeate (**13**),[2] lupeol (**14**),[2] 3β,20,28-trihydroxylupane (**15**),[3] 3β,20,28-trihydroxylupane 3-*O*-caffeate (**16**)[3] and acetyl oleanolic acid (**17**)[1] were isolated. All the compounds have been reported from the Russian material.[3] The content of betulin is about 5%, which is the same extent as that of *B. ermanii*.

Constituents of Inner Bark From the air-dried inner bark collected in June, two new diarylheptanoids, **19** and **20**, were isolated, together with acerogenin E (**18**),[2] lyoniresinol 3α-*O*-α-L-rhamnopyranoside (**21**),[2] 3,4,5-trimethoxyphenol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**22**),[2] benzyl alcohol β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (**23**),[10] (+)-catechin 7-*O*-β-D-xylopyranoside (**24**)[2] and monogynol A (**25**).[2]

Compound **19** was given the formula $C_{20}H_{22}O_4$ from the HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data for **19** were in good agreement with those of **18** except that one of the phenyl groups was substituted by not only a hydroxyl group but also a methoxyl group. By the ^{1}H–^{1}H and long-range ^{13}C–^{1}H COSY, the structure was determined to be 16-hydroxy-17-*O*-methylacerogenin E (Fig. 1).

Compound **20** was given the formula $C_{25}H_{32}O_9$ from the HR-FAB-MS. The ^{13}C-NMR data for **20** showed the presence of a biphenyl group as **18**, seven sp^3 carbons and a hexosyl group, indicating **20** to be a glycoside of diarylheptanoid. On enzymatic hydrolysis with a glycosidase mixture of turbo, **20** gave D-glucose and an aglycone (**20a**) which was identified as alnusdiol by comparison of the physical properties and spectral data with those previously reported.[11] Thus, the structure of **20** was determined to be alnusdiol β-D-glucopyranoside. The absolute configuration of C-9 and C-11, (*S*,*S*) or (*R*,*R*), which had remained to be confirmed in the previous report, was determined by application of the glycosylation shift

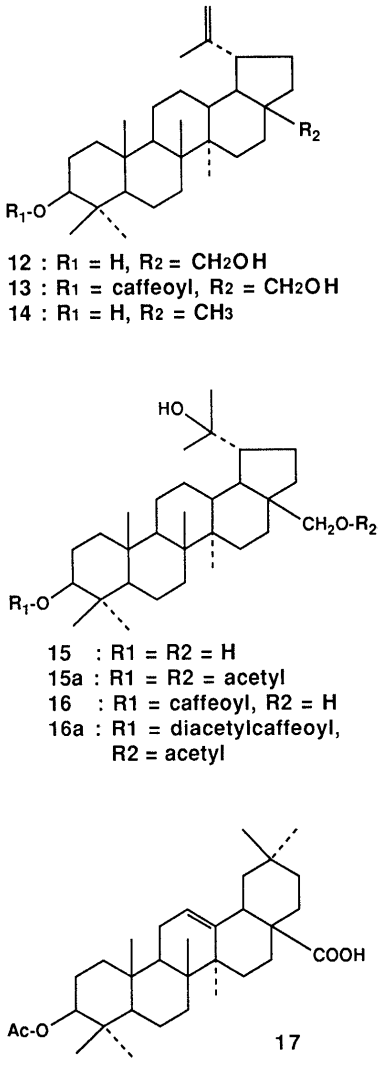


Chart 2. Constituents of Outer Bark

rule in ^{13}C-NMR spectroscopy.[9] When compared with alnusdiol (**20a**), **20** showed β-D-glucosylation shifts of -1.7 ppm for C-8 and -3.6 ppm for C-10, indicating an (*S*)-configuration at C-9.

Constituents of Root Bark From the air-dried root bark collected in June, two new acylated triterpenes, **27** and **28**, were isolated, together with dammarenediol II 3-*O*-caffeate (**26**)[2] and oleanolic acid 3-*O*-caffeate (**29**).[2]

Compound **27** was given a formula with one more oxygen atom than **26**, $C_{39}H_{58}O_6$, from the HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data for **27** were in good agreement with those of **26**, except for the signals around C-25. The differences between their data suggested the presence of a hydroxyl group at C-26. On alkaline methanolysis, **27** gave a triterpene **27a** which was identified as 3β,20(*S*),26-trihydroxydammar-24-ene by comparison of the physical properties and spectral data with those reported.[12] Thus, the structure of **27** was determined to be 3β,20(*S*),26-trihydroxydammar-24-ene 3-*O*-caffeate.

Compound **28** was given the formula $C_{39}H_{58}O_5$, which is one oxygen atom less than that of **27** from the HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data for **28** were in good agreement with those of **27** except for the signals for an acyl group, which were assignable to those of a *p*-coumaroyl group. Thus, the structure of **28** was determined to be 3β,20(*S*),26-trihydroxydammar-24-ene 3-*O*-*p*-coumarate.

In this study, 29 compounds including six new ones, were isolated. Their profiles resemble those of *B. ermanii* and *B. platyphylla* var. *japonica*, but they showed a tendency to have more oxygenated structures, *e.g.*, the 17α-hydroxyl of **1**, **2**, **5**, **6** and **7**, the 6-methoxyl of **9** and **10**, and the 26-hydroxyl of **27** and **28**.

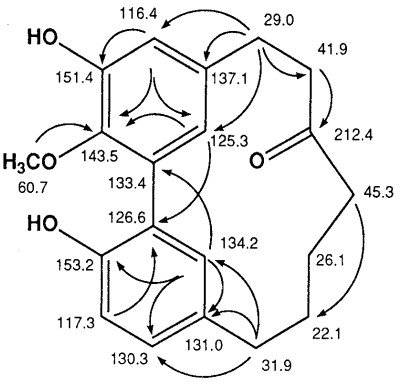


Fig. 1. ^{13}C-NMR Data and Long-Range ^{13}C–^{1}H COSY Connections for **19** in C_5D_5N

Experimental

The instruments, materials and experimental conditions were the same as described in Part 1 of this series.[2]

Isolation Materials of *B. maximowicziana* were collected in Morioka, Iwate Prefecture, in June.

Fresh Leaves: Fresh leaves (2 kg) were extracted with MeOH (30 l) at room temperature for 2 weeks. The extract and 10 l MeOH were passed over a column of activated charcoal (130 g). The resulting solution was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel using $CHCl_3$ and MeOH. The fractions containing **5** and **6** were rechromatographed on Sephadex LH-20 using 95% MeOH to obtain **5** (48 mg) and **6** (33 mg). The fractions containing triterpenes and flavonoids were rechromatographed on Sephadex LH-20 using MeOH and on silica gel using *n*-hexane–EtOAc or $CHCl_3$–MeOH to obtain **1** (54 mg), **2** (11 mg), **3** (14 mg), **4** (4 mg), **7** (10 mg), **8** (29 mg), **9** (60 mg), **10** (60 mg) and **11** (31 mg).

Outer Bark: Air-dried outer bark (368 g) was extracted with MeOH (1 l) under reflux for 6 h. The extract was concentrated to a syrup and chromatographed on silica gel using $CHCl_3$ and EtOAc to obtain **12** (18.6 g), **13** (2.9 g), **14** (3.4 g) and **17** (3.4 g). The fractions containing **15** and **16** were rechromatographed on silica gel using *n*-hexane–EtOAc and $CHCl_3$–MeOH–H_2O–AcOH (360 : 30 : 2 : 1) to obtain **15** (112 mg) and **16** (94 mg).

Inner Bark: Air-dried inner bark (1.6 kg) was extracted with MeOH (5 l) under reflux for 6 h. The extract was concentrated and partitioned between water (2 l) and ether (2 l), and then water (2 l) and *n*-BuOH (2 l). The ether layer was concentrated and chromatographed on silica gel using *n*-hexane–EtOAc and $CHCl_3$–EtOAc to obtain **18** (29 mg), **19** (32 mg) and **25** (201 mg). The *n*-BuOH layer was concentrated and chromatographed on silica gel using $CHCl_3$–MeOH, on Sephadex LH-20 using 80% MeOH, and on Chromatorex ODS using CH_3CN–H_2O to obtain **20** (75 mg), **21** (570 mg), **22** (660 mg), **23** (28 mg) and **24** (2.2 g).

Root Bark: Air-dried root bark (350 g) was extracted with MeOH (4 l) under reflux for 6 h. The extract was concentrated and partitioned between $CHCl_3$ (1 l), MeOH (1 l) and water (0.75 l). The lower layer

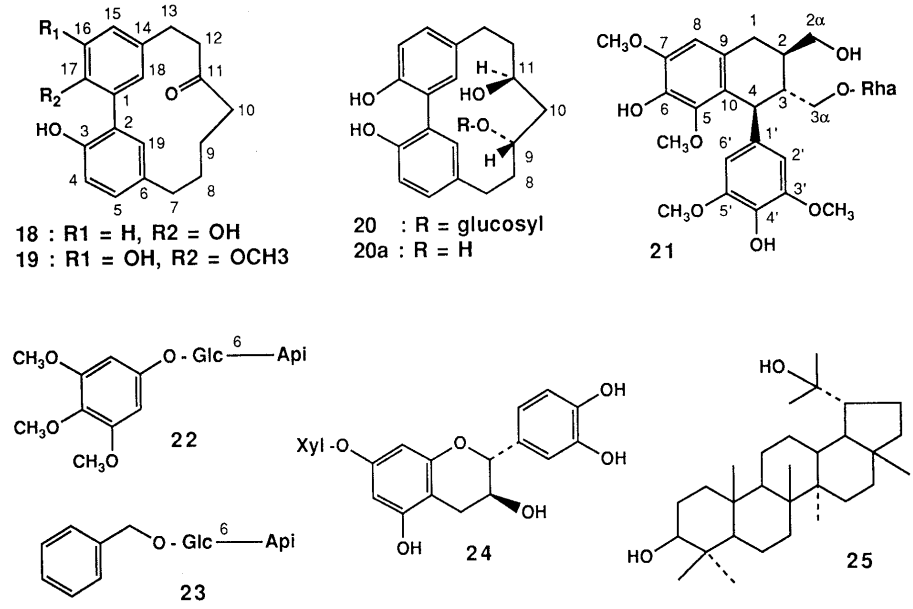
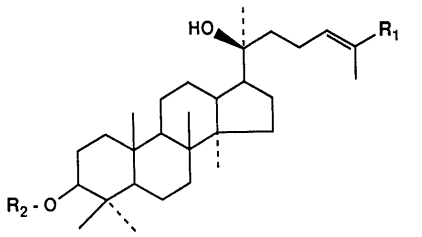


Chart 3. Constituents of Inner Bark



26 : R_1 = CH_3, R_2 = caffeoyl
27 : R_1 = CH_2OH, R_2 = caffeoyl
27a : R_1 = CH_2OH, R_2 = H
28 : R_1 = CH_2OH, R_2 = *p*-coumaroyl

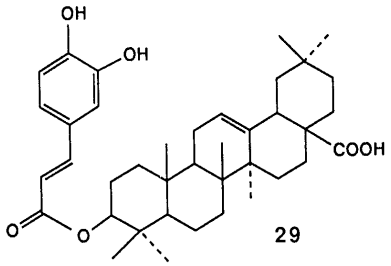


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Chart 4. Constituents of Root Bark

was concentrated and chromatographed on silica gel using $CHCl_3$–MeOH and *n*-hexane–EtOAc to obtain **26** (255 mg), **27** (27 mg), **28** (37 mg) and **29** (413 mg).

Compound 1 [12β-Acetoxy-20(*S*),24(*R*)-epoxy-3α,17,25-trihydroxydammarane] A colorless amorphous powder, $[\alpha]_D$ −9° (*c*=1.0, $CHCl_3$). ^{1}H-NMR (C_5D_5N) δ: 0.91, 0.92, 1.06, 1.20, 1.27, 1.29, 1.42, 1.51, 2.04 (each 3H, s), 3.60 (1H, t, *J*=2.5 Hz), 3.94 (1H, dd, *J*=7.9, 5.5 Hz), 5.50 (1H, td, *J*=11.0, 5.2 Hz). HR-FAB-MS (negative mode) *m/z*: 533.384 $[M-H]^-$. Calcd for $C_{32}H_{53}O_6$: 533.384.

Alkaline Methanolysis of 1 A mixture of **1** (29 mg) and 3% NaOMe in MeOH (10 ml) was refluxed for 2 h. The mixture was diluted with water (100 ml) and extracted with *n*-BuOH (100 ml). The *n*-BuOH solution was washed with water, evaporated, and the residue was chromatographed on silica gel using $CHCl_3$–EtOAc to obtain **1a** (22 mg).

Compound 1a [20(*S*),24(*R*)-Epoxy-3α,12β,17α,25-tetrahydroxydammarane] Colorless needles from acetone, mp 257—258 °C, $[\alpha]_D$ +6° (*c*=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3415, 2940, 1453, 1376, 1146, 1118, 1088, 1055, 1033, 1010, 982. ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.87, 0.94, 0.98, 1.12, 1.18, 1.30, 1.35 (each 3H, s), 3.40 (1H, br s), 3.77 (1H, td, *J*=10.5, 4.6 Hz), 3.84 (1H, dd, *J*=8.9, 5.9 Hz). HR-FAB-MS (negative mode) *m/z*: 491.371 $[M-H]^-$. Calcd for $C_{30}H_{51}O_5$: 491.374.

Compound 2 [20(*S*),24(*R*)-Epoxy-3α,17α,25-trihydroxydammarane] Colorless needles from *n*-hexane–acetone, mp 196—197 °C, $[\alpha]_D$ +8° (*c*=1.0, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3340, 2935, 1452, 1371, 1206, 1145, 1064, 1033, 982, 950, 913, 881. ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.86, 0.94, 0.96, 1.13, 1.16, 1.21, 1.24 (each 3H, s), 3.39 (1H, t, *J*=2.7 Hz), 3.76 (1H, t, *J*=7.3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 33.6 (C-1), 25.3 (C-2), 76.2 (C-3), 37.6 (C-4), 49.5 (C-5), 18.2 (C-6), 34.6 (C-7), 41.1 (C-8), 50.6 (C-9), 37.3 (C-10), 23.3 (C-11), 21.4 (C-12), 45.6 (C-13), 49.8 (C-14), 32.5 (C-15), 36.9 (C-16), 83.9 (C-17), 15.6 (C-18), 16.0 (C-19), 90.1 (C-20), 22.1 (C-21), 33.0 (C-22), 26.4 (C-23), 83.5 (C-24), 71.8 (C-25), 27.4 (C-26), 25.0 (C-27), 28.3 (C-28), 22.0 (C-29), 17.2 (C-30). HR-EI-MS *m/z*: 476.386 $[M]^+$. Calcd for $C_{30}H_{52}O_4$: 476.386.

Compound 3 (3-*epi*-Ocotillol II) Colorless needles from acetone, mp 167—168 °C, $[\alpha]_D$ +18° (*c*=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3425, 2940, 1643, 1616, 1447, 1371, 1322, 1293, 1249, 1127, 1065, 1031, 982. ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.85, 0.89, 0.94, 0.96, 1.12, 1.13, 1.21 (each 3H, s), 3.39 (1H, t, *J*=2.6 Hz), 3.73 (1H, t, *J*=7.3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 33.6 (C-1), 25.4 (C-2), 76.3 (C-3), 37.6 (C-4), 49.5 (C-5), 18.2 (C-6), 35.2 (C-7), 40.6 (C-8), 50.6 (C-9), 37.3 (C-10), 21.4 (C-11), 27.3 (C-12), 42.9 (C-13), 50.1 (C-14), 31.4 (C-15), 25.7 (C-16), 49.5 (C-17), 16.0 (C-18), 15.4 (C-19), 86.4 (C-20), 23.5 (C-21), 35.6 (C-22), 26.1 (C-23), 83.3 (C-24), 71.4 (C-25), 27.4 (C-26), 24.2 (C-27), 28.3 (C-28), 22.1 (C-29), 16.5 (C-30). HR-FAB-MS (negative mode) *m/z*: 459.384 $[M-H]^-$. Calcd for $C_{30}H_{51}O_3$: 459.384.

Compound 5 (Betulamaximoside A) A colorless amorphous powder, $[\alpha]_D$ −19° (*c*=1.0, MeOH). ^{1}H-NMR (C_5D_5N) δ: 0.86, 0.87, 1.01, 1.03, 1.24, 1.29, 1.39, 1.50, 2.04 (each 3H, s), 3.63 (1H, br s), 3.93 (1H, dd, *J*=8.1, 5.8 Hz), 4.82 (1H, d, *J*=7.6 Hz). HR-FAB-MS (negative mode) *m/z*: 695.438 $[M-H]^-$. Calcd for $C_{38}H_{63}O_{11}$: 695.437.

Alkaline Methanolysis of 5 Compound **5** (45 mg) was subjected to alkaline methanolysis in the same manner as **1** to obtain **5a** (40 mg).

Compound 5a [20(*S*),24(*R*)-Epoxy-3α,12β,17α,25-tetrahydroxydammarane 3-*O*-β-D-Glucopyranoside] A colorless amorphous powder, $[\alpha]_D$ −2° (*c*=1.0, MeOH). ^{1}H-NMR (C_5D_5N) δ: 0.83, 0.85, 1.07 (each 3H, s), 1.22 (6H, br s), 1.41 (6H, br s), 1.54 (3H, s), 3.46 (1H, dt, *J*=9.2, 6.3 Hz), 3.66 (1H, br s), 4.83 (1H, d, *J*=7.6 Hz).

Enzymatic Hydrolysis of 5a A solution of **5a** (40 mg) and a glycosidase mixture of turbo (100 mg, Seikagaku Kogyo Co., Ltd.) in 0.05 M citrate buffer (pH 4.0, 20 ml) was stirred at 40 °C for 17 h. The reaction mixture was extracted with EtOAc (100 ml). The EtOAc solution was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel using $CHCl_3$–MeOH to obtain **1a** (16 mg). The buffer solution was neutralized with 5% Na_2CO_3 solution and evaporated. The residue was chromatographed on silica gel using 20% MeOH in $CHCl_3$ to obtain D-glucose (1.4 mg), $[\alpha]_D$ +40° (*c*=0.14, MeOH). Its trimethylsilyl ether was identified by comparison with an authentic sample on GLC.

Compound 6 (Betulamaximoside B) A colorless amorphous powder, $[\alpha]_D$ −22° (*c*=1.0, MeOH). ^{1}H-NMR (C_5D_5N) δ: 0.88, 0.92, 1.01, 1.03, 1.27, 1.29, 1.39, 1.50, 2.02, 2.04 (each 3H, s), 3.58 (1H, br s), 3.93 (1H, dd, *J*=8.1, 5.8 Hz), 3.97 (1H, dd, *J*=8.9, 7.6 Hz), 3.98 (1H, ddd, *J*=8.9, 6.1, 1.8 Hz), 4.04 (1H, t, *J*=8.9 Hz), 4.19 (1H, t, *J*=8.9 Hz), 4.75 (1H, d, *J*=7.6 Hz), 4.81 (1H, dd, *J*=11.6, 6.1 Hz), 4.95 (1H, dd, *J*=11.6, 1.8 Hz), 5.42 (1H, td, *J*=11.0, 5.2 Hz). HR-FAB-MS (negative mode) *m/z*: 737.448 $[M-H]^-$. Calcd for $C_{40}H_{65}O_{12}$: 737.448.

Alkaline Methanolysis of 6 Compound **6** (29 mg) was subjected to alkaline methanolysis in the same manner as **1** to obtain **5a** (21 mg).

Compound 7 [12β-Acetoxy-3α,17α,20(*S*)-trihydroxydammar-24-ene] A colorless amorphous powder, $[\alpha]_D$ −6° (*c*=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.87, 0.95, 1.00, 1.19, 1.23, 1.65, 1.71, 2.04 (each 3H, s), 3.40 (1H, t, *J*=2.3 Hz), 4.97 (1H, td, *J*=10.9, 5.3 Hz), 5.14 (1H, t, *J*=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 33.5 (C-1), 25.3 (C-2), 76.0 (C-3), 37.6 (C-4), 49.4 (C-5), 18.2 (C-6), 33.9 (C-7), 40.8 (C-8), 49.7 (C-9), 37.3 (C-10), 28.2 (C-11), 73.0 (C-12), 48.1 (C-13), 52.2 (C-14), 32.1 (C-15), 36.6 (C-16), 85.2 (C-17), 15.8 (C-18), 16.0 (C-19), 77.8 (C-20), 20.5 (C-21), 37.7 (C-22), 22.5 (C-23), 124.7 (C-24), 131.8 (C-25), 25.7 (C-26), 17.7 (C-27), 28.3 (C-28), 22.0 (C-29), 17.3 (C-30), 170.0 ($CH_3\underline{C}O$), 21.6 ($\underline{C}H_3CO$). FAB-MS *m/z*: 518 $[M]^+$.

Compound 8 [3α,17α,20(*S*)-Trihydroxydammar-24-ene] Colorless needles from *n*-hexane, mp 129—130 °C, $[\alpha]_D$ +12° (*c*=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3445, 2935, 1448, 1370, 1058. ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.86, 0.94, 0.96, 1.15, 1.18, 1.64, 1.70 (each 3H, s), 3.40 (1H, t, *J*=2.4 Hz), 5.12 (1H, t, *J*=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 33.6 (C-1), 25.3 (C-2), 76.3 (C-3), 37.6 (C-4), 49.5 (C-5), 18.2 (C-6), 34.6 (C-7), 41.2 (C-8), 50.6 (C-9), 37.3 (C-10), 23.2 (C-11), 21.3 (C-12), 45.0 (C-13), 49.9 (C-14), 32.3 (C-15), 36.0 (C-16), 85.0 (C-17), 15.8 (C-18), 16.1 (C-19), 78.9 (C-20), 21.2 (C-21), 36.5 (C-22), 22.6 (C-23), 124.8 (C-24), 131.9 (C-25), 25.8 (C-26), 17.8 (C-27), 28.3 (C-28), 22.1 (C-29), 17.2 (C-30). HR-EI-MS *m/z*: 460.389. Calcd for $C_{30}H_{52}O_3$: 460.391.

Compound 9 (6-Methoxykaempferol) Yellow needles from MeOH, mp 268—270 °C. IR ν_{max}^{KBr} cm^{-1}: 3340, 3145, 1645, 1620, 1591, 1561, 1500, 1374, 1299, 1273, 1197, 1175, 1029, 927, 844, 803. UV λ_{max}^{MeOH} nm (log ε): 367 (4.15), 269 (4.36). ^{1}H-NMR (DMSO-d_6) δ: 3.77 (3H, s), 6.56 (1H, s), 6.94 (2H, d, *J*=8.9 Hz), 8.05 (2H, d, *J*=8.9 Hz). ^{13}C-NMR (DMSO-d_6) δ: 135.3 (C-2), 146.9 (C-3), 176.0 (C-4), 151.6 (C-5), 130.7 (C-6), 157.1 (C-7), 93.7 (C-8), 151.3 (C-9), 103.4 (C-10), 121.6 (C-1′), 115.3 (C-2′, 6′), 129.4 (C-3′, 5′), 159.1 (C-4′), 59.9 (CH_3O). HR-FAB-MS *m/z*: 317 $[M+H]^+$.

Compound 10 (6-Methoxy-3-*O*-methylkaempferol) Yellow needles from MeOH, mp 219—220 °C. IR ν_{max}^{KBr} cm^{-1}: 3270, 2815, 1645, 1601, 1541, 1468, 1429, 1353, 1274, 1224, 1167, 1085, 1033, 982. UV λ_{max}^{MeOH} nm (log ε): 340 (4.40), 270 (4.18). ^{1}H-NMR (DMSO-d_6) δ: 3.76 (3H, s), 3.79 (3H, s), 6.55 (1H, s), 6.95 (2H, d, *J*=8.9 Hz), 7.93 (2H, d, *J*=8.9 Hz). ^{13}C-NMR (DMSO-d_6) δ: 137.2 (C-2), 155.6 (C-3), 178.1 (C-4), 152.3 (C-5), 131.1 (C-6), 147.3 (C-7), 93.9 (C-8), 151.9 (C-9), 104.5 (C-10), 120.6 (C-1′), 115.5 (C-2′, 6′), 130.2 (C-3′, 5′), 160.1 (C-4), 59.9, 59.6 (CH_3O). FAB-MS *m/z*: 331 $[M+H]^+$.

Compound 15 (Lupane-3β,20,28-triol) A colorless crystalline powder

from $CHCl_3$–MeOH, mp 272—273 °C, $[\alpha]_D$ −6.0° (c=0.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3340, 2930, 1450, 1360, 1170, 1095, 1030. ^{1}H-NMR (C_5D_5N) δ: 0.87, 1.03, 1.06, 1.13, 1.22, 1.36, 1.45 (each 3H, s), 3.44 (1H, dd, J=8.6, 7.6 Hz), 3.68 (1H, d, J=10.6 Hz), 4.19 (1H, d, J=10.6 Hz). ^{13}C-NMR ($CDCl_3$) δ: 38.7 (C-1), 27.4 (C-2), 78.9 (C-3), 38.8 (C-4), 55.2 (C-5), 18.3 (C-6), 34.5 (C-7), 41.4 (C-8), 50.2 (C-9), 37.0 (C-10), 21.3 (C-11), 29.0 (C-12), 36.2 (C-13), 43.4 (C-14), 27.2 (C-15), 29.7 (C-16), 49.2 (C-17), 48.7 (C-18), 49.7 (C-19), 73.5 (C-20), 28.3 (C-21), 33.4 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.1 (C-26), 15.0 (C-27), 60.8 (C-28), 24.6 (C-29), 31.6 (C-30). EI-MS m/z: 460 $[M]^+$, 442 $[M^+ - H_2O]$, 411, 288, 234, 207, 189, 135. Acetylation (Ac_2O–pyridine) yielded **15a**: colorless leaves from acetonitrile, $[\alpha]_D$ *ca.* 0° (c=0.1, $CHCl_3$), mp 258—260 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.84, 0.85, 0.86, 0.98, 1.07, 1.13, 1.24, 2.04, 2.06 (each 3H, s), 3.83 (1H, d, J=11.2 Hz), 4.33 (1H, d, J=11.2 Hz), 4.47 (1H, dd, J=8.6, 7.3 Hz). ^{13}C-NMR ($CDCl_3$) δ: 38.3 (C-1), 23.7 (C-2), 80.9 (C-3), 37.8 (C-4), 55.2 (C-5), 18.2 (C-6), 34.4 (C-7), 41.4 (C-8), 50.1 (C-9), 36.9 (C-10), 21.3 (C-11), 28.9 (C-12), 36.6 (C-13), 43.4 (C-14), 27.2 (C-15), 30.3 (C-16), 47.8 (C-17), 48.8 (C-18), 49.6 (C-19), 73.4 (C-20), 28.2 (C-21), 34.0 (C-22), 27.9 (C-23), 16.5 (C-24), 16.1, 16.2 (C-25 and C-26), 15.0 (C-27), 62.9 (C-28), 24.7 (C-29), 31.6 (C-30), 21.0, 21.3 ($\underline{C}H_3CO \times 2$), 171.0, 171.5 ($CH_3\underline{C}O \times 2$).

Compound 16 (Lupane-3β,20,28-triol 3-*O*-Caffeate) Colorless needles from AcOEt–*n*-hexane, mp 241—245 °C, $[\alpha]_D$ +22° (c=0.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1680, 1620, 1590, 1510, 1260, 1170. UV λ_{max}^{MeOH} nm (log ε): 218 (4.21), 236 (4.04), 244 (4.05), 302 (4.17), 329 (4.28). ^{1}H-NMR (C_5D_5N) δ: 0.83, 0.93, 0.95, 1.03, 1.12, 1.34, 1.43 (each 3H, s) 3.63 (1H, d, J=10.6 Hz), 4.14 (1H, d, J=10.6 Hz), 4.81 (1H, dd, J=10.9, 5.0 Hz), 6.60 (1H, d, J=15.8 Hz), 7.18 (2H, s), 7.57 (1H, s), 7.95 (1H, d, J=15.8 Hz). EI-MS m/z: 604 $[M^+ - H_2O]$, 573, 424, 393, 355, 203, 289, 163. Acetylation (Ac_2O–pyridine) yielded **16a**: a colorless amorphous powder, $[\alpha]_D$ +10.2° (c=0.8, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3520, 2930, 1775, 1730, 1700, 1635, 1500, 1370, 1230, 1200, 1170. UV λ_{max}^{THF} nm (log ε): 219 (4.21), 277 (4.24). ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, s), 0.91, 1.00, 1.08, 1.14, 1.24 (each 3H, s), 2.06, 2.297, 2.300 (each 3H, s), 3.84 (1H, d, J=11.2 Hz, 28-H), 4.34 (1H d, J=11.2 Hz, 28-H), 4.61 (1H, dd, J=9.6, 5.6 Hz), 6.38 (1H, d, J=15.8 Hz), 7.21 (1H, d, J=8.3 Hz), 7.36 (1H, d, J=2.0 Hz), 7.40 (1H, dd, J=8.3, 2.0 Hz), 7.59 (1H, d, J=15.8 Hz). ^{13}C-NMR ($CDCl_3$) δ: 38.3 (C-1), 23.7 (C-2), 81.2 (C-3), 38.0 (C-4), 55.2 (C-5), 18.1 (C-6), 33.9 (C-7), 41.4 (C-8), 50.1 (C-9), 36.9 (C-10), 21.2 (C-11), 28.9 (C-12), 36.5 (C-13), 43.3 (C-14), 27.2 (C-15), 30.2 (C-16), 47.8 (C-17), 48.8 (C-18), 49.5 (C-19), 73.3 (C-20), 28.1 (C-21), 34.3 (C-22), 27.9 (C-23), 16.6 (C-24), 16.2 (C-25), 16.1 (C-26), 15.0 (C-27), 62.8 (C-28), 24.7 (C-29), 31.6 (C-30), 133.4 (C-1′), 123.8 (C-2′), 142.2 (C-3′), 143.3 (C-4′), 122.6 (C-5′), 126.3 (C-6′), 120.0 (C-7′), 142.4 (C-8′), 167.9 (C-9′), 20.5, 20.6, 21.0 ($\underline{C}H_3CO$), 166.3, 168.0 ($C\underline{C}H_3\underline{C}O$). EI-MS m/z: 730 $[M^+ - H_2O]$, 646, 466, 205, 163. On alkaline methanolysis with 5% KOH/MeOH, **16** gave **15**.

Compound 19 (16-Hydroxy-17-*O*-methylacerogenin E) Pale brown prisms from MeOH, mp 167—170 °C, $[\alpha]_D$ 0° (c=1.0, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350, 3000, 2930, 1695, 1580, 1495. UV λ_{max}^{MeOH} nm (log ε): 250 (4.02), 298 (3.73). ^{1}H-NMR (270 MHz, C_5D_5N) δ: 1.75—1.95 (4H, m), 2.60—2.70 (4H, m), 2.72—2.90 (2H, m), 3.02—3.10 (2H, m), 3.94 (3H, s), 7.04—7.20 (6H, m). ^{13}C-NMR (C_5D_5N) δ: 133.4 (C-1), 126.6 (C-2), 153.2 (C-3), 117.3 (C-4), 130.3 (C-5), 131.0 (C-6), 31.9 (C-7), 22.1 (C-8), 26.1 (C-9), 45.3 (C-10), 212.4 (C-11), 41.9 (C-12), 29.0 (C-13), 137.1 (C-14), 116.4 (C-15), 151.4 (C-16), 143.5 (C-17), 125.3 (C-18), 60.7 (CH_3O). HR-EI-MS m/z: 326.153 $[M^+]$. Calcd for $C_{20}H_{22}O_4$: 326.152.

Compound 20 (Alnusdiol β-D-Glucopyranoside) A pale brown amorphous powder, $[\alpha]_D$ −26° (c=0.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3360, 2910, 1600, 1505. UV λ_{max}^{MeOH} nm (log ε): 250 (4.05), 254 (4.03), 299 (3.77). ^{1}H-NMR (C_5D_5N) δ: 2.00—2.23 (2H, m), 2.40—2.60 (2H, m), 2.62—3.05 (3H, m), 3.30—3.80 (3H, m), 4.00—4.30 (5H, m), 4.53 (1H, t, J=9.9 Hz), 4.65 (1H, t, J=9.9 Hz), 4.94 (1H, d, J=7.6 Hz), 7.10—7.30 (6H, m). ^{13}C-NMR (CD_3OD : $CDCl_3$ = 5 : 1) δ: 127.0 (C-1), 127.0 (C-2), 152.0 (C-3), 116.8 (C-4), 130.3 (C-5), 132.3 (C-6), 27.0 (C-7), 33.8 (C-8), 71.3 (C-9), 47.8 (C-10), 66.8 (C-11), 35.3 (C-12), 27.3 (C-13), 131.6 (C-14), 130.1 (C-15), 116.7 (C-16), 151.9 (C-17), 134.5 (C-18), 102.0 (Glc-1), 74.5 (Glc-2), 77.7 (Glc-3), 71.4 (Glc-4), 76.7 (Glc-5), 62.6 (Glc-6). HR-FAB-MS (negative mode) m/z: 475.198 $[M-H]^-$. Calcd for $C_{25}H_{31}O_9$: 475.197.

Enzymatic Hydrolysis of 20 A solution of **20** (18 mg) and a glycosidase mixture of turbo (100 mg, Seikagaku Kogyo Co., Ltd.) in 0.05 M citrate buffer (pH 4.0, 5 ml) was stirred at 37 °C for 23 h. The reaction mixture was extracted with EtOAc (100 ml). The EtOAc solution was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The residue was chromatographed on silica gel using $CHCl_3$–MeOH to obtain **20a** (6 mg). The buffer solution was neutralized with 5% Na_2CO_3 solution and evaporated. The residue was chromatographed on silica gel using 20% MeOH in $CHCl_3$ to obtain D-glucose (2 mg), $[\alpha]_D$ +20° (c=0.04, MeOH). Its trimethylsilyl ether was identified by comparison with an authentic sample on GLC. Alnusdiol (**20a**)[11]: colorless needles from AcOEt, mp > 300 °C, $[\alpha]_D$ −46.3° (c=0.26, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3150, 2920, 1570, 1495, 1450, 1430, 1400, 1225. UV λ_{max}^{EtOH} nm (log ε) nm: 248 sh (4.04), 302 (3.95). ^{1}H-NMR (CD_3OD : $CDCl_3$ = 5 : 1) δ: 1.70—1.85 (2H, m), 1.94 (2H, dd, J=7.6, 4.3 Hz), 2.38 (2H, ddd, J=16.2, 11.6, 5.0 Hz), 2.80—3.04 (4H, m), 3.95—4.06 (2H, m), 6.82 (2H, d, J=8.6 Hz), 7.03—7.08 (4H, m). ^{13}C-NMR (CD_3OD : $CDCl_3$ = 5 : 1) δ: 126.9 (C-1, 2), 151.9 (C-3, 17), 116.7 (C-4, 16), 130.2 (C-5, 15), 131.6 (C-6, 14), 27.2 (C-7, 13), 35.5 (C-8, 12), 67.0 (C-9, 11), 51.4 (C-10), 134.6 (C-18, 19). MS m/z: 314 $[M^+]$, 255, 225, 211, 181, 165.

Compound 23 (Benzyl Alcohol β-D-Apiofuranosyl-(1→6)-β-D-glucopyranoside)[10] A colorless amorphous powder, $[\alpha]_D$ −75.2° (c=1.0, MeOH). ^{1}H-NMR (CD_3OD) δ: 3.78 (1H, d, J=9.9 Hz), 3.94 (1H, d, J=2.3 Hz), 4.33 (1H, d, J=7.3 Hz), 4.65 (1H, d, J=11.9 Hz), 4.87 (1H, d, J=11.9 Hz), 5.05 (1H, d, J=2.6 Hz), 7.23—7.50 (5H, m). ^{13}C-NMR (C_5D_5N) δ: 138.8 (C-1), 128.6 (C-2), 128.6 (C-3), 127.8 (C-4), 128.6 (C-5), 128.6 (C-6), 70.9 (C-α), 103.7 (Glc-1), 75.1 (Glc-2), 78.5 (Glc-3), 71.9 (Glc-4), 77.3 (Glc-5), 69.0 (Glc-6), 111.2 (Api-1), 77.9 (Api-2), 80.5 (Api-3), 75.0 (Api-4), 65.6 (Api-5).

Compound 27 [Dammar-24-ene-3β,20(*S*),26-triol 3-*O*-Caffeate] A colorless crystalline powder from $CHCl_3$, mp 215—218 °C, $[\alpha]_D$ +38.0° (c=0.14, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1680, 1625, 1592, 1505, 1260, 1165. UV λ_{max}^{MeOH} (log ε) nm: 221 sh (4.18), 298 sh (4.23), 312 (4.27). ^{1}H-NMR (C_5D_5N) δ: 0.85, 0.98, 0.99, 1.00, 1.02, 1.44, 1.88 (each 3H, s), 4.33 (2H, s), 4.87 (1H, dd, J=11.2, 5.3 Hz), 5.85 (1H, t, J=7.3 Hz), 6.72 (1H, d, J=15.8 Hz), 7.19 (2H, d, J=8.6 Hz), 7.67 (2H, d, J=8.6 Hz), 8.04 (1H, d, J=15.8 Hz). ^{13}C-NMR (C_5D_5N) δ: 38.9 (C-1), 24.3 (C-2), 80.5 (C-3), 38.4 (C-4), 56.2 (C-5), 18.5 (C-6), 35.5 (C-7), 40.7 (C-8), 50.9 (C-9), 37.3 (C-10), 21.9 (C-11), 25.4 (C-12), 42.6 (C-13), 50.7 (C-14), 31.7 (C-15), 28.1 (C-16), 50.4 (C-17), 16.8 (C-18), 15.7 (C-19), 74.1 (C-20), 26.2 (C-21), 41.8 (C-22), 22.9 (C-23), 125.5 (C-24), 136.1 (C-25), 68.2 (C-26), 14.0 (C-27), 28.2 (C-28), 16.4 (C-29), 16.9 (C-30), 126.2 (C-1′), 130.7 (C-2′), 116.9 (C-3′), 161.5 (C-4′), 116.9 (C-5′), 130.7 (C-6′), 145.0 (C-7′), 115.9 (C-8′), 167.3 (C-9′). HR-FAB-MS (negative mode) m/z: 605.419 $[M-H]^-$. Calcd for $C_{39}H_{57}O_5$: 605.421. On alkaline methanolysis with 5% NaOMe, **27** gave dammar-24-ene-3β,20(*S*),26-triol (**27a**) and methyl caffeate. Dammar-24-ene-3β,20(*S*),26-triol (**27a**): colorless needles from MeOH, mp 194—197 °C, $[\alpha]_D$ +20° (c=0.1, MeOH). ^{1}H-NMR (C_5D_5N) δ: 0.89, 0.99, 1.00, 1.07, 1.26, 1.45, 1.88 (each 3H, s), 3.47 (1H, t-like), 4.34 (2H, s), 5.86 (1H, t-like).

Compound 28 [Dammar-24-ene-3β,20(*S*),26-triol 3-*O*-*p*-Coumarate] A colorless crystalline powder from $CHCl_3$, mp 215—218 °C, $[\alpha]_D$ +38.0° (c=0.14, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1680, 1625, 1592, 1505, 1260, 1165. UV λ_{max}^{MeOH} (log ε) nm: 221 sh (4.18), 298 sh (4.23), 312 (4.27). ^{1}H-NMR (C_5D_5N) δ: 0.85, 0.98, 0.99, 1.00, 1.02, 1.44, 1.88 (each 3H, s), 4.33 (2H, s), 4.87 (1H, dd, J=11.2, 5.3 Hz), 5.85 (1H, t, J=7.3 Hz), 6.72 (1H, d, J=15.8 Hz), 7.19 (2H, d, J=8.6 Hz), 7.67 (2H, d, J=8.6 Hz), 8.04 (1H, d, J=15.8 Hz). ^{13}C-NMR (C_5D_5N) δ: 38.9 (C-1), 24.3 (C-2), 80.5 (C-3), 38.4 (C-4), 56.2 (C-5), 18.5 (C-6), 35.5 (C-7), 40.7 (C-8), 50.9 (C-9), 37.3 (C-10), 21.9 (C-11), 25.4 (C-12), 42.6 (C-13), 50.7 (C-14), 31.7 (C-15), 28.1 (C-16), 50.4 (C-17), 16.8 (C-18), 15.7 (C-19), 74.1 (C-20), 26.2 (C-21), 41.8 (C-22), 22.9 (C-23), 125.5 (C-24), 136.1 (C-25), 68.2 (C-26), 14.0 (C-27), 28.2 (C-28), 16.4 (C-29), 16.9 (C-30), 126.2 (C-1′), 130.7 (C-2′), 116.9 (C-3′), 161.5 (C-4′), 116.9 (C-5′), 130.7 (C-6′), 145.0 (C-7′), 115.9 (C-8′), 167.3 (C-9′). HR-FAB-MS (negative mode) m/z: 605.419 $[M-H]^-$. Calcd for $C_{39}H_{57}O_5$: 605.421. On alkaline methanolysis with 5% NaOMe, **28** gave dammar-24-ene-3β,20(*S*),26-triol (**27a**) and methyl *p*-coumarate.

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