Terpenoids from Two *Dicranopteris* Species

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Two new compounds, (6*S*,13*S*)-6-{[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}cleroda-3,14-dien-13-ol (**1**) and kadsuric acid 3-methyl ester (**2**), together with nine known compounds, (6*S*,13*E*)-6-{[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}cleroda-3,13-dien-15-ol (**3**), (6*S*,13*S*)-6-[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}-13-{[α-L-rhamnopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}cleroda-3,14-diene (**4**), (6*S*,13*S*)-6-{[6-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}-13-{[α-L-rhamnopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}cleroda-3,14-diene (**5**), 15-hydroxydehydroabietic acid (**6**), 15-hydroxylabd-8(17)-en-19-oic acid (**7**), junicedric acid (**8**), (4β)-kaur-16-en-18-oic acid (**9**), (4β)-16-hydroxykauran-18-oic acid (**10**), and (4β,16β)-16-hydroxykauran-18-oic acid (**11**) were isolated from the fronds of *Dicranopteris linearis* or *D. ampla*. Their structures were established by extensive 1D- and 2D-NMR spectroscopy. Compounds **1** and **3**–**8** showed no anti-HIV activities.

Introduction. – *Dicranopteris* species (ferns, family Gleicheniaceae) are widely distributed in China. In our previous research on *D. dichotoma*, we have reported the isolation and structural elucidation of two new highly oxygenated phenolic derivatives, dichotomains A and B, and of some tetranorclerodanes and clerodane-type diterpene glycosides, and some of them were tested for their anti-HIV activities [1][2]. To search for bioactive compounds from *Dicranopteris* species, we studied the fronds of *D. linearis* (BURM.) UNDERW and *D. ampla* CHING et CHIU, which led to the isolation of two new compounds, including a clerodane-type diterpene glycoside, (6*S*,13*S*)-6-{[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}cleroda-3,14-dien-13-ol[1]) (**1**), and a 3,4-secolanostane triterpenoid, kadsuric acid 3-methyl ester[1]) (**2**), together with nine known compounds, (6*S*,13*E*)-6-{[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]-oxy}cleroda-3,13-dien-15-ol[2]) (= (1*S*,3*R*,4*S*,4a*R*,8a*R*)-1,2,3,4,4a,5,6,8a-octahydro-4-[(3*E*)-5-hydroxy-3-methylpent-3-en-1-yl]-3,4,8,8a-tetramethylnaphthalen-1-yl 6-deoxy-4-*O*-β-D-glucopyranosyl-α-L-mannopyranoside; **3**) [2], (6*S*,13*S*)-6-{[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}-13-{[α-L-rhamnopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}cleroda-3,14-diene[2]) (= (1*S*)-1-{2-{(1*S*,2*R*,4*S*,4a*R*,8a*R*)-4-{[4-*O*-(6-*O*-acetyl-β-D-glucopyranosyl)-6-deoxy-α-L-mannopyranosyl]oxy}-1,2,3,4,4a,7,

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

[2]) Notice that here the systematic name (1*S*,2*R*,4a*S*,5*R*,8a*S*)-decahydro-1,2,4a,5-tetramethyl-1-[(3*S*)-3-methylpentyl]naphthalene is used for clerodane.

8,8a-octahydro-1,2,4a,5-tetramethylnaphthalen-1-yl}ethyl}-1-methylprop-2-en-1-yl 6-deoxy-4-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-galactopyranoside; **4**) [3], (6*S*,13*S*)-6-{[6-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}-13-{[α-L-rhamnopyranosyl-(1 → 4)-β-D-fucopyranosyl]oxy}cleroda-3,14-diene[2]) (**5**) [4], 15-hydroxydehydroabietic acid (=(1*R*,4a*S*,10a*R*)-1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-hydroxy-1-methylethyl)phenanthrene-1-carboxylic acid; **6**) [5], 15-hydroxylabd-8(17)-en-19-oic acid (=(1*S*,4a*R*,5*S*,8a*R*)-Decahydro-5-[(3*S*)-5-hydroxy-3-methylpentyl]-1,4a-dimethyl-6-methylenenaphthalene-1-carboxylic acid; **7**) [6], junicedric acid (=(β*S*,1*S*,4a*R*,5*S*,8a*R*)-5-carboxydecahydro-β,5,8a-trimethyl-2-methylenenaphthalene-1-pentanoic acid; **8**) [7], (4β)-kaur-16-en-18-oic acid[3]) (**9**) [8], (4β)-16α-hydroxy-18-kauran-18-oic acid[3]) (**10**) [9], and (4β,16β)-16-hydroxykauran-18-oic acid[3]) (**11**) [10] from *D. linearis* and *D. ampla*. In addition, compounds **1** and **3**–**8** showed no anti-HIV activities.

Results and Discussion. – Compound **1** was obtained as a pale yellow powder. Its molecular formula was determined as $C_{32}H_{54}O_{11}$ by HR-ESI-MS (M^+ at m/z 613.3593), which revealed 6 unsaturation degrees. The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table 1*) showed the presence of a diterpene and two hexose moieties. Assignment of each glycoside was achieved by the analysis of ^{1}H,^{1}H-COSY and HMBC experiments (*Fig. 1*). Acid hydrolysis of **1** by the method described in [2] led to decomposition of the aglycone, but gave a mixture of glucose and rhamnose. The two monosaccharides were identified as having D- and L-configuration, respectively, by comparison of their R_f values with those of authentic samples; comparisons of the ^{1}H- and ^{13}C-NMR data of the glucopyranosyl unit with those of compound **3** [2] and considering that all glucose and rhamnose units in structures of compounds isolated from fern species have D- and L-configuration, respectively, confirmed these attributions. The relative configuration of **1** was determined by a ROESY experiment (*Fig. 2*). Thus, the structure of **1** was established as 6-{[β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]cleroda-3,14-dien-13-ol[1]).

The diterpene moiety of **1** was composed by five Me groups (δ(C) 27.5, 23.2, 18.4, 16.6, and 16.2), five CH_2 groups (δ(C) 36.1, 36.1, 33.1, 27.5, and 18.7), four olefinic C-atoms (δ(C) 146.2, 144.5, 123.7, and 112.3), three CH groups (δ(C) 87.9, 47.0, and 35.3), and three quarternary C-atoms (δ(C) 72.6, 45.0, and 38.8) according to the ^{1}H- and ^{13}C-NMR spectra. In the HMBC plot, the correlation of the anomeric H-atom (δ(H) 4.58) of the glucopyranosyl unit with C(4) (δ(C) 83.5) of the rhamnopyranosyl unit established a glucopyranosyl(1 → 4)rhamnopyranosyl linkage. Moreover, the sugar chain was linked to C(6) of the aglycone by the correlation of the anomeric H-atom (δ(H) 4.77) of the rhamnopyranosyl unit with C(6) (δ(C) 87.9) of the aglycone. The rhamnopyranosyl and glucopyranosyl units were in α- and β-configurations as established by the coupling constants of their anomeric H-atoms (J = 1.3 and 7.8 Hz). The above evidence suggested that compound **1** was similar to (6*S*,13*S*)-6-{[6-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl]oxy}cleroda-3,14-diene-13-ol, except for the absence of an Ac group in **1** [2]. The ROESY correlations of H–C(6) with H–C(8) and H–C(10) established that H–C(6) and H–C(10) were both β-oriented; accordingly, the glycosyloxy group at C(6) was α-oriented (*Fig. 2*). The ROESY correlation between Me(19) and Me(20) indicated that Me(20) was α-oriented.

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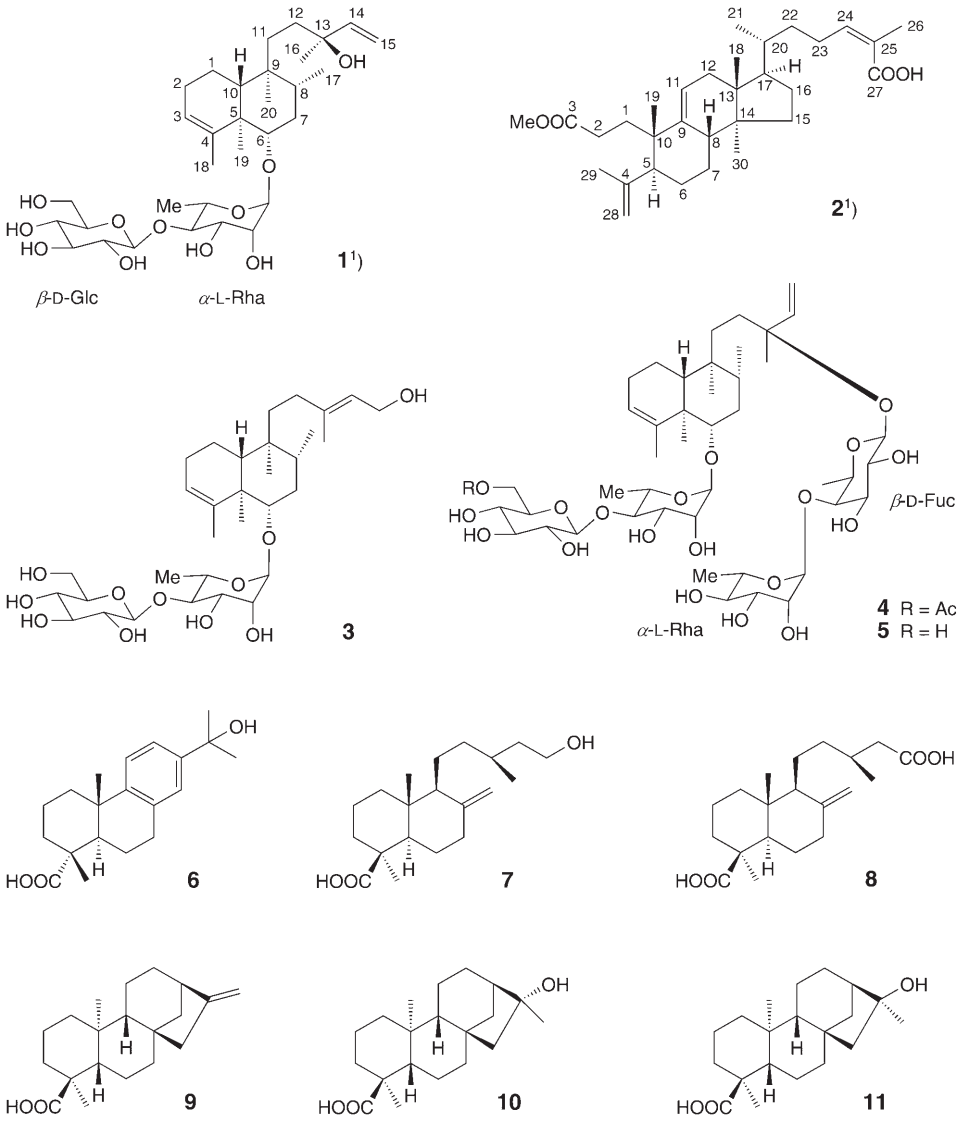
[3]) The name kaurane implies (5β,8α,9β,10α,13α,16β) configuration, according to *Chem. Abstr.*



Compound **2**, a colorless oil, had a molecular formula $C_{31}H_{48}O_4$, as deduced from, its HR-ESI-MS (M^+ at 485.3624), corresponding to eight unsaturation degrees. In the IR spectrum, the absorptions at 3424, 1740, 1689, and 1637 cm^{-1} indicated the presence of a COOH group and of C=C bonds. The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table 2*) were very similar to those of kadsuric acid [11], except that the former possesses an additional MeO group, suggesting that **2** was a monomethyl ester derivative of kadsuric acid. The HMBC (*Fig. 1*) and ROESY data (*Fig. 2*) confirmed the structure of **2** as kadsuric acid 3-methyl ester.

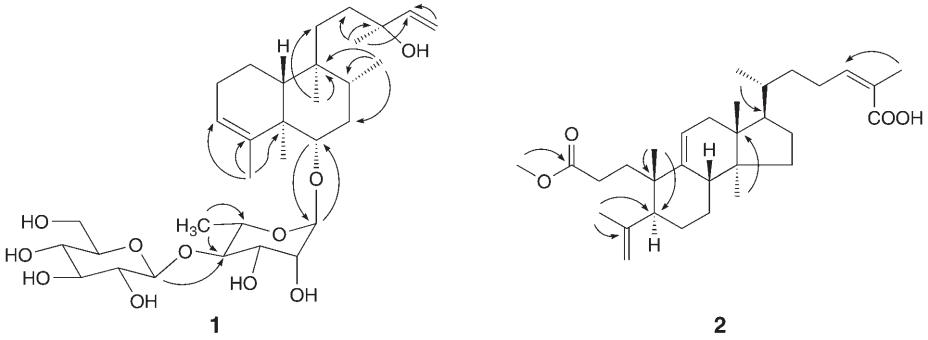


Fig. 1. *HMBC Data of compounds* **1** *and* **2**

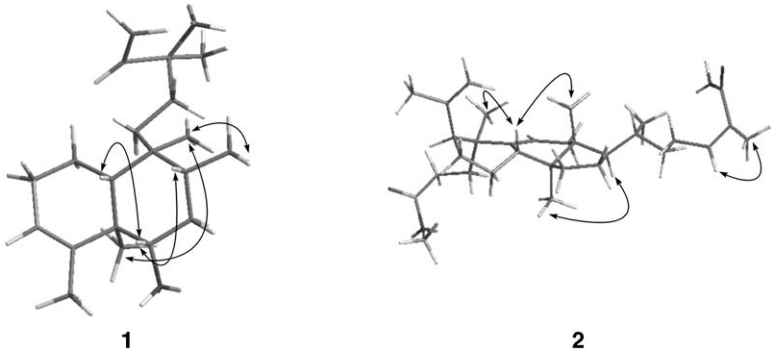


Fig. 2. *ROESY Correlations of the aglycone of compounds* **1** *and* **2**

The ^{1}H-NMR spectrum of **2** shows the presence of an isopropenyl group at δ(H) 4.87 and 4.71 (2*s*, 1 H each, CH_2(28)) and 1.77 (*s*, Me(29), three tertiary Me groups at δ(H) 0.66, 1.07, and 0.76 (3*s*, Me(18), Me(19), and Me(30)), besides an angelic acid-type terminal moiety at δ(H) 6.10 (*t*, $J = 6.7$ Hz, H–C(24)) and 1.93 (*s*, Me(26)), and a secondary Me group at δ(H) 0.91 (*d*, $J = 7.4$ Hz, Me(21)). Furthermore, the HMBC correlations between δ(H) 3.67 (MeO) and δ(C) 174.9 (C(3)) of **2** indicated that the methyl ester group was located at C(3). The relative configuration of **2** was determined by a ROESY experiment. The ROESY correlation H–C(8)/Me(19) suggested that H–C(8) was in β-orientation, the correlation H–C(17)/Me(30) indicated that H–C(17) was in α-configuration, and the correlation H–C(24)/Me(26) suggested that the C(24)=C(25) bond was in (*Z*) configuration.

Compounds **1** and **3**–**8** were tested for their anti-HIV activities. But none of them showed obvious activities.

Experimental Part

General. Column chromatography (CC): silica gel (100–200 mesh; *Qingdao Marine Chemical Inc.*, China) and silica gel *H* (10–40 μm, *Qingdao*). TLC: silica gel; visualization by spraying with 10% H_2SO_4 in EtOH followed by heating. Optical rotations: *Horiba-SEPA-300* spectro-polarimeter. UV Spectra: *Shimadzu-210A* double-beam spectrophotometer; λ_{max} log (ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrophotometer; KBr discs; $\tilde{\nu}_{max}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AM-400* and *-DRX-500*

Table 1. *^{13}C- and ^{1}H-NMR* (CD_3OD) *Assignments of Compound* **1**[1]). δ in ppm, J in Hz.

	δ (C)	δ (H)		δ (C)	δ (H)
CH_2(1)	18.7 (*t*)	1.57–1.59 (*m*)	Me(17)	16.2 (*q*)	0.80 (*d*, J = 5.8)
CH_2(2)	27.5 (*t*)	1.97–2.01 (*m*)	Me(18)	23.2 (*q*)	1.71 (*s*)
H–C(3)	123.7 (*d*)	5.11–5.15 (*m*)	Me(19)	16.6 (*q*)	1.05 (*s*)
C(4)	144.5 (*s*)		Me(20)	18.4 (*q*)	0.73 (*s*)
C(5)	45.0 (*s*)		Rha:		
H–C(6)	87.9 (*d*)	3.37–3.41 (*m*)	H–C(1′)	103.3 (*d*)	4.77 (*d*, J = 1.3)
CH_2(7)	36.1 (*t*)	1.97–2.01 (*m*)	H–C(2′)	72.5 (*d*)	3.87–3.93 (*m*)
H–C(8)	35.3 (*d*)	1.56–1.60 (*m*)	H–C(3′)	72.6 (*d*)	3.79–3.83 (*m*)
C(9)	38.8 (*s*)		H–C(4′)	83.5 (*d*)	3.61 (*t*, J = 8.3)
H–C(10)	47.0 (*d*)	1.29–1.33 (*m*)	H–C(5′)	68.7 (*d*)	3.75–3.79 (*m*)
CH_2(11)	33.1 (*t*)	1.35, 1.32 (both overlapped)	Me(6′)	17.9 (*q*)	1.30 (*d*, J = 6.2)
			Glc:		
CH_2(12)	36.1 (*t*)	1.38–1.42, 1.28–1.32 (2*m*)	H–C(1″)	105.7 (*d*)	4.58 (*d*, J = 7.8)
C(13)	72.6 (*s*)		H–C(2″)	76.0 (*d*)	3.18–3.22 (*m*)
H–C(14)	146.2 (*d*)	5.86 (*dd*, J = 9.8, 17.4)	H–C(3″)	78.1 (*d*)	3.33–3.37 (*m*)
CH_2(15)	112.3 (*t*)	5.03 (*dd*, J = 1.5, 7.3), 5.17 (*dd*, J = 1.5, 17.4)	H–C(4″)	71.4 (*d*)	3.26–3.30 (*m*)
			H–C(5″)	74.0 (*d*)	3.84–3.88 (*m*)
Me(16)	27.5 (*q*)	1.23 (*s*)	CH_2(6″)	62.7 (*t*)	3.85 (overlapped), 3.68 (*d*, J = 11.0)

Table 2. *^{13}C- and ^{1}H-NMR* ($CDCl_3$) *Assignments of Compound* **2**[1]). δ in ppm, J in Hz.

	δ (C)	δ (H)		δ (C)	δ (H)
CH_2(1)	32.2 (*t*)	1.99–2.01, 1.77–1.81 (2*m*)	CH_2(16)	28.8 (*t*)	1.28–1.32 (*m*)
CH_2(2)	28.8 (*t*)	2.46–2.50, 2.37–2.41 (2*m*)	H–C(17)	50.8 (*d*)	1.57–1.63 (*m*)
C(3)	174.9 (*s*)		Me(18)	14.6 (*q*)	0.66 (*s*)
C(4)	147.6 (*s*)		Me(19)	26.7 (*q*)	1.07 (*s*)
H–C(5)	49.3 (*d*)	2.06–2.10 (*m*)	H–C(20)	36.0 (*d*)	1.40–1.44 (*m*)
CH_2(6)	28.0 (*t*)	1.73–1.75, 1.63–1.67 (2*m*)	Me(21)	18.1 (*q*)	0.91 (*d*, J = 7.4)
CH_2(7)	27.9 (*t*)	1.54–1.56 (*m*)	CH_2(22)	35.8 (*t*)	1.50–1.54, 1.12–1.16 (*m*)
H–C(8)	42.5 (*d*)	2.08–2.12 (*m*)	CH_2(23)	26.9 (*t*)	2.55–2.59, 2.44–2.48 (*m*)
C(9)	142.4 (*s*)		H–C(24)	147.3 (*d*)	6.10 (*t*, J = 6.7)
C(10)	42.5 (*s*)		C(25)	125.8 (*s*)	
H–C(11)	118.5 (*d*)	5.33 (*d*, J = 5.7)	Me(26)	20.5 (*q*)	1.93 (*s*)
CH_2(12)	37.6 (*t*)	2.13–2.17, 1.93–1.97 (2*m*)	C(27)	173.2 (*s*)	
C(13)	43.9 (*s*)		CH_2(28)	113.7 (*t*)	4.87, 4.71 (2*s*)
C(14)	47.2 (*s*)		Me(29)	23.3 (*q*)	1.77 (*s*)
CH_2(15)	33.7 (*t*)	1.36–1.40 (*m*)	Me(30)	18.1 (*q*)	0.76 (*s*)
			MeO	51.5 (*s*)	3.67 (*s*)

instruments; chemical shifts δ in ppm rel. to residual solvent signals, J in Hz. ESI-MS and HR-ESI-MS: *VG AutoSpec-3000* spectrometers; in m/z.

Plant Material. The fronds of *D. linearis* and *D. ampla* were collected from Hekou county, Yunnan Province, P. R. China, in March 2006. The specimens were identified by Prof. *Xia Cheng* and deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dry fronds of *D. linearis* (12 kg) were ground and extracted 3 × with acetone (each time with 10 l for 24 h) at r.t. The acetone extract was concentrated and the crude extract suspended in H_2O/AcOEt. The AcOEt extract was subjected to CC (MCI, 95% EtOH). The fraction obtained with 95% EtOH was concentrated, and the residue (190 g) was subjected to CC (silica gel (200–300 mesh), $CHCl_3$/MeOH 1:0 → 0:1): *Frs. A–E. Fr. B* was subjected to repeated CC (silica gel) and semiprep. HPLC: **6** (16 mg), **7** (84 mg), and **8** (16 mg). *Fr. D* was subjected repeatedly to CC (*RP-18* and silica gel): **3** (2 g) and **4** (10 mg). Further purification by CC (*Sephadex LH-20*, *RP-18*) and semiprep. HPLC yielded **1** (4 mg) and **5** (1.5 mg).

The dry fronds of *D. ampla* (13 kg) were extracted and separated in the same manner as those of *D. linearis*. Compounds **2** (11 mg), **9** (6 mg), **10** (5 mg), and **11** (22 mg) were isolated from *Fr. B*. Compounds **4** (2 g) and **5** (11 mg) were obtained from *Fr. D*.

*(1*S*,3*R*,4*S*,4a*R*,8a*R*)-1,2,3,4,4a1a,5,6,8a-Octahydro-4-[3*S*)-3-hydroxy-3-methylpent-4-en-1-yl]-3,4,8,8a-tetramethylnaphthalen-1-yl 6-Deoxy-4-*O*-β-*D*-glucopyranosyl-α-*L*-mannopyranoside* (**1**): Pale yellow powder. M.p. 190–191°. $[\alpha]_D^{18} = -30.5$ ($c = 2.2$, MeOH). UV (MeOH): 202 (1.4). IR (KBr): 3420, 2965, 2920, 2873, 1641. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (neg.): 613 ($[M-H]^-$). HR-ESI-MS: 613.3593 ($C_{32}H_{53}O_{11}^-$; calc. 613.3587).

Acid Hydrolysis of **1**. A soln. of **1** (3 mg) in 2M HCl (3 ml) was heated in a water bath at 70° for 6 h. After cooling, the mixture was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. TLC Comparison (SiO_2, $CHCl_3$/MeOH 8:2) with authentic samples revealed the presence of glucose and rhamnose in the water layer (R_f 0.16 and 0.43, resp.). Unfortunately, the aglycone of **1** was not obtained because the TLC inspection of the water layer and the $CHCl_3$ part indicated that there were at least four products, which could not be identified due to their limited amount.

*(3*R*,3a*R*,6*S*,7*S*,9a*S*,9b*S*)-3-[(1*R*,4*Z*)-5-Carboxy-1-methylhex-4-en-1-yl]-2,3,3a,4,6,7,8,9,9a,9b-decahydro-3a,6,9b-trimethyl-7-(1-methylethenyl)-1*H*-benz[e]indene-6-propanoic Acid Methyl Ester* (**2**): Colorless oil. $[\alpha]_D^{24} = +50$ ($c = 0.240$, $CHCl_3$). UV (MeOH): 240 (0.30), 201 (0.13). IR (KBr): 3424, 2930, 1740, 1689, 1637, 1460, 1436, 1172, 895. NMR: *Table 2*. HR-ESI-MS: 485.3624 ($C_{31}H_{48}O_4^+$; calc. 485.3630).

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