Diterpene Glycosides from the Dry Fronds of *Conyza japonica*

by **Jiang Hu**[a])[1]), **Yan Song**[b])[1]), **Hui Li**[c]), **Xia Mao**[a]), **Xiaodong Shi**[a]), and **Zhiming Liu***[d])

[a]) College of Biological Resources and Environmental Science, Qujing Normal University, Sanjiang Avenue, Unicorn District, Qujing 655011, P. R. China (phone/fax: +86-874-8998627; e-mail: hujiang@ustc.edu)

[b]) Department of Pharmacy, 455 Hospital of People's Liberation Army, Shanghai 200052, P. R. China

[c]) College of Physics and Electronic Engineering, Qujing Normal University, Qujing 655011, P. R. China

[d]) The First People's Hospital of Qujing, Qujing 655000, P. R. China

Phytochemical investigation of the 95% EtOH extract of the dry fronds of *Conyza japonica* (THUNB.) LESS. resulted in the isolation of three new labdane diterpene glycosides, (3*β*,13*S*)-13-*O*-*α*-L-rhamnopyranosyllabda-8(17),14-dien-3-yl *α*-L-rhamnopyranoside (**1**), (3*β*,13*S*)-13-*O*-*α*-L-rhamnopyranosyllabda-8(17),14-diene-3-yl 2-*O*-acetyl-*α*-L-rhamnopyranoside (**2**), and (3*β*,13*S*)-13-*O*-*α*-L-rhamnopyranosylabda-8(17),14-dien-3-yl 6-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-rhamnopyranoside (**3**), together with their aglycone, (13*S*)-labda-8(17),14-diene-3,13-diol (**4**). Their structures were characterized by spectroscopic analyses and chemical correlations, including 1D- and 2D-NMR, and HR-ESI-MS. Furthermore, compounds **1** – **3** appeared to be promising as active agents against the tested pathogen fungi and oral pathogens as they possessed moderate cytotoxic properties.

Introduction. – The genus *Conyza*, which belongs to the family Asteraceae, widely grows in tropical and subtropical areas [1]. Among 50 species, over 20 species have been investigated phytochemically. Diverse types of diterpenes, including labdane, clerodane, *seco*-clerodane, and alicyclic types, have been reported as the characteristic constituents of *Conyza* and are considered to be major responsible for the *in vitro* and *in vivo* pharmacological effects [2 – 15]. *Conyza japonica* (THUNB.) LESS., a perennial herbaceous plant, is distributed mainly at an altitude of 700 – 2500 m above sea level in the southern regions of China. In folk medicine, its aerial parts are used to treat some kinds of inflammatory disease, especially laryngitis, paradentitis, amygdalitis, and chronic bronchitis [16]. We have reported the isolation of phenylpropanoid glycosides from the titled plant [17]. In continuation of our search for pharmacologically and structurally interesting substances, the present study was undertaken to systematically examine the EtOH extract of the dry fronds of *C. japonica* from Caojian Town of Yunnan Province and afforded three new labdane diterpenes glycosides, (3*β*,13*S*)-13-*O*-*α*-L-rhamnopyranosyllabda-8(17),14-dien-3-yl *α*-L-rhamnopyranoside (**1**), (3*β*,13*S*)-13-*O*-*α*-L-rhamnopyranosyllabda-8(17),14-dien-3-yl 2-*O*-acetyl-*α*-L-rhamnopyranoside (**2**), and (3*β*,13*S*)-13-*O*-*α*-L-rhamnopyranosylabda-8(17),14-dien-3-yl 6-*O*-acetyl-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-rhamnopyranoside (**3**) as well as their aglycone, (13*S*)-labda-8(17),14-diene-3,13-diol (**4**; *Fig. 1*). This article deals with the isolation and

[1]) These two authors contributed equally to this work.

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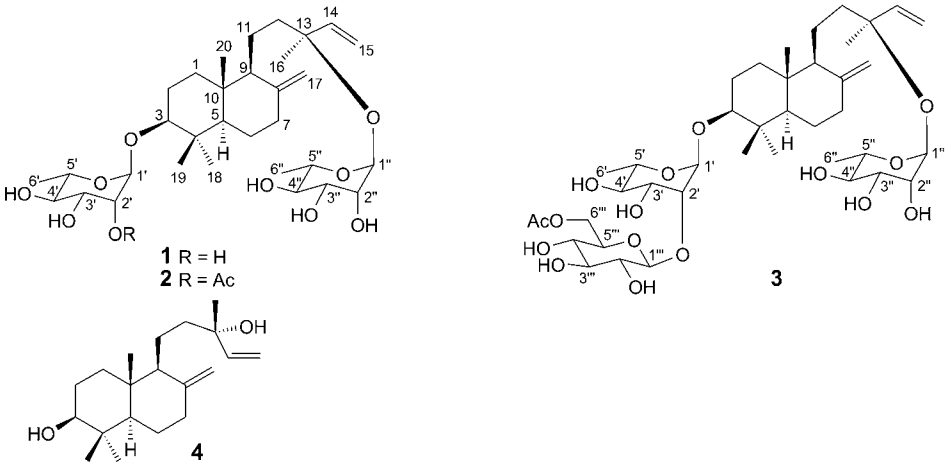

Fig. 1. *Structures of compounds* **1**–**4**

structure elucidation of the three new diterpene glycosides on the basis of the spectroscopic analysis and acid hydrolysis. Furthermore, all the isolated compounds were evaluated *in vitro* as antimicrobial and cytotoxic agents.

Results and Discussion. – Compound **1** was obtained as colorless oil, and its molecular formula was deduced as $C_{32}H_{54}O_{10}$ by HR-ESI-MS (*m/z* 597.3632 ($[M-H]^-$; calc. 597.3639)), corresponding to six degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra of **1** showed the characteristic resonances of a diterpene and two hexoses (*Tables 1* and *2*). Assignment of each glycosidic H-atom system was achieved by ^{1}H,^{1}H-COSY and HMQC experiments. The diterpene moiety of **1** contained four Me groups (δ(C) 28.6, 23.3, 16.9, and 15.1), six CH_2 groups (δ(C) 42.1, 39.3, 38.5, 27.8, 25.1, and 18.9), four olefinic C-atoms (δ(C) 149.6, 143.8, 115.8, and 107.6), two CH groups (δ(C) 59.3 and 49.5), two quarternary C-atoms (δ(C) 40.6 and 40.4), an O-bearing CH group (δ(C) 90.8), and an O-bearing quaternary C-atom (δ(C) 80.9) according to the NMR data, which suggested that it was similar to (3*β*,13*S*)-labda-8(17),14-diene-3,13-diol (**4**), a common aglycone of the labdane-type diterpene glycosides of *Conyza* [2]. Acid hydrolysis of **1** with 1M HCl in dioxane/H_2O (1:1) gave diterpene **4** ($C_{20}H_{34}O_2$) and L-rhamnose which was identified directly by GC analysis [18]. The HMBCs between the anomeric H-atoms (δ(H) 4.77 and 4.79) of the two rhamnopyranosyl units with C(3) (δ(C) 90.8) and C(13) (δ(C) 80.9) of the aglycone indicated the sugar moieties were located at C(3) and C(13), respectively (*Fig. 2*).

The relative configuration was determined by a ROESY experiment. The ROESY correlations between H–C(3) with H_α–C(1) and H–C(5) confirmed that H–C(3) was α-oriented. From a biogenetic perspective, (3*β*,13*S*)-labda-8(17),14-diene-3,13-diol (**4**) should be the aglycone of **1** because it was isolated from the same genus, and its specific rotation ($[\alpha]_D^{18} = -19$ ($c = 0.65$, MeOH)) coincided with the reported value. Thus, the structure of **1** was determined as (3*β*,13*S*)-13-*O*-*α*-L-rhamnopyranosyllabda-8(17),14-dien-3-yl *α*-L-rhamnopyranoside.

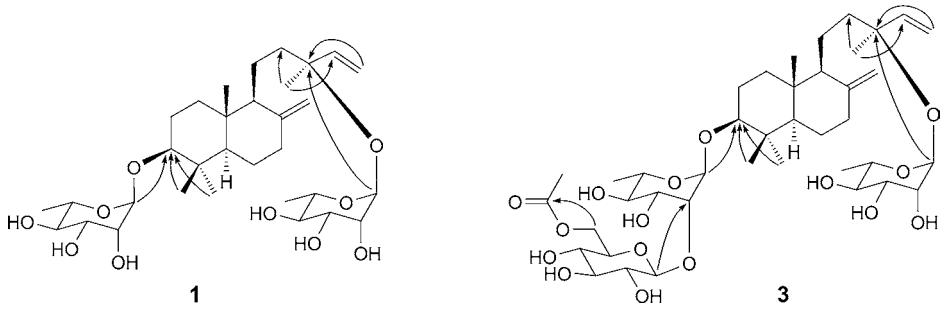

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **3**

Compound **2**, colorless oil, exhibited a molecular-ion peak at *m*/*z* 639.3742 ($[M-H]^-$; calc. 639.3744) in the high-resolution mass spectrometry, which corresponded to the molecular formula $C_{34}H_{56}O_{11}$ with seven degrees of unsaturation. The ^{1}H- and ^{13}C-NMR data of **2** and **1** were very similar, indicating that **2** was a derivative of the same labdane-type aglycon. Comparing the NMR data of **2** with those of compound **1**, the only significant difference was the presence of an AcO group in **2**. The position of the AcO group at C(2′) in one of the rhamnopyranosyl units was established by the HMBCs of H–C(2′) (δ(H) 3.80 – 3.82) with the C=O C-atom (δ(C) 172.8) of the AcO group, which was further confirmed by the HMBCs of H–C(3′) (δ(H) 3.70 – 3.72) with AcO–C(2′) (δ(C) 75.0). The relative configuration of **2** was deduced from its analog **1** and substantiated by its ROESY spectrum. From these results and the spectral data, the structure of **2** was determined as (3β,13*S*)-13-*O*-α-L-rhamnopyranosyllabda-8(17),14-dien-3-yl 2-*O*-acetyl-α-L-rhamnopyranoside.

Compound **3** was obtained as colorless oil. The negative-ion-mode HR-ESI-MS spectrum displayed a *pseudo*-molecular ion at *m*/*z* 837.4035 ($[M+Cl]^-$, $C_{40}H_{66}ClO_{16}^-$; calc. 837.4039) consistent with a molecular formula of $C_{40}H_{66}O_{16}$, corresponding to eight degrees of unsaturation. The ^{1}H- and ^{13}C-NMR spectra of **3** revealed the presence of a diterpene, three hexose moieties, and one AcO group. The resemblance of the NMR spectra (*Tables 1* and *2*) to those of **1** suggested that **3** was a diterpene glycoside of (3β,13*S*)-labda-8(17),14-diene-3,13-diol. Acid hydrolysis of **3** with 1M HCl in dioxane/H_2O (1:1) gave the aglycone (**4**), D-glucose, and L-rhamnose, which were identified directly by GC analysis [18]. The HMBC between the anomeric H-atom (δ(H) 4.32) of the glucopyranosyl unit and C(2′) (δ(C) 83.5) of the rhamnopyranosyl unit identified a glucopyranosyl-(1 → 2)-rhamnosyl linkage. Furthermore, the position of the sugar chain at C(3) of the aglycone was elucidated by the HMBC of the anomeric H-atom (δ(H) 5.09) of the rhamnopyranosyl unit and C(3) (δ(C) 89.6) of the aglycone. The HMBCs of the anomeric H-atom (δ(H) 4.82) of another rhamnopyranosyl unit with C(13) (δ(C) 80.1) suggested the location of the rhamnopyranosyl unit at C(13) of the aglycone. The HMBCs of H–C(6″) (δ(H) 4.38 and 4.26) of the glucopyranosyl unit with the C=O C-atom (δ(C) 171.0) of the AcO group indicated that the AcO group was positioned at C(6″) of the glucopyranosyl unit (*Fig. 2*). The ROESY correlations of H–C(3)/H_α–C(1) and H–C(3)/H–C(5) confirmed that H–C(3) is α-oriented. Thus, the structure of **3** was determined as (3β,13*S*)-13-*O*-α-L-rhamnopyranosylabda-8(17),14-dien-3-yl 6-*O*-acetyl-β-D-glucopyranosyl-(1 → 2)-α-L-rhamnopyranoside.

Table 1. *^{13}C-NMR Data of Compounds* **1**–**3** (125 MHz; in CD_3OD). δ in ppm.

Position	**1**	**2**	**3**
1	38.5 (*t*)	38.4 (*t*)	37.8 (*t*)
2	25.1 (*t*)	25.0 (*t*)	24.2 (*t*)
3	90.8 (*d*)	90.8 (*d*)	89.6 (*d*)
4	40.6 (*s*)	40.5 (*s*)	40.1 (*s*)
5	49.5 (*d*)	49.3 (*d*)	48.7 (*d*)
6	27.8 (*t*)	27.6 (*t*)	27.2 (*t*)
7	39.3 (*t*)	39.1 (*t*)	38.7 (*t*)
8	149.6 (*s*)	149.5 (*s*)	149.1 (*s*)
9	59.3 (*d*)	59.1 (*d*)	57.7 (*d*)
10	40.4 (*s*)	40.2 (*s*)	39.8 (*s*)
11	18.9 (*t*)	18.8 (*t*)	18.3 (*t*)
12	42.1 (*t*)	42.0 (*t*)	41.7 (*t*)
13	80.9 (*s*)	80.8 (*s*)	80.1 (*s*)
14	143.8 (*d*)	143.6 (*d*)	143.3 (*d*)
15	115.8 (*t*)	115.8 (*t*)	115.5 (*t*)
16	23.3 (*q*)	23.1 (*q*)	23.1 (*q*)
17	107.6 (*t*)	107.5 (*t*)	106.2 (*t*)
18	16.9 (*q*)	16.8 (*q*)	16.5 (*q*)
19	28.6 (*q*)	28.5 (*q*)	28.3 (*q*)
20	15.1 (*q*)	15.0 (*q*)	14.8 (*q*)
3-*O*-α-L-Rha			
1′	106.4 (*d*)	106.2 (*d*)	105.4 (*d*)
2′	73.5 (*d*)	75.0 (*d*)	83.5 (*d*)
3′	72.6 (*d*)	70.0 (*d*)	72.2 (*d*)
4′	74.9 (*d*)	78.0 (*d*)	74.5 (*d*)
5′	70.6 (*d*)	69.8 (*d*)	69.6 (*d*)
6′	18.2 (*q*)	18.4 (*q*)	18.2 (*q*)
13-*O*-α-L-Rha			
1″	96.9 (*d*)	96.8 (*d*)	96.1 (*d*)
2″	72.6 (*d*)	72.4 (*d*)	74.6 (*d*)
3″	71.9 (*d*)	72.6 (*d*)	72.5 (*d*)
4″	78.2 (*d*)	78.3 (*d*)	78.8 (*d*)
5″	69.9 (*d*)	70.0 (*d*)	69.0 (*d*)
6″	18.9 (*q*)	18.0 (*q*)	17.8 (*q*)
β-D-Glc			
1‴			106.6 (*d*)
2‴			75.0 (*d*)
3‴			75.2 (*d*)
4‴			73.2 (*d*)
5‴			78.0 (*d*)
6‴			64.0 (*t*)
Ac		172.8 (*s*),	171.0 (*s*),
		20.8 (*q*)	19.1 (*q*)

Table 2. *^{1}H-NMR Data of Compounds* **1**–**3** (600 MHz; in CD_3OD). δ in ppm, J in Hz.

Position	**1**	**2**	**3**
1	1.15 (*ddd*, J = 13.2, 13.0, 4.0, H_{ax}), 1.75 (*ddd*, J = 13.0, 4.0, 3.4, H_{eq})	1.14 (*ddd*, J = 13.2, 13.0, 4.0, H_{ax}), 1.75 (*ddd*, J = 13.0, 4.0, 3.4, H_{eq})	1.18–1.21 (overlapped, H_{ax}), 1.80 (*ddd*, J = 13.0, 4.0, 3.4, H_{eq})
2	1.63–1.67 (overlapped, H_{ax}), 1.90–1.92 (*m*, H_{eq})	1.63–1.68 (overlapped, H_{ax}), 1.89–1.91 (*m*, H_{eq})	1.68–1.70 (overlapped, H_{ax}), 1.95–1.98 (*m*, H_{eq})
3	3.16 (*dd*, J = 13.2, 4.5, H_{ax})	3.15 (*dd*, J = 13.2, 4.5, H_{ax})	3.22 (*dd*, J = 13.2, 4.5, H_{ax}),
5	1.12 (*dd*, J = 13.5, 4.0)	1.12 (*dd*, J = 13.5, 4.0)	1.17 (*dd*, J = 13.5, 4.0)
6	1.36–1.39 (overlapped, H_{ax}), 1.63–1.67 (overlapped, H_{eq})	1.36–1.39 (overlapped, H_{ax}), 1.63–1.68 (overlapped, H_{eq})	1.43–1.45 (*m*, H_{ax}), 1.68–1.70 (overlapped, H_{eq})
7	2.01 (*ddd*, J = 13.5, 13.0, 4.0, H_{ax}), 2.38 (*ddd*, J = 13.0, 4.0, 3.4, H_{eq})	1.99 (*ddd*, J = 13.5, 13.0, 4.0, H_{ax}), 2.37 (*ddd*, J = 13.0, 4.0, 3.4, H_{eq})	2.06 (*ddd*, J = 13.5, 13.0, 4.0, H_{ax}), 2.42 (*ddd*, J = 13.0, 4.0, 3.4, H_{eq})
9	1.52 (*t*, J = 7.0)	1.51 (*t*, J = 7.0)	1.57 (*t*, J = 7.0)
11	1.36–1.39 (overlapped, H_a), 1.55–1.58 (*m*, H_b)	1.36–1.39 (overlapped, H_a), 1.55–1.58 (*m*, H_b)	1.39–1.42 (*m*, H_a), 1.60–1.63 (*m*, H_b)
12	1.28 (*td*, J = 13.5, 7.0, H_a), 1.70 (*td*, J = 13.5, 7.0, H_b)	1.27 (*td*, J = 13.5, 7.0, H_a), 1.70 (*td*, J = 13.5, 7.0, H_b)	1.30 (*td*, J = 13.5, 7.0, H_a), 1.72 (*td*, J = 13.5, 7.0, H_b)
14	5.76 (*dd*, J = 17.0, 10.5)	5.79 (*dd*, J = 17.0, 10.5)	5.80 (*dd*, J = 17.0, 10.5)
15	5.15 (*dd*, J = 17.0, 1.5, H_a), 5.20 (*dd*, J = 10.5, 1.5, H_b)	5.15 (*dd*, J = 17.0, 1.5, H_a), 5.21 (*dd*, J = 10.5, 1.5, H_b)	5.19 (*dd*, J = 17.0, 1.5, H_a), 5.27 (*dd*, J = 10.5, 1.5, H_b)
16	1.31 (*s*)	1.30 (*s*)	1.35 (*s*)
17	4.56 (*d*, J = 1.5, H_a), 4.84 (*d*, J = 1.5, H_b)	4.53 (*d*, J = 1.5, H_a), 4.81 (*d*, J = 1.5, H_b)	4.59 (*d*, J = 1.5, H_a), 4.84 (*d*, J = 1.5, H_b)
18	0.81 (*s*)	0.80 (*s*)	0.86 (*s*)
19	1.04 (*s*)	1.02 (*s*)	1.06 (*s*)
20	0.70 (*s*)	0.69 (*s*)	0.73 (*s*)
3-*O*-*α*-L-Rha			
1′	4.77 (*d*, J = 1.3)	4.93 (*d*, J = 1.3)	5.09 (*d*, 1.3)
2′	3.66–3.72 (overlapped)	3.80–3.82 (*m*)	4.09–4.10 (*m*)
3′	3.26–3.34 (overlapped)	3.70–3.72 (*m*)	3.81–3.82 (*m*)
4′	3.34–3.38 (overlapped)	3.30–3.32 (overlapped)	3.33–3.35 (*m*)
5′	3.71–3.73 (overlapped)	3.74–3.76 (overlapped)	3.76–3.78 (overlapped)
6′	1.21–1.23 (overlapped)	1.21–1.23 (overlapped)	1.18–1.21 (overlapped)

Table 2 (cont.)

Position	**1**	**2**	**3**
13-*O*-*α*-L-Rha			
1″	4.79 (*d*, *J* = 1.3)	4.78 (*d*, *J* = 1.3)	4.82 (*d*, *J* = 8.0)
2″	3.66 – 3.72 (overlapped)	3.68 – 3.70 (*m*)	3.68 – 3.70 (overlapped)
3″	3.26 – 3.34 (overlapped)	3.24 – 3.26 (*m*)	3.71 – 3.73 (*m*)
4″	3.34 – 3.38 (overlapped)	3.30 – 3.32 (overlapped)	3.31 – 3.33 (*m*)
5″	3.71 – 3.73(overlapped)	3.74 – 3.76 (overlapped)	3.76 – 3.78 (overlapped)
6″	1.21 – 1.23 (overlapped)	1.21 – 1.23 (overlapped)	1.18 – 1.21 (overlapped)
β-D-Glc			
1‴			4.32 (*d*, *J* = 8.0)
2‴			3.18 – 3.20 (*m*)
3‴			3.35 – 3.38 (*m*)
4‴			3.27 – 3.31 (*m*)
5‴			3.68 – 3.70 (overlapped)
6‴			4.26 (*dd*, *J* = 13.0, 7.0)
			4.38 (*dd*, *J* = 13.0, 7.0)
Ac		2.07 (*s*)	2.09 (*s*)

Experimental Part

General. All solvents were distilled before use. TLC: silica gel GF_{254} (10–40 μm; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China). *MCI Gel CHP20P* (75–150 μm; *Mitsubishi Kasei Chemical Industries*), C_{18} reversed-phase silica gel (20–45 μm; *Fuji Silysia Chemical Ltd.*). Column chromatography (CC): silica gel (SiO_2, 200–300 mesh, 10–40 μm; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China), C_{18} reversed-phase SiO_2 (60 mm; *Merck*), *Sephadex LH-20* (*Amersham Pharmacia Biotech,* Sweden). HPLC (anal. and prep.): *Shimadzu* model *LC-8A* on *YMC-Pack*, *R&D ODS* column (250 × 4.6 mm i.d. and 250 × 20 mm i.d.) with a *Shimadzu SPD-10AVP* UV detector. Optical rotations: *JASCO-20C* digital polarimeter. UV Spectra: *Hewlett-Packard-8452A* diode-array spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *PerkinElmer 577* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS: *VG AutoSpec-3000* mass spectrometer; in m/z. HR-ESI-MS: *API QSTAR Pulsar-1* mass spectrometer; in m/z.

Plant Material. The fronds of *C. japonica* were collected in the Yuanjiang, Yunnan Province, P. R. China, in August 2012. A specimen (CJ20120801), identified by one of the authors (*X. M.*), was deposited with the Herbarium of the College of Biological Resources and Environmental Science, Qujing Normal University, Qujing, P. R. China.

Extraction and Isolation. The dry fronds of *C. japonica* (1.0 kg) were ground into powder and extracted with 70% aq. EtOH (3×). After evaporation of EtOH, the crude extract (115 g) was partitioned between H_2O and AcOEt. The AcOEt-soluble portion (35 g) was chromatographed on a SiO_2 column eluted with $CHCl_3$/MeOH (from 100:1 to 1:1) to afford *Frs. 1–6*. *Fr. 2* (4.1 g) was separated by repeated CC on SiO_2 and then purified by *ODS* and *Sephadex LH-20* to afford **4** (211 mg). *Fr. 4* (6.3 g) was chromatographed on a *MCI* gel column eluted with MeOH/H_2O (from 60% to 95%) to yield five subfractions, *Frs. 4A–4E*. *Subfr. 4D* (1.3 g) was separated by repeated CC over *Sephadex LH-20* ($CHCl_3$/MeOH, MeOH), SiO_2, and then prep. HPLC (MeOH/H_2O 45% to 70%), yielding **1** (73 mg) and **2** (69 mg). *Fr. 5* (1.3 g) was divided into three subfractions (*Frs. 5A–5C*) by *MCI* gel CC eluting with MeOH/H_2O (from 50% to 95%). *Fr. 5C* (186 mg) was further separated by prep. HPLC (MeOH/H_2O, from 30% to 55%) to afford **3** (67 mg).

*(3β,13*S*)-13-*O-α-L-*Rhamnopyranosyllabda-8(17),14-dien-3-yl* α-L-*Rhamnopyranoside* (= *(2*S*,4a*R*,5*S*,8a*R*)-5-{(3*S*)-3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-3-methylpent-4-en-1-yl}decahydro-1,1,4*a*-trimethyl-6-methylidenenaphthalen-2-yl 6-Deoxy-α-L-mannopyranoside*; **1**). Colorless oil. $[\alpha]_D^{23.3} = -21.12$ ($c = 0.037$, MeOH). UV (MeOH): 204 (3.70). IR (KBr): 3420, 2935, 1720, 1642, 1387, 1042. ^{1}H-NMR (600 MHz, CD_3OD): *Table 2*. ^{13}C-NMR (CD_3OD, 125 MHz): *Table 1.* FAB-MS (neg.): 597 ($[M-H]^-$). HR-ESI-MS: 597.3632 ($[M-H]^-$, $C_{32}H_{53}O_{10}^-$; calc. 597.3639).

*(3β,13*S*)-13-*O-α-L-*Rhamnopyranosyllabda-8(17),14-dien-3-yl 2-*O-*Acetyl-α-L-rhamnopyranoside* (= *(2*S*,4a*R*,5*S*,8a*R*)-5-{(3*S*)-3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-3-methylpent-4-en-1-yl}decahydro-1,1,4*a*-trimethyl-6-methylidenenaphthalen-2-yl 2-*O-*Acetyl-6-deoxy-α-L-mannopyranoside*; **2**). Colorless oil. $[\alpha]_D^{23.3} = -23.17$ ($c = 0.030$, MeOH). UV (MeOH): 205 (2.73). IR (KBr): 3450, 2940, 1745, 1631, 1060. ^{1}H-NMR (CD_3OD, 600 MHz): *Table 2*. ^{13}C-NMR (CD_3OD, 125 MHz): *Table 1.* FAB-MS (neg.): 639 ($[M-H]^-$). HR-ESI-MS: 639.3742 ($[M-H]^-$, $C_{34}H_{55}O_{11}^-$; calc. 639.3744).

*(3β,13*S*)-13-*O-α-L-*Rhamnopyranosylabda-8(17),14-dien-3-yl 6-*O-*Acetyl-β-D-glucopyranosyl-(1→2)-α-L-rhamnopyranoside* (= *(2*S*,4a*R*,5*S*,8a*R*)-5-{(3*S*)-3-[(6-Deoxy-α-L-mannopyranosyl)oxy]-3-methylpent-4-en-1-yl}decahydro-1,1,4*a*-trimethyl-6-methylidenenaphthalen-2-yl 2-*O-*(6-O-Acetyl-β-D-glucopyranosyl)-6-deoxy-α-L-mannopyranoside*; **3**). Colorless oil. $[\alpha]_D^{23.3} = -17.17$ ($c = 0.025$, MeOH). UV (MeOH): 202 (3.51). IR (KBr): 3432, 2935, 1742, 1630, 1060. ^{1}H-NMR (CD_3OD, 600 MHz): *Table 2*. ^{13}C-NMR (CD_3OD, 125 MHz): *Table 1.* FAB-MS (neg.): 801 ($[M-H]^-$). HR-ESI-MS: 837.4035 ($[M+Cl]^-$, $C_{40}H_{66}ClO_{16}^-$; calc. 837.4039).

Acid Hydrolysis of **1**–**3**. A soln. of compound **1**, **2**, or **3** (*ca.* 10.0 mg) in 1M HCl (dioxane/H_2O 1:1, 1 ml) was heated at 95° for 2 h [19]. After cooling, the reaction mixture was neutralized by passage through an *Amberlite IRA-93ZU* (*Organo*, Tokyo, Japan) column and subjected to CC (SiO_2; $CHCl_3$/MeOH 19:1, 9:1, 1:1) to give an aglycone fraction and a sugar fraction (3.0 mg). The aglycone fraction was purified by CC (SiO_2; hexane/acetone 4:1) to give **4**. The solns. of the sugar parts, obtained as described above, in pyridine (2 ml) were added to L-cysteine methyl ester hydrochloride (1.5 mg) and

kept at 60° for 1 h each. Then, 1-(trimethylsilyl)-1*H*-imidazole (1.5 ml) was added to the mixture and kept at 60° for 30 min. Supernatant was subjected to GC analysis under the following conditions: column temp.: 180–280°, programmed: 3°/min, carrier gas: N_2 (1 ml/min), injector and detector temp.: 250°, injection volume: 4 µl, split ratio: 1/50. Configuration identification of D-glucose and L-rhamnose was carried out by comparison with the retention times of their corresponding derivatives. Retention times in GC of standard D-/L-glucose and L-/D-rhamnose derivatives were 19.450/19.856 and 15.850/16.313 min, resp. By comparing the retention time of the authentic sugars in the form of derivatives under the same condition, the sugar moieties of compounds **1**–**3** were determined to be D-glucose for **3**, and L-rhamnose for **1**–**3**. All chemical reagents and standard sugars were purchased from *Sigma–Aldrich*.

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