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TRITERPENES AND TRITERPENOID SAPONINS FROM *MUSSAENDA PUBESCENS*

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Key Word Index—*Mussaenda pubescens*; Rubiaceae; triterpenes; saponins; mussaendosides U and V.

Abstract—Two novel triterpenoid saponins, named mussaendosides U and V, together with one known saponin and four known triterpenes were isolated from the aerial parts of *Mussaenda pubescens* (Rubiaceae). The structures were determined on the basis of chemical analysis and spectral methods. All these compounds were identified for the first time from the genus *Mussaenda*. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Mussaenda pubescens Ait. f. is a liana-like shrub, distributed widely in shady hillsides, valleys and shrub jungle of east, south and southwest China. It has been used as a Chinese folk medicine for treatment of common cold, diarrhoea and some inflammatory diseases, such as laryngopharyngitis and acute gastroenteritis [1]. It is also used to detoxify mushroom poisoning and terminate early pregnancy in some districts of southeast China [2, 3]. In previous papers, we have reported the isolation and structure determination of several saponins with novel triterpenoid aglycones from the plant, some of them exhibited significant immuno-stimulating activity and antagonistic activity towards the acetylcholine receptor [4–7]. In continuation of our chemical study on this medicinal plant, two novel triterpenoid saponins, named mussaendosides U (**1**) and V (**2**) were isolated. Their structures were determined on the basis of chemical analysis and various spectral methods. One known saponin (**3**) and four α-amyrin type triterpenes (**4**–**7**) were also identified. All these compounds were reported for the first time from the genus *Mussaenda*.

RESULTS AND DISCUSSION

The ethanol extract of the aerial parts of *Mussaenda pubescens* was partitioned between water and ethyl acetate, and between water and *n*-butanol successively. The *n*-butanol fraction was subjected to polyporous resin DA-201, eluted with water, 40 and 90% ethanol, successively. From the 90% ethanol fraction, compounds **1**–**3** were purified through a series of chromatographies on silica gel columns and RP-18 Lobar columns [6].

Compound **1** was obtained as a white amorphous powder. In the positive ion mode FAB-mass spectrum, two molecular ion adducts were observed at *m/z* 1529 $[M+Na]^+$ and 1545 $[M+K]^+$; while in the negative ion mode FAB-mass spectrum, a deprotonated molecular ion peak appeared at *m/z* 1505 $[M-H]^-$. In the ^1H NMR spectrum, three characteristic signals were observed at δ 9.11 (1H, *d*, 7.7, NH), δ 0.29 (1H, *br s*, H-19a) and δ 0.55 (1H, *br s*, H-19b), respectively, suggesting that **1** possessed an amide group and a cyclopropane group in its aglycone as found in several saponins from the same genus [4, 6]. Comparison of the ^{13}C NMR spectral data of **1** with those of reported saponins confirmed heinsiagenin A to be its aglycone and with a saccharide chain connected to its C-3 position [4, 6]. Furthermore, on the basis of the HSQC spectrum, all the proton signals belonging to the aglycone were assigned (Table 1). According to the mass spectrum and ^{13}C NMR data, the molecular formula was deduced to be $C_{72}H_{115}NO_{32}$.

In its ^{13}C NMR spectrum, six anomeric carbon signals were observed at δ 106.5, 105.1, 102.8, 102.8, 102.1 and 101.9 ppm. Therefore, compound **1** should be a saponin with six sugar units in the saccharide moiety. Acidic hydrolysis of **1** yielded only glucose and rhamnose as its sugar components. According to the HSQC spectrum, six anomeric proton signals were

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Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **1** (C_5D_5N)

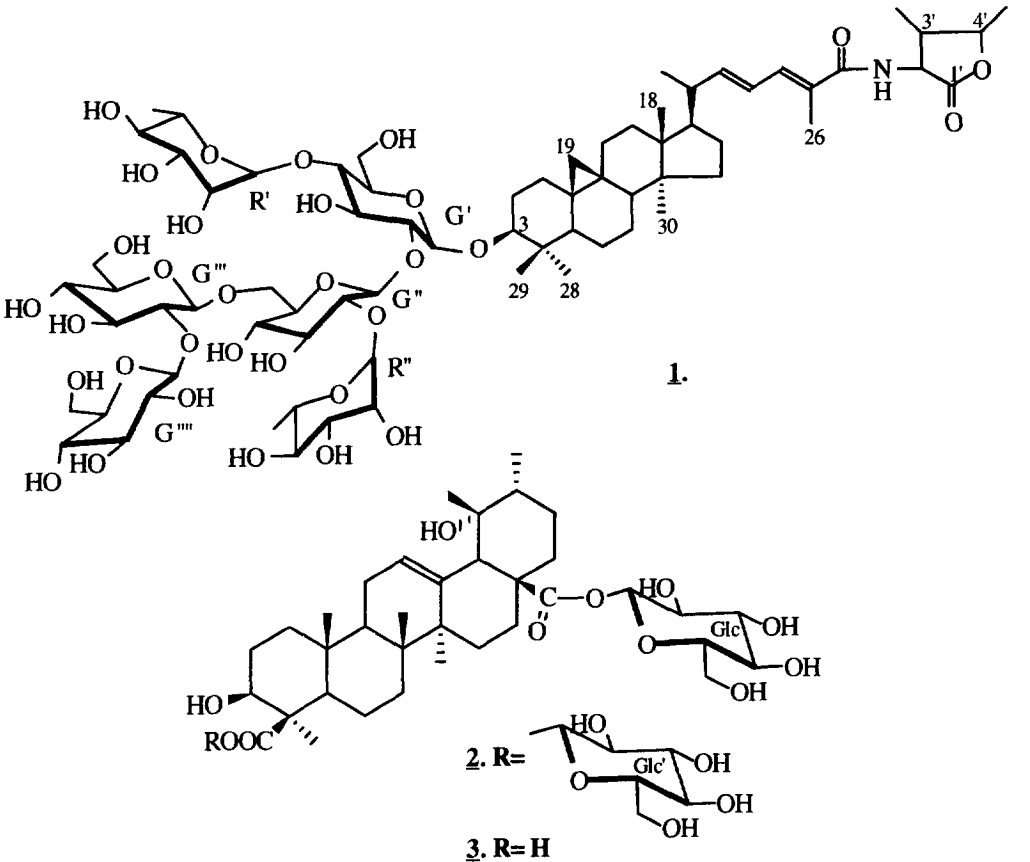
No.	^1H	^{13}C
1	1.13, 1.50, *m*	32.2, *t*
2	1.98, 2.32, *m*	29.8, *t*
3	3.45, *dd*, 10.8, 4.8	90.5, *d*
4		41.4, *s*
5	1.34, *m*	47.8, *d*
6	0.82, 1.62, *m*	21.2, *t*
7	1.07, 1.27, *m*	26.4, *t*
8	1.45, *m*	48.1, *d*
9		19.8, *s*
10		26.3, *s*
11	1.05, 1.95, *m*	26.6, *t*
12	1.56, 1.56, *m*	33.0, *t*
13		45.6, *s*
14		49.2, *s*
15	1.27, 1.27, *m*	35.8, *t*
16	1.23, 1.58, *m*	28.8, *t*
17	1.60, *m*	52.0, *d*
18	0.99, *s*	18.5, *q*
19	0.29, 0.55, *br s*	29.9, *t*
20	2.18, *m*	41.3, *d*
21	1.01, *d*, 6.5	19.9, *q*
22	5.65, *dd*, 14.8, 8.9	147.9, *d*
23	6.44, *dd*, 14.8, 11.1	123.8, *d*
24	7.29, *d*, 11.1	134.8, *d*
25		129.1, *s*
26	2.21, br s	13.5, *q*
27		170.8, *s*
28	1.27, *s*	15.6, *q*
29	1.47, *s*	26.2, *q*
30	0.90, *s*	19.5, *q*
1′		175.8, *s*
2′	5.69, *dd*, 7.4, 7.4	55.4, *d*
3′	2.94, *m*	38.6, *d*
4′	4.70, *m*	77.0, *d*
3′-CH_3	0.88, *d*, 7.2	8.1, *q*
4′-CH_3	1.19, *d*, 6.5	15.5, *q*
NH	9.11, *d*, 7.7	
G′-1	4.89, *d*, 7.6	105.1, *d*
G′-2	4.60, *m*	78.5, *d*
G′-3	4.49, *m*	78.0, *d*
G′-4	4.38, *m*	79.4, *d*
G′-5	3.64, *m*	76.3, *d*
G′-6	4.07, 4.17, *m*	61.6, *t*
G″-1	5.76, *d*, 7.4	101.9, *d*
G″-2	4.32, *m*	79.3, *d*
G″-3	4.32, *m*	78.3, *d*
G″-4	4.28, *m*	72.3, *d*
G″-5	4.18, *m*	76.0, *d*
G″-6	4.31, 4.64, *m*	70.3, *t*
G‴-1	5.37, *d*, 7.7	102.8, *d*
G‴-2	4.14, *m*	84.5, *d*
G‴-3	4.50, *m*	77.8, *d*
G‴-4	4.24, *m*	71.2, *d*
G‴-5	4.08, *m*	77.8, *d*
G‴-6	4.41, 4.55, *m*	62.5, *t*
G⁗-1	5.34, *d*, 6.8	106.5, *d*
G⁗-2	4.20, *m*	76.5, *d*
G⁗-3	4.21, *m*	78.1, *d*
G⁗-4	4.23, *m*	71.1, *d*
G⁗-5	3.95, *m*	78.8, *d*
G⁗-6	4.35, 4.55, *m*	62.3, *t*
R′-1	5.80, *br s*	102.8, *d*
R′-2	4.63, *m*	72.5, *d**
R′-3	4.51, *m*	72.7, *d*
R′-4	4.28, *m*	73.9, *d*
R′-5	4.82, *m*	70.7, *d*
R′-6	1.69, *d*, 6.1	18.7, *q*
R″-1	6.39, *br s*	102.1, *d*
R″-2	4.74, *br s*	72.4, *d*
R″-3	4.63, *m*	72.7, *d**
R″-4	4.33, *m*	74.2, *d*
R″-5	5.02, *m*	69.5, *d*
R″-6	1.86, *d*, 6.0	19.1, *q*

* Data may be interchanged.

found at δ 4.89 (1H, *d*, 7.6), 5.34 (1H, *d*, 6.8), 5.37 (1H, *d*, 7.7), 5.76 (1H, *d*, 7.4), 5.80 (1H, *br s*) and 6.39 (1H, *br s*). Among the six anomeric proton signals, the first four doublets were due to glucosyl units, while the last two broad singlets should belong to those of the rhamnosyl units. All the four glucosyl units should be in β-glycosidic linkage according to the coupling constants of their anomeric protons (J = 6.8–7.7 Hz) [7]. By analysing the ^1H-^1H DQF-COSY and TOCSY spectra, all proton signals belonging to the saccharide moiety of **1** were assigned by starting from the anomeric proton signals of each sugar unit and also from the characteristic 6-CH_3 doublet signals of the rhamnosyl units. The carbon signals of the saccharide moiety were further assigned according to the HSQC spectrum (Table 1). The C-5 signals of the two rhamnosyl units were found at δ 70.7 and 69.5, respectively. Therefore, the two rhamnosyl units should be both in α-glycosidic linkage [7].

The structure of the saccharide moiety of **1** was based upon the following information. The two sugar carbon signals at relative low fields at δ 70.3 (*t*) and δ 84.5 (*d*), suggested that glycosidation might take place at C-6 and C-2 of one or two glycosyl units. In the negative ion mode FAB mass spectrum, a series of fragments was observed at *m/z* 1359 $[M-rha-H]^-$, 1343 $[M-glu-H]^-$, 1213 $[M-rha-rha-H]^-$, 1197 $[M-rha-glu-H]^-$, 1181 $[M-glu-glu-H]^-$, 1051 $[M-rha-rha-glu-H]^-$, 1035 $[M-rha-glu-glu-H]^-$, 889 $[M-rha-rha-glu-glu-H]^-$, 873 $[M-rha-glu-glu-H]^-$, 727 $[M-rha-rha-glu-glu-glu]^-$ and 565 $[M-rha-rha-glu-glu-glu-glu-H]^-$. From these fragments, it can be deduced that at least one rhamnosyl unit and one glucosyl unit were at terminal positions. According to the fragment peak at *m/z* 727, the sugar unit that was attached directly to the aglycone should be a glucosyl unit.

In order to determine definitely the sequence and linking sites among the sugar units and aglycone, NOESY and HMBC experiments were performed. In the NOESY spectrum, cross peak signals could be



observed between anomeric protons and the protons to which they were connected. In the NOESY experiment of **1**, cross peak signals were observed between $H_{G'-1}$ and H-3, $H_{G''-1}$ and $H_{G'-2}$, $H_{G'''}$-1 and $H_{G''-6a,b}$, $H_{G''''}$-1 and $H_{G'''}$-2, $H_{R'-1}$ and $H_{G'-4}$ and between $H_{R''-1}$ and $H_{G''-2}$ (Fig. 1). Therefore, the interglycosidic linkages among all the six sugar units were determined. The above results were further confirmed by a HMBC experiment in which cross peak signals were observed between the anomeric carbon signal of one sugar unit and the proton signal to which the sugar was connected. In the HMBC experiment of **1**, cross peaks were observed between H-3 and $C_{G'-1}$, $H_{G'-1}$ and C-3, $H_{G'-2}$ and $C_{G''-1}$, $H_{G''-2}$ and $C_{R''-1}$, $H_{G'-4}$ and $C_{R'-1}$, $H_{G'''}$-1 and $C_{G''-6}$, $H_{G''-6a,6b}$ and $C_{G'''}$-1, $H_{G''''}$-1 and $C_{G'''}$-2, and between $H_{G'''}$-2 and $C_{G''''}$-1 (Fig. 1). The above results were consistent with those derived from a NOESY experiment. Therefore, the structure of **1** was determined to be heinsiagenin A 3-*O*-{[*β*-D-glucopyranosyl(1 → 2)-*O*-*β*-D-glucopyranosyl(1 → 6)]-*O*-*α*-L-rhamnopyranosyl(1 → 2)-*O*-*β*-D-glucopyranosyl (1→2)}-*O*-*α*-L-rhamnopyranosyl(1→4)-*O*-*β*-D-glucopyranoside. To our knowledge, this is a new natural compound and has been named mussaendoside U.

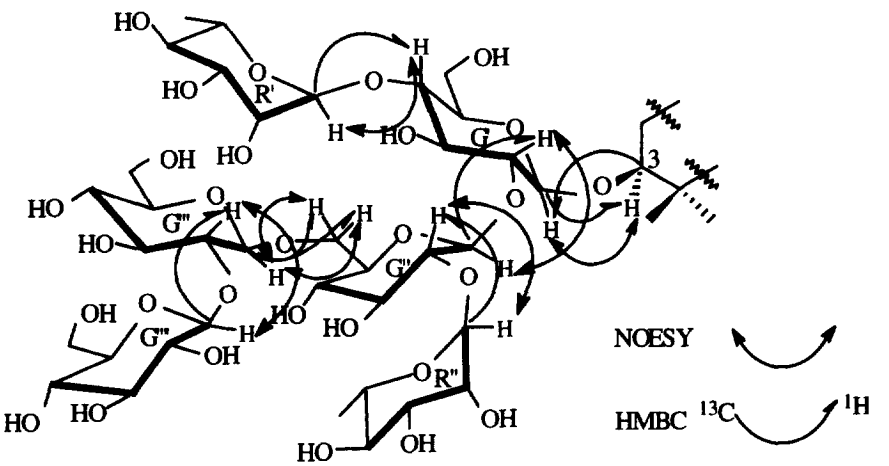


Fig. 1. Correlation signals among sugar units and aglycone in NOESY and HMBC spectra of **1**.

Compound **2** was obtained as a white amorphous powder. The positive ion mode FAB mass spectrum gave a sodium molecular adduct at *m/z* 850 $[M+Na]^+$, while the negative ion mode FAB-mass spectrum gave a deprotonated molecular ion at *m/z* 826 $[M-H]^-$. Therefore, its molecular weight was determined to be 827. In the negative ion mode FAB mass spectrum, significant fragments were also observed at *m/z* 663 and 501, indicating the sequential losses of two hexosyl units. In the ^{13}C NMR spectrum, 42 carbon signals were observed and among them were six methyl and two carboxylic groups. Therefore compound **2** was considered to be a triterpenoid saponin. From its mass spectrum and ^{13}C NMR data, the molecular formula of **2** was deduced to be $C_{42}H_{66}O_{16}$.

The ^1H NMR spectrum showed six methyl signals at δ 1.77 (*s*), 1.71 (*s*), 1.42 (*s*), 1.27 (*s*), 1.27 (*s*) and 1.08 (*d*, 6.4), one olefinic proton signal at δ 5.22 (*br s*), one broad singlet at δ 2.94 and one double doublet at δ 3.40 (*dd*, 11.2, 4.0). The ^{13}C NMR spectrum revealed the presence of one pair of trisubstituted olefinic carbon signals at δ 128.3 and 139.0 and one oxygen-connecting quaternary carbon signal at δ 72.5. From the above evidence, the aglycone of **2** was deduced to possess an α-amyrin type skeleton with a hydroxyl group at C-19. The existence of 19-OH was evidenced by the characteristic chemical shifts of C-12 and C-13, and by the H-18 proton as a broad singlet. The 3-hydroxyl was in the β-configuration according to the coupling constants of H-3 with the two H-2 protons. A comparison of the ^{13}C NMR data of **2** with those of similar structures in the literature suggested that the two carboxyls were located at C-24 and C-28 [5, 8]. Therefore, the aglycone of **2** was determined to be 3β,19α-dihydroxyl urs-12-en-24,28-dioic acid.

Acidic hydrolysis of **2** only yielded glucose as its unique sugar component, which indicated that the two hexosyl units should be glucosyl units. In the ^1H NMR spectrum, the two anomeric proton signals were observed at δ 6.35 (1H, *d* 7.6) and δ 6.26 (1H, *d*, 7.4), and in the ^{13}C NMR spectrum, the two anomeric carbon signals overlapped at δ 95.7. From the above evidence, the two glucosyl units should be both connected by β-glycosidic linkage to the aglycone through ester bonds, which meant that the two glucosyl units of **2** were linked to C-24 and C-28 carboxyl groups, respectively. A comparison of the ^{13}C NMR data with those of 3β,19α-dihydroxyl urs-12-en-24,28-dioic acid 28-*O*-β-D-glucopyranoside (**3**), a known saponin also isolated from the plant, showed significant variation of chemical shifts around C-24 due to glycosidation. This confirmed that the two glucosyl moieties were at the C-24 and C-28 positions (Table 2). Therefore compound **2** was identified as 3β,19α-dihydroxyl urs-12-en-24,28-dioic acid 24,28-di-*O*-β-D-glucopyranoside. It is a new saponin and has been named mussaendoside V.

Compound **2** was similar to another saponin mussaendoside K isolated from the same species in our previous report [8]. Mussaendosides K and V were both diesterified glycosides with two glucosyl units linking to the C-24 and C-28 carboxylic groups. To the best of our knowledge, saponins with sugar units attached to the C-24 carboxylic group are novel in the plant kingdom.

Table 2. ^{13}C NMR data of compounds (**2**–**4**) (C_5D_5N, 50 MHz)

No.	**2**	**3**	**4**
1	39.9 *t*	39.8 *t*	33.7 *t*
2	28.8 *t*	29.1 *t*	26.3 *t*
3	78.3 *d*	78.4 *d*	69.8 *d*
4	50.1 *s*	49.3 *s*	46.0 *s*
5	57.4 *d*	57.0 *d*	45.0 *d*
6	20.8 *t*	21.1 *t*	19.1 *t*
7	33.8 *t*	33.9 *t*	33.7 *t*
8	40.4 *s*	40.4 *s*	40.4 *s*
9	47.3 *d*	47.2 *d*	47.8 *d*
10	37.9 *s*	37.9 *s*	37.2 *s*
11	24.3 *t*	24.5 *t*	24.2 *t*
12	128.3 *d*	128.6 *d*	127.9 *d*
13	139.0 *s*	139.3 *s*	139.9 *s*
14	42.3 *s*	42.2 *s*	42.0 *s*
15	29.2 *t*	29.1 *t*	29.2 *t*
16	26.3 *t*	26.2 *t*	26.9 *t*
17	48.7 *s*	48.7 *s*	48.2 *s*
18	54.5 *d*	54.6 *d*	54.5 *d*
19	72.5 *s*	72.6 *s*	72.6 *s*
20	42.1 *d*	42.2 *d*	42.3 *d*
21	27.0 *t*	27.0 *t*	26.3 *t*
22	37.7 *t*	37.7 *t*	38.4 *t*
23	24.5 *q*	24.3 *q*	69.1 *t*
24	176.1 *s*	181.0 *s*	64.5 *t*
25	14.2 *q*	14.0 *q*	15.6 *q*
26	17.2 *q*	17.4 *q*	17.1 *q*
27	24.7 *q*	24.7 *q*	24.5 *q*
28	176.8 *s*	177.0 *s*	180.8 *s*
29	27.0 *q*	27.0 *q*	27.0 *q*
30	16.7 *q*	16.7 *q*	16.7 *q*
G-1 (G′-1)	95.7 (95.7) *d*	95.9 *d*	
G-2 (G′-2)	74.0 (74.0) *d*	74.0 *d*	
G-3 (G′-3)	79.1 (79.1) *d*	79.3 *d*	
G-4 (G′-4)	71.2[a] (71.0[a]) *d*	71.2 *d*	
G-5 (G′-5)	78.7 (78.7) *d*	79.0 *d*	
G-6 (G′-6)	62.3[b] (62.2[b]) *t*	62.3 *t*	

[a,b] Data may be interchanged.

Compound **3** (ilexsaponin A) was obtained before by Qin *et al.* [9] from the root bark of *Ilex pubescens* (Aquifoliaceae), but its ^{13}C NMR data were not reported. For the convenience of comparison, the ^{13}C NMR data of **3** are also listed in Table 2.

Compounds (**4**–**7**) were purified from the ethyl acetate fraction by repeated chromatography on a silica gel and RP-18 Lobar columns. Compound **4** (clethric acid) was previously identified by Takanashi *et al.* [10] from the leaves of *Clethra barbinervis* (Clethraceae). In the structure of **4**, two hydroxyl groups were located at C-23 and C-24. Triterpenes with both C-23 and C-24

hydroxyl groups were also found in several other plants [11–13]. The ^{13}C NMR data of **4** were not reported in the literature. Methylation of **4** gave **4a**. The ^{1}H NMR and ^{13}C NMR data of **4a** were identical to those reported for clethric acid methyl ester, thus confirming the structure of **4** [10]. The ^{13}C NMR data of **4** are also listed in Table 2.

Compounds (**5**–**7**) were triterpenes with an α-amyrin type skeleton. Their structures were established on the basis of the ^{1}H NMR, ^{13}C NMR and mass spectra. The data were identical with those of authentic samples reported in the literature [14, 15].

EXPERIMENTAL

General. $[\alpha]_D$ were measured with a Perkin–Elmer 241 MC polarimeter. MS were recorded with Finnigan TSQ 700 instrument. NMR spectra were obtained using Varian VXR 200 MHz and Bruker DRX 500 MHz instruments. Chemical shifts were reported in δ ppm, with TMS as internal standard. Polyporous resin was produced from Shanghai Institute of Medicinal Industry. Silica gel (230–400 mesh) and RP-18 Lobar column were produced from Merck Company.

Plant material. The aerial parts of *Mussaenda pubescens* were collected from Yongtai District, southeast China in December 1993. The plant was identified by Mr Rentong Chen, Fujian Institute of Traditional Chinese Medicine and a voucher specimen is deposited in the Herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and isolation. Dried plant material 4.0 kg was percolated (4×) with 95% EtOH at room temp. After evapn of EtOH *in vacuo*, the residual aq. soln was extracted with EtOAc and *n*-BuOH, successively.

The EtOAc fr. (250 g) was chromatographed repeatedly on a silica gel column, with a $CHCl_3$–Me_2CO (8:1–1:1) gradient as eluent. The resulting frs were further subjected to sepn on a RP-18 Lobar column, with EtOH–H_2O (8:2). Compounds **4** (120 mg), **5** (40 mg), **6** (65 mg) and **7** (35 mg) were obtained.

The *n*-BuOH fr. (150 g) was subjected to sepn on polyporous resin DA-201, eluted with H_2O, 40 and 90% EtOH, successively. The 90% EtOH fr. (40 g) was chromatographed repeatedly on silica gel columns, with $CHCl_3$–MeOH–H_2O (10:3:0.3–9:3:0.5–7:3:0.5). Frs were further subjected to chromatography on RP-18 Lobar columns, with a MeOH–H_2O (4:6–7:3) gradient. Compounds **1** (150 mg), **2** (10 mg) and **3** (20 mg) were obtained.

Compound **1**. White amorphous powder (150 mg). $[\alpha]_d^{25}$ −14.0° (CH_3OH, *c* 0.27). UV λ_{max}^{MeOH}: 265 nm. Negative ion mode FAB-MS *m/z*: 1505 $[M-H]^-$, 1359 $[M-rha-H]^-$, 1343 $[M-glu-H]^-$, 1213 $[M-rha-rha-H]^-$, 1197 $[M-rha-glu-H]^-$, 118 $[M-glu-glu-H]^-$, 1051 $[M-rha-rha-glu-H]^-$, 1035 $[M-rha-glu-glu-H]^-$, 889 $[M-rha-rha-glu-glu-H]^-$, 873 $[M-rha-glu-glu-glu-H]^-$ 727 $[M-rha-rha-glu-glu-glu]^-$ and 565 $[M-rha-rha-glu-glu-glu-glu-H]^-$. Positive ion mode FAB MS: *m/z* 1507 $[M+H]^+$, 1529 $[M+Na]^+$, 1545 $[M+K]^-$. ^{1}H and ^{13}C NMR data: see Table 1.

Acidic hydrolysis of **1**. Compound **1** (10 mg) was dissolved in 2 N HCl (5 ml) and then heated at 80° in a water bath for 4 hr. After extraction with $CHCl_3$, the aq. residue was evapd to dryness. Sugar components were identified on TLC together with authentic sugar samples, with a mixt. of EtOAc–MeOH–H_2O–HOAc (65:15:15:20) as developing solvent.

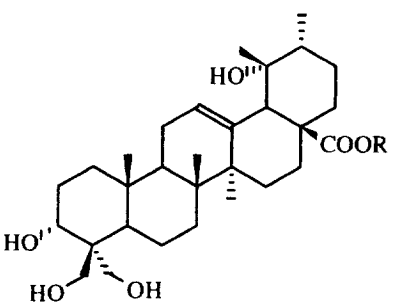
Compound **2**. White amorphous powder (10 mg). $[\alpha]_D^{25}$ −22.8° (CH_3OH, *c* 0.26). Positive ion mode FAB-MS *m/z*: 850 $[M+Na]^+$. Negative ion mode FAB-MS *m/z*: 826 $[M-H]^-$, 633 $[M-glu-H]^-$, 501 $[M-glu-glu-H]^-$. ^{1}H NMR (200 MHz, C_5D_5N): δ 6.35 (1H, *d*, 7.6), 6.26 (1H, *d*, 7.4), 5.22 (1H, *br s*), 3.40 (1H, *dd*, 11.2, 4.0), 2.94 (1H, *br s*), 1.77 (3H, *s*), 1.71 (3H, *s*), 1.42 (3H, *s*), 1.27 (3H, *s*), 1.27 (3H, *s*) and 1.08 (3H, *d* 6.4). ^{13}C NMR (50 MHz, C_5D_5N) data: see Table 2.

Acidic hydrolysis of **2**. Same procedure as above.

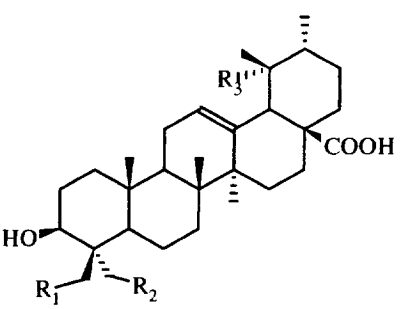
Compound **3**. White amorphous powder (20 mg). DCI MS *m/z*: 683 $[M+NH_4]^+$, 521 $[M-glu+NH_4]^+$. ^{13}C NMR: see Table 2.

Acidic hydrolysis of **3**. Same procedure as above.

Compound **4**. White amorphous powder (120 mg). DCI MS *m/z*: 522 $[M+NH_4]^+$. ^{13}C NMR: see Table 2.



4. R=H
4a. R=CH_3.



5. R1=H, R2=R3=OH
6. R1=R3=OH, R2=H
7. R1=R3=H, R2=OH

Methylation of **4**. Compound **4** (20 mg) was dissolved in 10 ml MeOH, and then mixed with 5 ml of a diazomethane–Et_2O soln. After being kept at room temp. for 24 h, the solvent was evapd *in vacuo*, and then the residue was subjected to chromatography on a silica gel column, with a mixt. of petrol–Me_2CO (1.5:1) as eluent. Pure **4a** (18 mg) was obtained.

Compound **4a**. White amorphous powder. TSP MS *m/z*: 537 $[M+NH_4]^+$, 542 $[M+Na]^+$. ^1H and ^{13}C NMR data were identical to those reported in the literature [9].

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REFERENCES

1. Jiangsu New Medical College, *Dictionary of Chinese Traditional Medicine*. Shanghai Science and Technology Press, Shanghai, 1986, p. 176.
2. Fujian Institute of Medicine, *Encyclopedia of Fujian Plant Medicines*, Vol. 1. Fujian People's Press, Fuzhou, 1979, p. 447.
3. Liu, X. J., Liang, G. J., Cai, X., Chao, Q., Chu, Y. H., Bao, Y. M., Long, X. H. and Wang, G. Q., *Acta Academy of Medicine Shanghai*, 1986, **13**, 273.
4. Zhao, W. M., Xu, J. P., Qin, G. W., Xu, R. S., Wu, H. M. and Weng, G. H., *Journal of Natural Products*, 1994, **57**, 1613.
5. Zhao, W. M., Xu, J. P., Qin, G. W. and Xu, R. S., *Phytochemistry*, 1995, **39**, 191.
6. Zhao, W. M., Xu, R. S., Qin, G. W., Tang, X. C. and Li, X. Y., *Natural Products Science*, 1995, **1**, 61.
7. Pawan, K. A., *Phytochemistry*, 1992, **31**, 3307.
8. Zhao, W. M., Xu, R. S., Qin, G. W., Vaisar, T. and Lee, M. S., *Phytochemistry*, 1996, **42**, 1131.
9. Qin, G. W., Chen, Z. X., Xu, R. S., Jiang, Z. F. and Liang, J. G., *Acta Chimica Sinica*, 1987, **45**, 249.
10. Takahashi, K. and Takani, M., *Chemical and Pharmaceutical Bulletin*, 1978, **26**, 2689.
11. Nandy, A. K., Podder, G., Sahu, N. P. and Mahato, S. B., *Phytochemistry*, 1989, **28**, 2769.
12. Mahato, S. B., Nandy, A. K. and Kundu, A. P., *Tetrahedron*, 1992, **48**, 2483.
13. Fang, S. Y., He, Z. S., Fan, G. J., Wu, H. M. and Xu, J. F., *Journal of Natural Products*, 1996, **59**, 304.
14. Nakatani, M., Miyazaki, Y., Iwashita, T., Naoki, H. and Hase, T., *Phytochemistry*, 1989, **28**, 1579.
15. Srivastava, S. K. and Jain, D. C., *Phytochemistry*, 1989, **28**, 644.