New Steroidal Saponins from the Rhizomes of *Anemarrhena asphodeloides* BUNGE (Liliaceae)

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From the rhizome of *Anemarrhena asphodeloides* BUNGE (Liliaceae), four new steroidal saponins named anemarrhenasaponins I—IV (1—4) were isolated, together with known saponins, timosaponin A-III (5), marcogenin diglycoside (6) and timosaponin B-II (7) and a xanthone C-glycoside, mangiferin. These saponins are coprostane type steroidal glycosides. Their structures were established on the basis of spectroscopic and chemical evidence.

Keywords *Anemarrhema asphodeloides*; Liliaceae; anemarrhenasaponin; coprostane glycoside; spirostanol; furostanol

The rhizome of *Anemarrhena asphodeloides* BUNGE (Liliaceae; Chimo in Japanese) has been known to exhibit anti-diabetic activity,[1] platelet aggregation activity[2] and diuretic activity,[3] and has been widely used in Chinese and Japanese folk medicines. Chemical constituents of the rhizome have been studied by several groups, and the isolation and identification of steroidal saponins,[2,4-7] xanthone C-glycosides[8] and polysaccharides[1] were reported. In this paper, we will report the isolation and structure elucidation of four new coprostane type saponins, named anemarrhenasaponins I—IV (**1**—**4**), isolated together with known saponins, timosaponin A-III (**5**), marcogenin diglycoside (**6**), timosaponin B-II (**7**), and mangiferin (xanthone C-glycoside).

A hot 50% aqueous methanol extract of the rhizomes of *A. asphodeloides* was roughly separated into CH_2Cl_2-, AcOEt- and 1-BuOH-soluble fractions. The 1-BuOH-soluble fraction was chromatographed on a silica-gel column, and followed by repeated preparative HPLC on reversed phase columns of ODS-4251-D and Capcell Pak C_8 to isolate **1**—**7** and mangiferin. The known compounds were identified by comparison of the spectral data with previously reported data.

Anemarrhenasaponin I (**1**), $C_{39}H_{66}O_{14} \cdot H_2O$, obtained as colorless needles, was positive to Ehrlich's reagent,[9] which suggested that **1** was a furostanol glycoside. The fast-atom bombardment mass spectrum (FAB-MS) of **1** revealed a quasimolecular ion peak at *m/z* 781 $[M+Na]^+$. The ^1H-NMR spectrum of **1** exhibited two singlet signals at δ 0.97 and 1.02 and three doublet signals at δ 0.89 ($J=7.3$ Hz), 0.90 ($J=7.3$ Hz) and 1.33 ($J=6.8$ Hz) due to steroidal methyl groups, and two doublet signals at δ 4.85 ($J=7.6$ Hz) and 5.21 ($J=7.7$ Hz), attributable to the anomeric protons of two β-pyranose residues. Furthermore, the H-16 signal observed at δ 5.05 (d, $J=8.8$, 3.6 Hz) of **1** shifted at a lower field, by 0.37 ppm, compared with that of timosaponin A-III (**5**) (δ, 4.68, ddd, $J=11.6$, 7.6, 0.2 Hz), which indicated that an electron attracting group such as an OH group linked to the position of C-15 on the aglycon. Acid hydrolysis of **1** gave glucose and galactose (sugar ratio = 1 : 1) and compound (**8**). Compound **8** showed a quasimolecular ion peak at *m/z* 437 $[M+Na]^+$. The ^1H-NMR spectrum of **8** exhibited a singlet vinyl methyl signal at δ 1.59 attributable to 21-CH_3, together with two singlet methyl signals at δ 0.68 (18-CH_3) and 0.98 (19-CH_3) and two doublet methyl signals at δ 0.88 (6H, $J=6.6$ Hz, 26- and 27-CH_3). The existence of the vinyl group in **8** was further confirmed by the ^{13}C-NMR spectrum which displayed carbon signals attributable to enol carbons at δ 103.4 (C-20) and 152.0 (C-22). In the H–H correlation spectroscopy (COSY) spectrum of **8**, H-15, H-16 and H-17 observed at δ 4.12 (dd, $J=9.5$, 3.3 Hz), 4.52 (dd, $J=10.6$, 3.3 Hz) and 2.62 (d, $J=10.6$ Hz), respectively, correlated with each other. From both the chemical shift and coupling constant of the H-15, it was indicated that a hydroxyl group was linked to the C-15 position in **8**. A signal enhancement (6.8%) of the H-15 of **8** was observed in the nuclear Overhauser effect (NOE) with irradiation at δ 0.68 (18-CH_3 arranged in β), which indicated that the H-15 was arranged in β as well as the 18-CH_3; thus the 15-OH group was arranged in α. The ^{13}C-NMR spectrum of **1** displayed a total of 39 signals, in which a signal at δ 110.3 was assignable to the C-22 of the furostanol, and 12 were assignable to the carbons, due to two pyranoses. However, no vinyl carbon appeared in the spectrum, so it could therefore be presumed that the vinyl group in **8** was formed by an elimination between a hydroxyl group at C-22 and a hydrogen at C-20 on the acid hydrolysis of **1**. The arrangement of β-gluco- and β-galactopyranose was elucidated by the comparison of ^1H- (Table I) and ^{13}C-NMR spectra (Table II) of **1** with those of **5**. The spectral data suggested that the same disaccharide, 2-*O*-(β-D-glucopyranosyl)-β-D-galactopyranose, as that in **5** was linked to the position of *O*-3 of the aglycon of **1**. From these data, the structure of anemarrhenasaponin I was determined to be **1**.

Anemarrhenasaponin II (**2**), $C_{39}H_{66}O_{14} \cdot H_2O$, obtained as colorless needles was also positive to Ehrlich's test. The FAB-MS of **2** showed the same quasimolecular ion peak at *m/z* 781 $[M+Na]^+$ as that of **1**. The ^1H-NMR spectrum of **2** exhibited two singlet methyl signals at δ 0.92 and 1.02 due to 18-CH_3 and 19-CH_3, respectively, and three doublet methyl signals at δ 1.33 (3H, d, $J=6.8$ Hz, 21-CH_3) and 0.92 (6H, d, $J=7.3$ Hz, 26- and 27-CH3). H-15 and H-16 were observed at δ 4.00—4.35 (overlapped with H-6′ and H-6″ on sugar residues) and 5.07 (dd, $J=7.8$, 2.7 Hz), respectively. The ^{13}C-NMR spectrum of **2** displayed almost similar signals to those of **1** for carbons on both pyranoses and aglycon. The NOE

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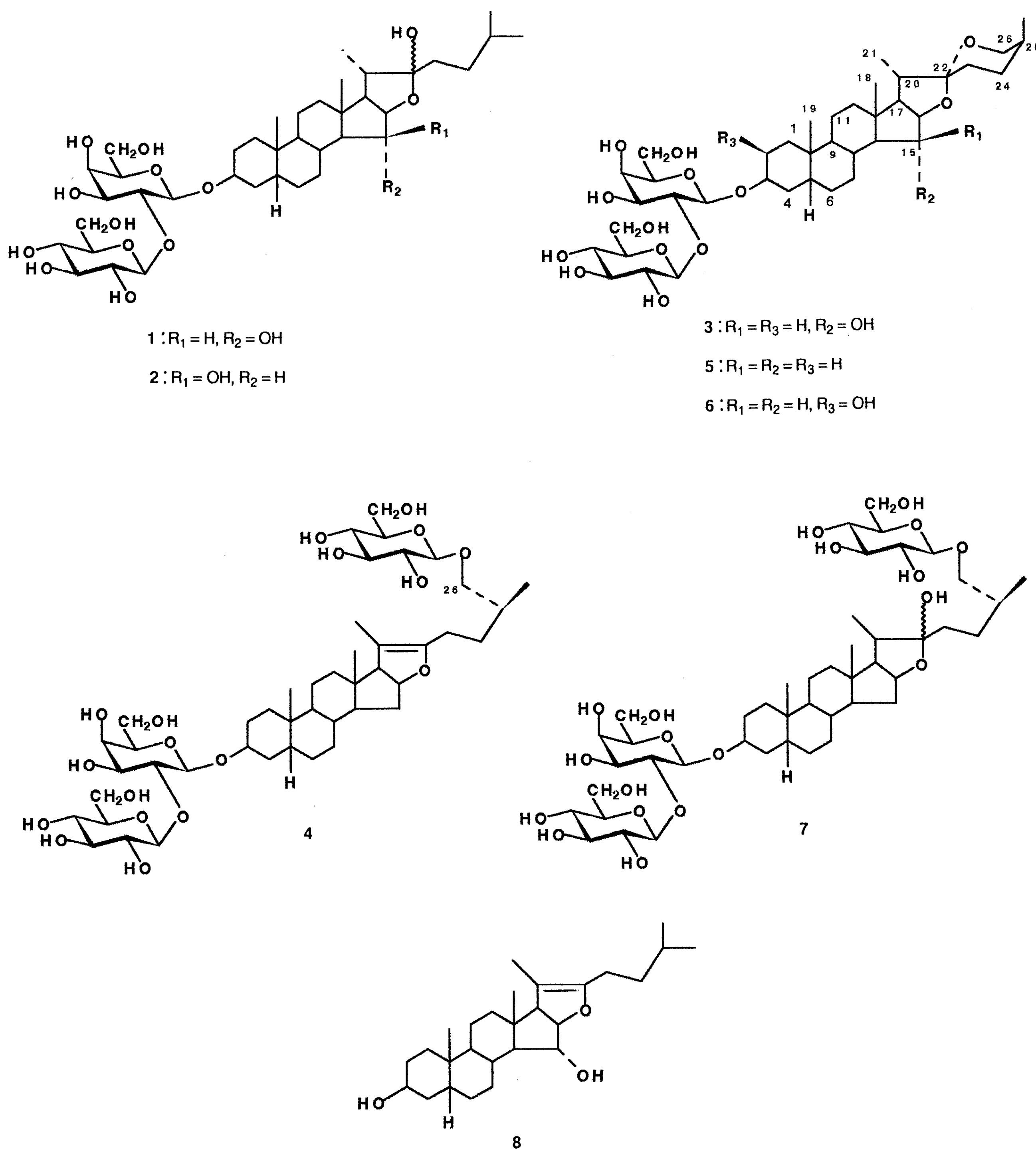

Chart 1

with irradiation with β-18-CH_3 at δ 0.92 showed no remarkable enhancement of the H-15 of **2**, different from that in the case of **1**, which suggested that the H-15 of **2** was arranged in α; thus, the 15-OH group was arranged in β. So, the structure of anemarrhenasaponin II was determined to be **2**.

Anemarrhenasaponin III (**3**), $C_{39}H_{64}O_{14} \cdot H_2O$, revealed a quasimolecular ion peak at m/z 779 $[M+Na]^+$ in the FAB-MS. The ^{1}H-NMR spectrum of **3** displayed two singlet methyl signals at δ 0.87 (18-CH_3) and 0.99 (19-CH_3), and two doublet methyl signals at δ 1.04 (J=7.0 Hz, 27-CH_3) and 1.12 (J=6.7 Hz, 21-CH_3), as well as that of **5**, together with two anomeric protons at δ 4.83 (d, J=7.6 Hz) and 5.18 (d, J=7.7 Hz). H-15 and H-16 were observed at δ 4.00—4.30 (overlapped with the protons of sugar residues) and 4.91 (dd, J=8.9, 3.7 Hz), respectively, similarly to those of **1**. The H-26 signals were exhibited at δ 3.27 (1H, d, J=11.0 Hz, H-26a) and 3.91 (1H, dd, J=11.0, 2.5 Hz, H-26b) similarly to **5**. A signal enhancement (4.8%) of the H-15 of **3** was observed in the NOE with irradiation at δ 0.87 (β-arranged 18-CH_3). These spectral data indicated that **3** was spirostanol and had an

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TABLE I. ^{1}H-NMR Spectra of Compounds **1**—**3** and **5**[a)]

	1	2	3	5
Aglycon[b)]				
18-CH_3	0.97 (s)	0.92 (s)	0.87 (s)	0.82 (s)
19-CH_3	1.02 (s)	1.02 (s)	0.99 (s)	0.96 (s)
21-CH_3	1.33 (d, 6.8)	1.33 (d, 6.8)	1.12 (d, 6.7)	1.15 (d, 7.1)
26-CH_3	0.89 (d, 7.3)	0.92 (d, 7.3)	—	—
27-CH_3	0.90 (d, 7.3)	0.92 (d, 7.3)	1.04 (d, 7.0)	1.08 (d, 7.0)
26-$C\underline{H}_aH_b$–	—	—	3.27 (d, 11.0)	3.37 (d, 11.0)
26-$CH_a\underline{H}_b$–	—	—	3.91 (dd, 11.0, 2.5)	4.07 (11.0, 2.4)
H-3	4.26 (br s)	4.28 (br s)	4.25 (br s)	4.30 (br s)
H-15	5.05[c)]	4.00—4.35[c)]	4.00—4.30[c)]	1.25—2.25[d)]
H-16	5.05 (dd, 8.8, 3.6)	5.07 (dd, 7.8, 2.7)	4.91 (dd, 8.9, 3.7)	4.68 (ddd, 11.6, 7.6, 0.2)
H-17	2.00—3.00[d)]	2.00—3.00[d)]	2.00—3.00[d)]	2.00—3.00[d)]
Sugar				
H-1′	4.85 (d, 7.6)	4.87 (d, 7.6)	4.83 (d, 7.6)	4.88 (d, 7.6)
H-2′	4.60 (dd, 8.6, 7.6)	4.63 (dd, 9.1, 7.6)	4.56 (dd, 9.2, 7.6)	4.62 (dd, 9.5, 7.6)
H-3′	4.22 (dd, 8.6, 3.1)	4.23 (dd, 9.1, 3.0)	4.20 (dd, 9.2, 3.0)	4.23 (dd, 9.5, 3.1)
H-4′	4.52 (d, 3.1)	4.54 (d, 3.0)	4.49 (d, 3.0)	4.53 (d, 3.1)
H-5′	3.98 (dd, 6.7, 6.7)	3.99 (dd, 6.7, 6.7)	4.00—4.32	4.00 (dd, 7.3, 6.4)
H-6′$_a$, 6′$_b$	4.20—4.36	4.35—4.45	4.00—4.32	4.32—4.48
H-1″	5.21 (d, 7.7)	5.24 (d, 7.6)	5.18 (d, 7.7)	5.24 (d, 7.7)
H-2″	4.01 (dd, 8.7, 7.7)	4.05 (dd, 8.9, 7.6)	3.99 (dd, 8.8, 7.7)	4.03 (dd, 8.7, 7.7)
H-3″	4.13 (dd, 9.1, 8.7)	4.16 (dd, 9.2, 8.9)	4.10 (dd, 9.2, 8.8)	4.15 (dd, 9.1, 8.7)
H-4″	4.25 (dd, 9.4, 9.1)	4.28 (dd, 9.2, 9.2)	4.22 (dd, 9.2, 9.2)	4.26 (dd, 9.4, 9.1)
H-5″	3.78 (m)	3.81 (m)	3.75 (ddd, 9.2, 4.0, 2.4)	3.81 (ddd, 9.2, 4.0, 2.4)
H-6″$_a$, 6″$_b$	3.79—4.00	4.25—4.45	4.30—4.62	4.32—4.48

a) Spectra run in pyridine-d_5. The signal assignments were based on H–H COSY, and multiplicities and coupling constants (*J* in Herz) are given in parentheses. *b*) Only assignable signals for aglycons are listed. *c*) Overlapped with H-6′ and H-6″. *d*) Overlapped with other signals on the aglycon.

α-OH group at the position of C-15. This was confirmed by the ^{13}C-NMR spectrum of **3**; signals of C-15, C-16 and C-22 of **3** shifted at δ 78.7, 91.3 and 109.3, respectively, similarly to the corresponding ones of **1**, and that of C-26 shifted at δ 65.0, similarly to the corresponding one of **5**. The signals due to 12 carbons on the two pyranoses of **3** were very close to those of **1**, **2** and **5**, which suggests that the same oligosaccharide as that in **1**, **2** and **5** linked to the *O*-3 position on the aglycon with the same glycosidic linkage. These data led to the proposed structure **3** for anemarrhenasaponin III.

Anemarrhenasaponin IV (**4**), $C_{45}H_{74}O_{18}\cdot H_2O$, was obtained as an amorphous powder. Compound **4** showed a quasimolecular ion peak at *m/z* 925 $[M+Na]^+$. The ^{13}C-NMR spectrum of **4** exhibited, together with a signal at δ 84.5 assignable to C-16, two vinyl carbon signals at δ 105.3 and 152.3 attributable to C-20 and C-22 respectively, similarly to those of **8**, and three anomeric carbon signals at δ 102.3, 103.4 and 105.0, which suggests that **4** is a furostanol triglycoside having an enolate group on the E ring. Treatment of **4** with β-glucosidase gave compound **5**, which was confirmed by FAB-MS and ^{1}H- and ^{13}C-NMR. So, the structure of anemarrhenasaponin IV was determined to be **4**.

As compounds **1**—**3** were obtained as crystals, signals of only one of the isomers with respect to the configuration at the position of C-22 were observed in each ^{13}C-NMR spectrum in pyridine-d_5. However, their ^{13}C-NMR spectra in D_2O revealed very complex signal patterns due to a mixture of both isomers. At present, the exact configuration at the position of C-22 of **1**, **2** and **4** remains unresolved.

Experimental

Materials and General Procedure Rhizomes of *Anemarrhema asphodeloides* (grown in Shan Xi Sheng prefecture in China) were purchased from Nakai-Kohshindo, Kobe, Hyogo prefecture, Japan. β-Glucosidase (from almond) was purchased from Wako Pure Chemical Industries, Ltd., Japan. Other chemicals and solvents were of reagent grade, and were obtained from commercial sources. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The thin-layer chromatography (TLC) utilized Kieselgel HF_{254} (Merck), and spots were detected by spraying the plates with $Ce(SO_4)_2$–10% H_2SO_4 (1 : 9) reagent followed by heating at 100 °C for 10 min. Column chromatography was carried out on Wakogel C-200 and the eluates were monitored by TLC. An SSC-6300 (Senshu Scientific Co., Ltd.) equipped with an SSC autoinjector 6310 and an SSC fraction collector 6320 for preparative HPLC using ODS-4521-D and Capcell Pak C_8 (Shiseido, 10 mm i.d. × 250 mm; flow rate, 1.0 ml/min; column temp. 40 °C) was used. Gas liquid chromatography (GLC) was run on a Shimadzu GC-9A with a flame ionization detector using a glass column (2.0 m × 4 mm i.d.) packed with 3% ECNSS-M (column temperature, gradient from 180° up to 240 °C (5 °C/min); H_2 60 ml/min, N_2 80 ml/min). ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL Alhpa NMR spectrometer at 500 and 125 MHz, respectively, and chemical shifts were given in ppm with tetramethylsilane as an internal standard. Multiplicities of ^{1}H- and ^{13}C-NMR signals were indicated as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). Fast-atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-DX 300 mass spectrometer. Optical rotations were measured at 25 °C with a JASCO J-20A spectropolarimeter.

Isolation Pulverized rhizomes of *Anemarrhena asphodeloides* (1.3 kg) were extracted three times with hot aqueous 50% MeOH (2.0 l). After removing the solvent under reduced pressure, the concentrated material (500 g) was suspended in H_2O (2.0 l) and extracted with CH_2Cl_2 (800 ml × 4), then with AcOEt (800 ml × 3) and finally with 1-BuOH (800 ml × 5). The combined 1-BuOH extracts were evaporated to give a residue (125 g). The residue was subjected to column chromatography on silica-gel (20 × 100 cm), and eluted first with $CHCl_3$–MeOH–H_2O (65 : 35 : 20, lower layer, 6.0 l), then with $CHCl_3$–MeOH–H_2O (65 : 35 : 15, lower layer, 4.5 l) and finally with $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower

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TABLE II. ^{13}C-NMR Chemical Shifts of Compounds **1**—**5**[a)]

	1	**2**	**3**	**4**	**5**
Aglycon					
C-1	30.6 (t)[b)]	30.6 (t)[b)]	30.8 (t)[b)]	30.9 (t)[b)]	30.9 (t)[b)]
C-2	27.1 (t)[c)]	27.1 (t)[c)]	27.4 (t)[c)]	26.9 (t)	27.0 (t)[c)]
C-3	75.4 (d)	75.4 (d)	75.3 (d)	75.2 (d)	75.4 (d)
C-4	31.0 (t)[b)]	31.0 (t)[b)]	31.0 (t)[b)]	31.3 (t)[b)]	30.9 (t)[b)]
C-5	36.3 (d)	36.2 (d)	36.3 (t)	38.8 (d)	36.8 (t)
C-6	26.9 (t)[c)]	26.9 (t)[c)]	27.1 (t)[c)]	26.9 (t)	26.7 (t)[c)]
C-7	26.7 (t)[c)]	26.7 (t)[c)]	26.8 (t)[c)]	26.9 (t)	26.7 (t)[c)]
C-8	36.9 (d)	36.9 (d)	36.9 (d)	36.8 (d)	35.5 (d)
C-9	40.2 (d)	40.2 (d)	40.3 (d)	40.1 (d)	40.2 (d)
C-10	35.2 (s)	35.4 (s)	35.3 (s)	34.4 (s)	35.2 (s)
C-11	21.1 (t)	21.1 (t)	21.1 (t)	21.3 (s)	21.1 (t)
C-12	37.6 (t)	36.9 (t)	40.9 (t)	40.1 (t)	40.3 (t)
C-13	41.2 (s)	41.2 (s)	41.0 (s)	43.9 (s)	40.9 (s)
C-14	60.8 (d)	60.5 (d)	60.8 (d)	54.7 (d)	56.4 (d)
C-15	78.0 (d)	79.0 (d)	78.7 (d)	33.6 (t)	32.1 (t)
C-16	91.2 (d)	92.4 (d)	91.3 (d)	84.5 (d)	81.3 (d)
C-17	61.3 (d)	61.5 (d)	60.3 (d)	64.6 (d)	61.9 (d)
C-18	18.0 (q)	17.7 (q)	17.9 (q)	17.1 (q)	16.6 (q)
C-19	24.1 (q)	24.1 (q)	24.1 (q)	24.0 (q)	24.0 (q)
C-20	40.6 (d)	40.3 (d)	42.4 (d)	105.3 (s)	40.5 (d)
C-21	16.5 (q)	15.9 (q)	14.9 (q)	11.8 (q)	14.9 (q)
C-22	110.3 (s)	110.3 (s)	109.3 (s)	152.3 (s)	109.7 (s)
C-23	35.6 (t)	35.3 (t)	26.3 (t)	20.6 (t)	26.4 (t)[d)]
C-24	24.1 (t)	24.1 (t)	26.2 (t)	30.9 (t)	26.2 (t)[d)]
C-25	28.1 (d)	28.7 (d)	27.4 (t)	29.1 (d)	27.5 (d)
C-26	22.8 (q)[d)]	22.8 (q)[d)]	65.0 (t)	75.4 (t)	65.7 (t)
C-27	22.7 (q)[d)]	22.7 (q)[d)]	16.3 (q)	17.1 (q)	16.3 (q)
O-3 Galactose					
C-1	102.2 (d)	102.4 (d)	102.3 (d)	102.3 (d)	102.4 (d)
C-2	81.6 (d)	81.7 (d)	81.7 (d)	81.5 (d)	81.6 (d)
C-3	75.0 (d)	75.1 (d)	75.0 (d)	76.5 (d)	75.1 (d)
C-4	69.7 (d)	69.7 (d)	69.7 (d)	69.7 (d)	69.7 (d)
C-5	76.4 (d)	76.6 (d)	76.7 (d)	76.7 (d)	76.5 (d)
C-6	62.0 (d)	62.1 (d)	62.0 (d)	62.0 (d)	62.1 (d)
O-3 Glucose					
C-1	105.9 (d)	106.0 (d)	105.9 (d)	105.0	105.9 (d)
C-2	76.7 (d)	76.9 (d)	76.4 (d)	76.1 (d)	76.8 (d)
C-3	77.8 (d)	77.9 (d)	77.8 (d)	77.9 (d)	77.8 (d)
C-4	71.5 (d)	71.6 (d)	71.5 (d)	71.6 (d)	71.6 (d)
C-5	78.2 (d)	78.3 (d)	78.2 (d)	78.2 (d)	78.3 (d)
C-6	62.6 (t)	62.7 (t)	62.6 (t)	62.7 (t)	62.7 (t)
O-26 Glucose					
C-1				103.4 (d)	
C-2				76.2 (d)	
C-3				78.1 (d)	
C-4				71.6 (d)	
C-5				78.3 (d)	
C-6				62.7 (t)	

a) All spectra run in pyridine-d_5. The signal assignments of **1**—**3** and **5** were based on H–C COSY and distortionless enhancement by polarization transfer (DEPT) methods. The multiplicities in the parentheses were based on the DEPT method without proton decoupling. The chemical shifts of **4** were obtained by comparison with those of **1**—**3** and **5** and reported ones.[7)] *b*—*d*) These values are interchangeable in each column.

layer, 3.0 l) to give nine fractions (1—9) (each 1.5 l fraction). After removing the solvent, fractions 1—9 gave residues of 2.6, 1.7, 0.5, 47.8, 2.8, 10.7, 12.5, 5.5 and 10.6 g, respectively, in which frs. 4, 5 and 8 gave a positive trichloroantimony test.[8)] Rechromatography of the residue obtained from fr. 4 gave compound **5** (36.3 g). Successively preparative HPLC of the residue obtained from fr. 5 using an ODS-4251-D column with 30% H_2O in MeOH and Capcell Pac C_8 column with 25, 40 and 50% H_2O in CH_3CN afforded compounds **1** (350 mg), **2** (67.0 mg), **3** (160 mg) and **6** (1.04 g). Preparative HPLC of the residue obtained from fr. 8 using the ODS column with 40% H_2O in MeOH gave compounds **4** (315 mg), **7** (1.7 g) and mangiferin (1.8 g).

Compound 1 Colorless needles from EtOH, mp 202—204 °C, $[\alpha]_D$ −41.7° (c=1.08, pyridine). Ehrlich's reagent: positive. FAB-MS m/z: 781 $[M+Na]^+$. *Anal.* Calcd for $C_{39}H_{66}O_{14} \cdot H_2O$: C, 60.28; H, 8.56. Found: C, 60.25; H, 8.57. ^{1}H-NMR spectrum: Table I. ^{13}C-NMR spectrum: Table II.

Compound 2 Colorless needles from EtOH, mp 174—176 °C, $[\alpha]_D$ −39.1 (c=0.95, pyridine). Ehrlich's reagent: positive. FAB-MS m/z: 781 $[M+Na]^+$. *Anal.* Calcd for $C_{39}H_{66}O_{14} \cdot H_2O$: C, 60.28; H, 8.56. Found: C, 60.13; H, 8.60. ^{1}H-NMR spectrum: Table I. ^{13}C-NMR spectrum: Table II.

Compound 3 Colorless needles from EtOH, mp 260—262 °C, $[\alpha]_D$ −45.1° (c=1.12, pyridine). FAB-MS m/z: 779 $[M+Na]^+$. *Anal.* Calcd for $C_{39}H_{64}O_{14} \cdot H_2O$: C, 60.45; H, 8.58. Found: C, 60.15; H, 8.51. ^{1}H-NMR spectrum: Table I. ^{13}C-NMR spectrum: Table II.

Compound 4 A white amorphous powder, $[\alpha]_D$ −11.5° (c=1.05, pyridine). Ehrlich's reagent: positive. FAB-MS m/z: 925 $[M+Na]^+$. *Anal.* Calcd for $C_{45}H_{74}O_{18} \cdot H_2O$: C, 58.68; H, 8.10. Found: C, 58.56; H, 8.17. ^{13}C-NMR spectrum: Table II.

Acid Hydrolysis of Compound 1 A solution of **1** (80 mg) in 1 N H_2SO_4 (2 ml) in H_2O-dioxane (1 : 1) was heated at 100 °C for 1 h. After dilution with water, the reaction mixture was extracted twice with AcOEt, and the water layer was neutralized with aqueous $Ba(OH)_2$ solution, and centrifugated. The resulting supernatant was evaporated to give a residue. Reduction with $NaBH_4$ was followed by treatment with Ac_2O–pyridine, and the residue gave an acetylated mixture. GLC of the mixture showed two peaks of peracetylated glucitol and galactitol (t_R=13.4 and 12.6 min, respectively) at a ratio of 1 : 1. The AcOEt layer was concentrated and subjected to column chromatography (a gradient of 0—10% acetone in benzene) to give compound **8** (48 mg, 85%) as a white amorphous powder. FAB-MS of **8** m/z: 437 $[M+Na]^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.88 (6H, d, J=6.6 Hz, 26- and 27-CH_3), 0.98 (3H, s, 19-CH_3), 1.59 (3H, s, 21-CH_3), 2.07 (2H, dd, J=7.9, 7.0 Hz, 2×H-23), 2.62 (1H, d, J=10.6 Hz, H-17), 4.10 (1H, br s, H-3), 4.12 (1H, dd, J=9.5, 3.3 Hz, H-15), 4.51 (1H, dd, J=10.6, 3.3 Hz, H-16). ^{13}C-NMR ($CDCl_3$) δ: 31.1 (C-1), 27.2 (C-2), 67.0 (C-3), 31.9 (C-4), 36.2 (C-5), 28.6 (C-6), 26.5 (C-7), 36.9 (C-8), 40.6 (C-9), 35.3 (C-10), 21.1 (C-11), 38.7 (C-12), 43.3 (C-13), 59.0 (C-14), 81.2 (C-15), 96.1 (C-16), 61.4 (C-17), 15.5 (C-18), 23.9 (C-19), 103.4 (C-20), 11.5 (C-21), 152.0 (C-22), 23.7 (C-23), 25.9 (C-24), 28.9 (C-25), 22.4 (C-26), 22.3 (C-27). *Anal.* Calcd for $C_{27}H_{44}O_3$: C, 56.24, H, 7.69. Found: C, 56.15; H, 7.71.

Enzymic Degradation of Compound 4 To a solution of **4** (100 mg) in a Na_2HPO_4–NaH_2PO_4 buffer solution (pH 6.2)[10)] (1 ml) was added β-glucosidase (10 mg), and the mixture was incubated at 37 °C for 24 h. EtOH (0.5 ml) was added to the reaction mixture, which was then heated at 80 °C for 10 min, and filtered. The filtrate was evaporated to obtain a residue, which was subjected to column chromatography ($CHCl_3$–MeOH–H_2O, 65 : 35 : 10, lower layer) to give product **5** (56 mg) as colorless needles, mp >300 °C (lit. mp 317—322 °C) (dec.).[4)] This product was identified as **5** by FAB-MS (m/z: 763 $[M+Na]^+$) and ^{1}H- (Table I) and ^{13}C-NMR spectra (Table II).

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