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A PYRROLINE GLUCOSIDE ESTER AND STEROIDAL SAPONINS FROM *LILIUM MARTAGON*

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Key Word Index—*Lilium martagon*; Liliaceae; phenylpropanoid esters; pyrroline derivatives; jatropham derivatives; steroidal saponins.

Abstract—A new phenylpropanoid ester of a pyrroline derivative and two new steroidal saponins were isolated from the fresh bulbs of *Lilium martagon*, along with several previously known compounds. The structures of the new compounds were determined by spectroscopic data, hydrolysis, and by comparison with spectral data of known compounds to be (−)-5-hydroxy-3-methyl-3-pyrrolin-2-one 5-*O*-(6-*O*-*p*-coumaroyl-β-D-glucopyranoside), (25*S*)-spirost-5-ene-3β,17α,27-triol 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside} and (25*S*)-5α-spirostane-3β,17α,27-triol 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside}, respectively. *Lilium martagon* crosses well with *L. hansonii* to produce a valuable garden hybrid lily. In this study, the secondary metabolites of *L. martagon* were revealed to be closely related to those of *L. hansonii*, giving a good example of the correlation between the secondary metabolites and cross-compatibility.

INTRODUCTION

Lilium martagon is widely distributed throughout Europe and Asiatic Russia and known as a most adaptable lily, growing freely either in sun and shade, and in a wide variety of soils including both heavy clay and chalk [1]. A survey of the literature showed that previous phytochemical studies carried out on *L. martagon* had demonstrated the occurrence of γ-methyleneglutamic acid as the most abundant free amino acid in the roots, bulbs, leaves and petals [2], and 5-hydroxy-3-methyl-3-pyrrolin-2-one in the aerial parts and bulbs, the content of which was highest in April when vegetation growth was just beginning [3]. However, there has been no systematic exploration of the secondary metabolites of *L. martagon*. Our detailed investigation of the bulbs has led to the discovery of a new phenylpropanoid ester of a pyrroline derivative and two new spirostanol saponins, together with five known compounds. This paper reports the structural elucidation of the new compounds by spectroscopic data, hydrolysis, and by comparison with spectral data of known compounds.

RESULTS AND DISCUSSION

Fresh bulbs of *L. martagon* (3.5 kg) was extracted with hot methanol. The 1-butanol-soluble phase of the methanolic extract gave eight compounds: **1** (207 mg), **2** (50.7 mg), **3** (3.47 g), **4** (2.53 g), **5** (82.7 mg), **6** (43.1 mg), **7** (34.1 mg) and **8** (25.9 mg), after a series of chromatographic separations.

Compounds **1**–**5** are known constituents and the structures were identified as 3,6′-di-*O*-feruloylsucrose [4, 5], (2*S*)-1-*O*-caffeoyl-3-*O*-β-D-glucopyranosylglycerol (regaloside C) [6], (−)-5-hydroxy-3-methyl-3-pyrrolin-2-one (jatropham) [7, 8], jatropham 5-*O*-β-D-glucopyranoside [7, 8] and jatropham 5-*O*-[*O*-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranoside] [8]. The physical and spectral data are consistent with the indicated literature values. Copies of the original spectra are obtainable from the authors.

Compound **6** was obtained as a pale-yellow amorphous solid, $[\alpha]_D$ −26.3° (methanol). The molecular formula $C_{20}H_{23}NO_9$ was deduced from the ^{13}C NMR spectrum and high-resolution positive-ion FAB mass spectrum showing an accurate $[M]^+$ ion at *m/z* 422.1429 (calc. 422.1451). The spectral features of **6** were essentially analogous to those of **4**; the ^1H NMR spectrum in pyridine-d_5 showed signals for an olefinic proton at δ6.75 (*br s*), an oxymethine proton at δ5.87 (*br s*) and a methyl group attached on a double bond at δ1.86 (*br s*), and sequential signals due to a β-glucose moiety, among which an anomeric proton was assigned at δ5.09 (*d*, *J* = 7.9 Hz). In addition, two pairs of doublet signals due to *trans*-alkene protons at δ8.03 and 6.63 (each *d*, *J* = 15.9 Hz) and *p*-disubstituted aromatic protons at δ7.57 and 7.14 (each

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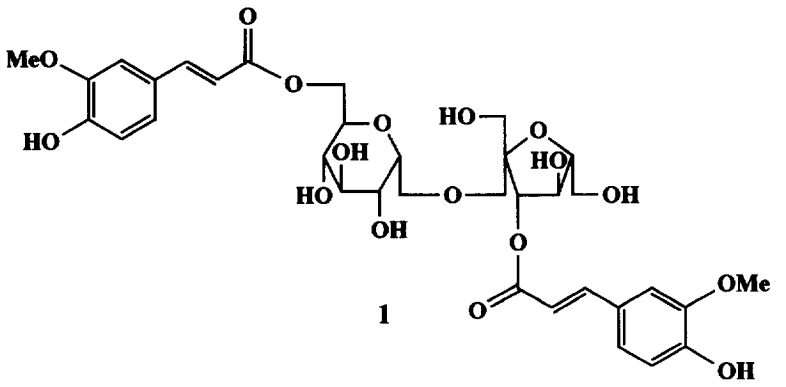
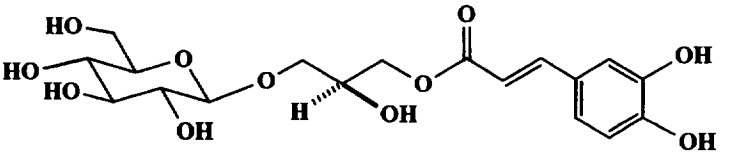


1



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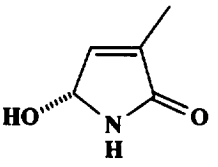

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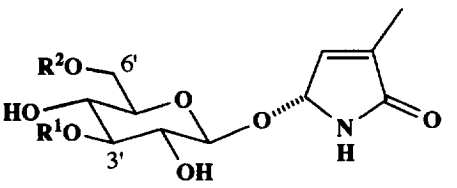
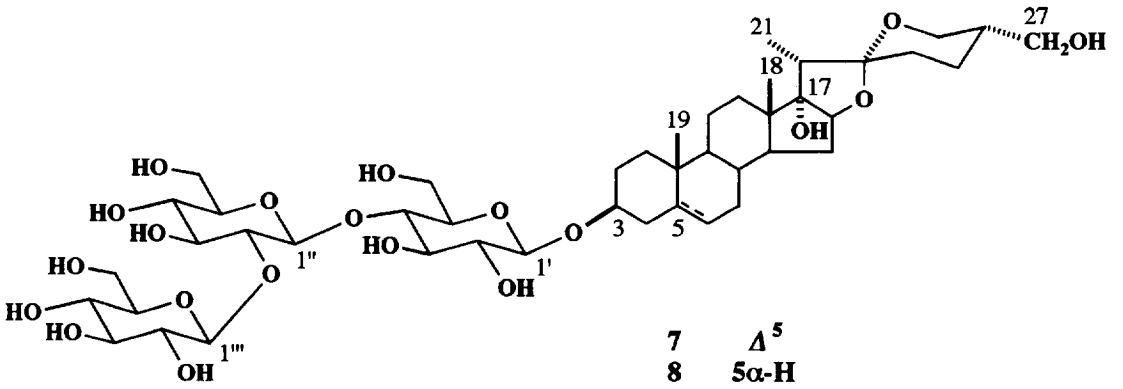


	R^1	R^2
4	H	H
5	β-D-Glucopyranosyl	H
6	H	*p*-Coumaroyl



7	Δ^5
8	5α-H

d, J = 8.6 Hz) were observed in the ^{1}H NMR spectrum, and an ester carbonyl carbon at δ167.9 in the ^{13}C NMR spectrum. Treatment of **6** with 3% sodium methoxide in methanol gave methyl *p*-coumarate. The above data were indicative of **6** being a *p*-coumaroyl ester of **4**. The ester linkage position in the C-6 hydroxyl group of the glucose was evident from the shifts due to *O*-acylation. On comparison of the whole ^{13}C NMR signals of **6** with those of **4**, the signal due to C-6 of the glucose was shifted to lower field by 1.5 ppm and that due to C-5 to upper field by 2.9 ppm, while all other signals remained almost unaffected. Furthermore, the signals assignable to the 6-H_2

Table 1. ^1H and ^{13}C NMR chemical shift assignments of compounds **4** and **6** in pyridine-d_5

	4			**6**		
Position	^1H	J (Hz)	^{13}C	^1H	J (Hz)	^{13}C
1	9.19 *br s*		—	9.01 *br s*		—
2	—		173.5	—		173.2
3	—		136.9	—		137.0
4	6.75 *br s*		139.4	6.75 *br s*		139.2
5	5.88 *br s*		85.4	5.87 *br s*		85.1
6	1.85 *br s*		10.5	1.86 *br s*		10.6
1′	5.12 *d*	7.8	103.0	5.09 *d*	7.9	102.4
2′	4.06 *dd*	8.4, 7.8	74.9	4.07 *dd*	8.8, 7.9	74.8
3′	4.24 *dd*	8.8, 8.4	78.4	4.23 *dd*	8.8, 8.8	78.3
4′	4.19 *dd*	9.1, 8.8	71.6	4.13 *dd*	9.5, 8.8	71.3
5′	3.94 *ddd*	9.1, 5.8, 2.4	78.7	4.03 *ddd*	9.5, 5.7, 2.0	75.8
6′	4.51 *dd*	11.8, 2.4	62.6	5.12 *dd*	11.7, 2.0	64.1
	4.30 *dd*	11.8, 5.8		4.88 *dd*	11.7, 5.7	
1″				—	—	126.1
2″				7.57 *d*	8.6	130.8
3″				7.14 *d*	8.6	116.8
4″				—		161.6
5″				7.14 *d*	8.6	116.8
6″				7.57 *d*	8.6	130.8
7″				8.03 *d*	15.9	145.8
8″				6.63 *d*	15.9	114.9
9″				—	—	167.9

methylene protons of the glucose were shifted to lower fields by 0.61 and 0.58 ppm to appear at δ5.12 (*dd*, J = 11.7 and 2.0 Hz) and 4.88 (*dd*, J = 11.7 and 5.7 Hz) as compared with those of **4** (Table 1). Thus, the structure of **6** was formulated as jatropham 5-*O*-(6-*O*-*p*-coumaroyl-β-D-glucopyranoside).

Compound **7** was obtained as an amorphous solid, $[\alpha]_D$ − 49.2° (methanol), and assigned the molecular formula $C_{45}H_{72}O_{20}$ by the negative ion FAB-mass spectrum which showed an $[M]^-$ion at *m/z* 932, and from the elemental analysis. The glycosidic nature of **7** was suggested by the intense IR absorptions at 3380 and 1065 cm^{-1}. The ^1H NMR spectrum exhibited signals for three methyl protons at δ1.26 (*d*, J = 7.2 Hz) and 0.97 and 0.91 (each *s*), three anomeric protons at δ5.32 (*d*, J = 7.5 Hz), 5.10 (*d*, J = 8.0 Hz) and 5.02 (*d*, J = 7.8 Hz), and an olefinic proton at δ 5.25 (*br d*, J = 4.7 Hz). Acid hydrolysis of **7** with 1 M hydrochloric acid in dioxane–H_2O (1 : 1) gave D-glucose, identified by converting it into the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative, followed by HPLC analysis [9, 10]. The aglycone decomposed under acid conditions. The ^{13}C NMR spectrum showed 45 resonance lines, supporting the molecular formula deduced from the FAB mass spectrum and elemental analysis, 18 of which could be assigned to three D-glucose units: three anomeric carbons at δ107.2, 102.9 and 102.0, and 27 to the aglycone part; one distinctive quaternary carbon signal at δ110.3 (C) was assumed to be assignable to C-22 of the spirostan skeleton, and two olefinic carbon signals were observed at δ140.8 (C) and 121.7 (CH). The data mentioned above implied **7** to be a spirostanol trisaccharide composed of three D-glucoses and a $C_{27}H_{42}O_5$ molecular formula for the aglycone.

Attempted comparison of the ^1H and ^{13}C NMR spectra of **7** with those of previously reported (25*R*)-spirost-5-en-3β-ol (diosgenin) 3-*O*-glycosides [11], and taking into account the molecular formula of the aglycone, suggested that **7** was a spirost-5-en-3β-ol 3-*O*-trisaccharide with hydroxyl groups at C-17 and C-27. The presence of the C-17 hydroxyl group was confirmed by the isolated AB_3 spin system due to 20-H and 21-Me [δ2.27 (1H, *q*, J = 6.5 Hz) and 1.21 (3H, *d*, J = 6.5 Hz)] in the ^1H NMR spectrum and two- or three-bond coupled C, H correlations from the quaternary carbon at δ90.2 assignable to C-17 to 18-Me at δ0.93, 15a-H at δ2.16, 20-H and 21-Me in the proton-detected heteronuclear multiple-bond correlation (HMBC) spectrum (Figs 1–3). The presence of the C-27 hydroxyl group was reinforced by tracing out the proton spin systems of the F-ring portion in the ^1H–^1H correlation spectroscopy (COSY) and homonuclear Hartmann–Hahn (HOHAHA) spectra. All assignments of the ^1H and ^{13}C NMR of the aglycone moiety established by interpretation of the two-dimensional (2D) NMR spectra are shown in Table 2. The 2D NMR spectra were measured in a mixed solvent of pyridine-d_5 and methanol-d_4 in a ratio of 11 : 1 to minimize signal overlap and remove exchangeable protons.

The NOE correlations between 1α(ax)-H and 3-H, 9-H and 14-H, 14-H and 16-H, 18-Me and 20-H, and 16-H and 26(ax)-H observed in the phase-sensitive NOE correlation spectroscopy (PHNOESY) gave evidence for the β-orientation of the C-3 oxygen atom and α-orientation of the C-17 hydroxyl group as well as the usual stereostructures

Table 2. ^1H and ^{13}C NMR chemical shift assignments of compound **7** in pyridine-d_5–methanol-d_4 (11 : 1)

Position	^1H	J (Hz)	^{13}C
1eq	1.66 *br d*	12.1	37.5
ax	0.84 *ddd*	12.1, 12.1, 2.4	
2eq	2.04		30.2
ax	1.68 *dddd*	12.1, 12.1, 12.1, 2.4	
3	3.79 *m*		78.4
4eq	2.64 *dd*	12.4, 4.7	39.3
ax	2.41 *dd*	12.4, 11.9	
5	—		140.9
6	5.26 *br d*	3.7	121.8
7eq	1.89 *br d*	12.9	32.5
ax	1.51		
8	1.58 *dddd*	10.5, 10.5, 10.5, 4.2	32.4
9	0.89		50.3
10	—		37.1
11a	1.54		21.0
b	1.43		
12eq	1.51		32.0
ax	2.08 *ddd*	13.9, 13.9, 4.2	
13	—		45.2
14	2.01		53.1
15α	2.16		31.8
β	1.45		
16	4.42		90.0
17	—		90.2
18	0.93 *s*		17.2
19	0.92 *s*		19.4
20	2.27 *q*	6.5	45.0
21	1.21 *d*	6.5	9.7
22	—		110.4
23 (2H)	1.77		31.8
24a	1.80		23.6
b	1.76		
25	2.02		39.0
26eq	4.02		64.0
ax	3.83 *dd*	11.4, 11.4	
27a	3.67 *dd*	10.6, 5.1	64.4
b	3.58 *dd*	10.6, 7.5	
1′	4.95 *d*	7.8	102.0
2′	3.94 *dd*	9.3, 7.8	74.6
3′	4.26 *dd*	9.3, 9.3	76.5
4′	3.89 *dd*	9.3, 9.3	83.0
5′	4.14 *m*		77.3
6′	4.95 *br d*	11.7	62.6
	4.21 *dd*	11.7, 5.1	
1″	5.03 *d*	7.7	103.0
2″	4.01 *dd*	10.1, 7.7	85.2
3″	4.20 *dd*	10.1, 10.1	78.1
4″	4.00 *dd*	10.1, 10.1	71.1
5″	3.94 *m*		78.2
6″	4.47 *br d*	11.0	62.4
	4.14 *dd*	11.0, 5.4	
1‴	5.20 *d*	7.7	107.1
2‴	4.04 *dd*	9.8, 7.7	76.5
3‴	4.11 *dd*	9.8, 9.8	78.1
4‴	4.18 *dd*	9.8, 9.8	70.7
5‴	3.79 *m*		78.8
6‴	4.42 *br d*	12.9	62.0
	4.29 *dd*	12.9, 4.3	

Table 3. Content of jatropham (**3**) and its glucoside (**4**) in the bulbs of *L. martagon* and *L. hansonii*

	3	**4**
L. martagon	0.099%*	0.072%
L. hansonii†	0.030%	0.110%

*Percentage of fresh weight.
†Data from ref. [7].

of spirostanol, i.e. B/C *trans*, C/D *trans* and D/E *cis*-ring fusions, and (20*S*)- and (22*R*)-configurations. The (25*S*)-configuration was confirmed by the ^1H NMR parameter of the 26(ax)-proton at δ3.83 (*dd*, $J_{26ax-H,26eq-H} = 11.4$ Hz and $J_{26ax-H,25ax-H} = 11.4$ Hz).

The presence of a terminal, a 2-substituted and a 4-substituted β-D-glucopyranose was shown by comparison of the ^{13}C NMR resonances for each glucose, which were assigned by means of a combined use of the ^1H–^1H COSY, HOHAHA and HMQC spectra, with those of an authentic methyl β-D-glucopyranoside [11, 12]. In the HMBC spectrum, the anomeric proton signals at δ5.20 (terminal glucose), 5.03 (2-substituted glucose) and 4.95 (4-substituted glucose) showed $^3J_{C,H}$ correlations traversing the glycosidic linkage with the carbon signals at δ85.2 (C-2 of 2-substituted glucose), 83.0 (C-4 of 4-substituted glucose) and 78.4 (C-3 of aglycone), respectively. Accordingly, the structure of **7** was elucidated as (25*S*)-spirost-5-ene-3β,17α,27-triol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside}.

All spectral features of **8** ($C_{45}H_{74}O_{20}$) showed a strong similarity to those of **7**. On comparison of the ^1H NMR spectrum of **8** with that of **7**, the olefinic proton signal due to 6-H disappeared in that of **8**, accompanied by an upfield shift of 19-Me by 0.23 ppm. Furthermore, two olefinic carbon signals at δ140.8 (C) and 121.7 (CH) observed in the ^{13}C NMR spectrum of **7** were displaced by the aliphatic signals at δ44.5 (CH) and 28.9 (CH_2) in that of **8**. These data indicated that **8** was a 5,6-dihydro derivative of **7**. The ^{13}C shift of the C-19 methyl at δ12.3 accounted for an A/B *trans*-ring junction (5α-H) [13]. Thus, the structure of **8** was shown to be (25*S*)-5α-spirostane-3β,17α, 27-triol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside}.

Compound **6** is a new pyrroline glucoside. Compounds **7** and **8** are new steroidal saponins with a new triglycoside sequence; the aglycone structure of the latter compound is also new.

Lilium martagon crosses well with *L. hansonii* to produce a valuable garden hybrid lily [1]. Our detailed inspection of the constituents revealed that both lilies contain a considerable amount of jatropham (**3**) and jatropham glucoside (**4**) as the characteristic components (Table 3), and also contain spirost-5-ene derivatives and the corresponding 5α-spirostan derivatives at the same time in their bulbs. These findings present a good example of the correlation between the secondary metabolites produced in the plants and their cross-compatibility.

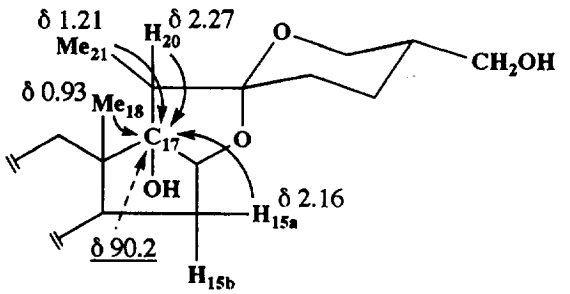


Fig. 1. HMBC correlations of the aglycone part of compound 7. $^{n}J_{C,H}$ was optimized for 8 Hz. Underlined figure indicates ^{13}C shift.

EXPERIMENTAL

General. NMR (ppm, *J* Hz): 1D (Bruker AM-400) and 2D (Bruker AM-500); CC: silica gel (Fuji-Silysia Chemical), octadecylsilanized (ODS) silica gel (Nacalai Tesque), Sephadex LH-20 (Pharmacia), Toyopearl HW-40 (Tosoh) and Diaion HP-20 (Mitsubishi-Kasei); TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 or 0.5 mm thick, Merck); HPLC: Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010 or UV-8000) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-Kasei-Kogyo, 10 mm i.d. × 250 mm, ODS, 5 μm) for prep. HPLC or with a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 μm) for analyt. HPLC.

Plant material. The bulbs of *L. martagon* were provided from Hokkaido Experiment Station of Medicinal Plants (Japan). The bulbs were cultivated and the plant specimen is on file in our laboratory.

Extraction and isolation. Fresh bulbs of *L. martagon* (3.5 kg) were cut into pieces and extracted with hot MeOH. The MeOH extract, after removal of the solvent under red. pres., was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble phase was fractionated on a silica gel column using a mobile phase composed of $CHCl_3$–MeOH with increasing amounts of MeOH (9:1; 4:1; 2:1; 1:1), and finally with MeOH alone to give 6 frs (I–VI). Fr. I was chromatographed on silica gel, eluting with $CHCl_3$–MeOH (13:1) and EtOAc–Me_2CO (19:1), and on Sephadex LH-20 with MeOH to give compound **3** (3.47 g). Fr. II was subjected to CC on silica gel, eluting with $CHCl_3$–MeOH–H_2O (120:20:1; 60:10:1; 100:20:1), and on an ODS silica gel column with MeOH–H_2O (1:1) to give **6** with a few impurities. Final purification of **6** was performed by prep. TLC, developing with $CHCl_3$–MeOH–H_2O (40:10:1) to give **6** (43.1 mg) as a pure compound. Fr. III was subjected to CC on silica gel, eluting with $CHCl_3$–MeOH–H_2O (50:10:1) and EtOAc–MeOH (8:1) to give **1** (207 mg). Fr. IV was dissolved in hot MeOH. After cooling, the ppt. was filtered off to give **4** (2.53 g). The filtrate was chromatographed on silica gel, eluting with $CHCl_3$–MeOH–H_2O (27:10:1; 25:10:1), and on Sephadex LH-20 with MeOH to give **2** (50.7 mg). Fr. V was dissolved in $CHCl_3$–MeOH and the ppt. was filtered off to give **5** (82.7 mg). Fr. VI was

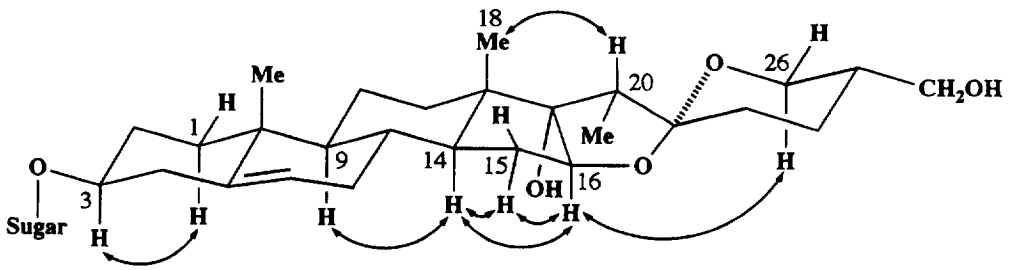


Fig. 2. NOE correlations of the aglycone part of compound **7**.

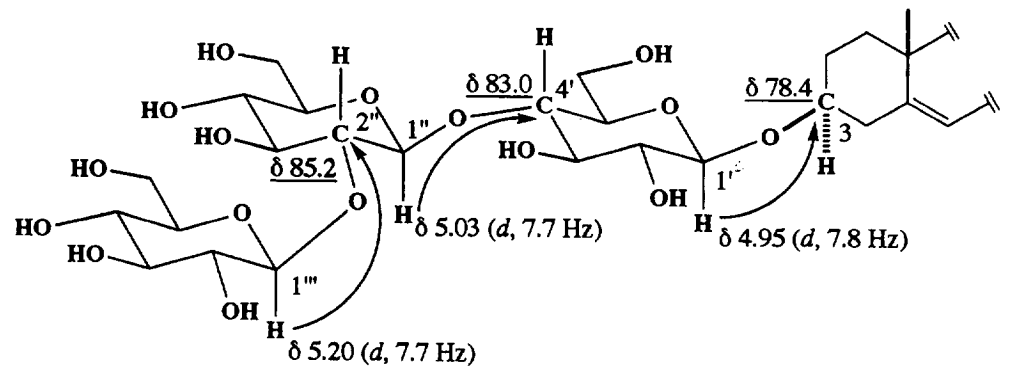


Fig. 3. HMBC correlations of the saccharide part of compound **7**.

shown by TLC analysis to contain steroidal saponins and abundant saccharides, from which the saccharides were removed by passage through a Diaion HP-20 column, eluting with H_2O gradually enriched with MeOH. The MeOH eluate fr. was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (20:10:1) and on ODS silica-gel with MeOH–H_2O (16:7) to give a mixt. of **7** and **8**, sepn of which was carried out by prep. HPLC using MeCN–H_2O (1:2) to give **7** (34.1 mg) and **8** (25.9 mg).

Compound **6**. A pale-yellow amorphous solid, $[\alpha]_D^{29}$ $-26.3°$ (MeOH: *c* 0.19). Positive-ion FABMS *m/z*: 422.1429 $[M]^+$ (calc. for $C_{20}H_{23}NO_9$: 422.1451); UV λ_{max}^{MeOH} nm (log ε): 312 (4.32), 300sh (4.26), 226sh (4.07); UV $\lambda_{max}^{MeOH+3\%NaOMe}$ nm 362; IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 2910 (CH), 1685 (C=O), 1625, 1595, 1580, 1505, 1435, 1320, 1255, 1195, 1165, 1045, 1010, 855, 825, 740, 690.

Alkaline methanolysis of **6**. Compound **6** (10.8 mg) was treated with 3% NaOMe in MeOH (2 ml) at room temp. for 1 hr. The reaction mixt. was neutralized by passing it through an Amberlite IR-120B (Organo) column and subjected to silica gel CC, eluting with $CHCl_3$ to yield methyl *p*-coumarate.

Compound **7**. Amorphous solid, $[\alpha]_D^{29}$ $-49.2°$ (MeOH; *c* 0.13). Anal. calc. for $C_{45}H_{72}O_{20}\cdot 3/2H_2O$: C, 56.30; H, 7.87. Found: C, 56.29; H, 7.95. Negative-ion FABMS *m/z*: 932 $[M]^-$, 769 $[M-\text{glucosyl}]^-$; IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2925 and 2980 (CH), 1445, 1375, 1240, 1165, 1150, 1065, 1030, 970, 900, 695; ^{1}H NMR (pyridine-d_5): δ5.32 (1H, *d*, *J* = 7.5 Hz, 1‴-H), 5.25 (1H, *br d*, *J* = 4.7 Hz, 6-H), 5.10 (1H, *d*, *J* = 8.0 Hz, 1″-H), 5.02 (1H, *d*, *J* = 7.8 Hz, 1′-H), 3.73 (1H, *dd*, *J* = 10.7, 5.1 Hz, 27a-H), 3.65 (1H, *dd*, *J* = 10.7, 7.2 Hz, 27b–H), 2.30 (1H, *q*, *J* = 7.2 Hz, 20-H), 1.26 (3H, *d*, *J* = 7.2 Hz, 21-Me), 0.97 (3H, *s*, 18-Me), 0.91 (3H, *s*, 19-Me); ^{13}C NMR (pyridine-d_5): δ37.4, 30.1, 78.3, 39.3, 140.8, 121.7, 32.4, 32.3, 50.1, 37.0, 20.9, 32.1, 45.1, 53.1, 31.8, 90.1, 90.1, 17.2, 19.4, 44.9, 9.7, 110.3, 31.8, 23.6, 39.0, 63.9, 64.4 (C-1–C-27), 102.0, 74.7, 76.7,[a] 83.5, 77.4, 62.9 (C-1′–C-6′), 102.9, 85.5, 78.3,[b] 71.3, 78.2,[b] 62.6 (C-1″–C-6″), 107.2, 76.6,[a] 78.3,[b] 70.7, 78.8, 62.0 (C-1‴–C-6‴). [a,b]Assignments may be interchanged.

Acid hydrolysis of **7**. A soln of **7** (7 mg) in 1 M HCl (dioxane–H_2O, 1:1, 2 ml) was refluxed for 2 hr under an Ar atmosphere. The reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column, and then transferred to a silica gel column, eluting with $CHCl_3$–MeOH (19:1; 1:1), and a Toyopearl HW-40 column to give a monosaccharide fr. (2.5 mg) and several unidentified artefactual sapogenols. The saccharide fr. in H_2O (1 ml) was treated with (−)-α-methylbenzylamine (16 mg) and Na $[BH_3CN]$ (8 mg) in EtOH (1 ml) for 4 hr at 40° followed by acetylation with Ac_2O (0.3 ml) in pyridine (0.3 ml). The reaction mixt. was passed through a Sep-Pak C_{18} cartridge (Waters), initially eluting with H_2O–MeCN (1:1, 10 ml), and then with MeCN (10 ml). The MeCN eluate fr. was further passed through a Toyopak IC-SP M (Tosoh) cartridge with EtOH (10 ml) to give the corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative of D-glucose, which was then analysed by HPLC. HPLC: R_t 24.02 min. [column: TSK-gel ODS-Prep column (Tosoh), 4.6 mm i.d. × 250 mm, ODS, 5 μm; solvent, MeCN–H_2O (2:3); flow rate: 0.8 ml min^{-1}; detection: UV 230 nm].

Compound **8**. Amorphous solid, $[\alpha]_D^{29}$ $-27.0°$ (MeOH; *c* 0.13). Anal. calc. for $C_{45}H_{74}O_{20}\cdot 1/2H_2O$: C, 57.25; H, 8.01. Found: C, 57.29; H, 7.98. Negative-ion FABMS *m/z*: 934 $[M]^-$, 771 $[M-\text{glucosyl}]^-$; IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2920 and 2870 (CH), 1445, 1375, 1245, 1165, 1150, 1065, 1025, 975, 905, 695; ^{1}H NMR (pyridine-d_5): δ5.31 (1H, *d*, *J* = 7.5 Hz, 1‴-H), 5.10 (1H, *d*, *J* = 7.8 Hz, 1″-H), 5.01 (1H, *d*, *J* = 7.8 Hz, 1′-H), 3.72 (1H, *dd*, *J* = 10.6, 5.1 Hz, 27a-H), 3.64 (1H, *dd*, *J* = 10.6, 7.2 Hz, 27b-H), 2.30 (1H, *q*, *J* = 7.2 Hz, 20-H), 1.25 (3H, *d*, *J* = 7.2 Hz, 21-Me), 0.96 (3H, *s*, 18-Me), 0.68 (3H, *s*, 19-Me); ^{13}C NMR (pyridine-d_5): δ37.1, 29.9, 78.3, 34.8, 44.5, 28.9, 32.4[a], 35.7, 52.8, 35.8, 21.1, 32.3[a], 45.4, 54.2, 31.6, 90.1, 90.0, 17.4, 12.3, 44.9, 9.7, 110.2, 31.8, 23.6, 39.0, 63.9, 64.4 (C-1–C-27), 101.6, 74.7, 76.6,[b] 83.6, 77.3, 62.9 (C-1′–C-6′), 102.9, 85.5, 78.3, 71.3, 78.3, 62.5 (C-1″–C-6″), 107.2, 76.7,[b] 78.3, 70.7, 78.8, 61.9 (C-1‴–C-6‴). [a,b]Assignments may be interchanged.

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