Phytochemistry, Vol. 31, No. 8, pp. 2767–2775, 1992
Printed in Great Britain.

0031-9422/92 $5.00+0.00

JATROPHAM DERIVATIVES AND STEROIDAL SAPONINS FROM THE BULBS OF *LILIUM HANSONII*

KAZUTOMO ORI, YOSHIHIRO MIMAKI, KYOKO MITO, YUTAKA SASHIDA,* TAMOTSU NIKAIDO,† TAICHI OHMOTO† and AKIYOSHI MASUKO‡

Tokyo College of Pharmacy, 1432-1, Horinouchi, Hachioji, Tokyo 192-03, Japan; †Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan; ‡Central Technical Research Laboratory, Nippon Oil Company Ltd, 8, Chidori-cho, Naka-ku, Yokohama, Kanagawa 231, Japan

(*Received* 25 *November* 1991)

Key Word Index—*Lilium hansonii*; Liliaceae; bulbs; jatropham derivatives; steroidal saponins; X-ray analysis; cyclic AMP phosphodiesterase; inhibitory activity.

Abstract—Two new jatropham derivatives and three new steroidal saponins were isolated from the fresh bulbs of *Lilium hansonii*, along with previously known compounds. The structures of the new compounds were elucidated, on the basis of spectroscopic data and chemical evidence, and by comparing them with those of known compounds, as (−)-5-hydroxy-3-methyl-3-pyrrolin-2-one (jatropham) 5-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside, (2*S**,4*R**)-1-(3-methyl-2-oxo-3-pyrrolinyl)-4-methyl-5-oxo-2-pyrrolidinecarboxylic acid, 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-3β,22ξ-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside, (25*R*)-5α-spirostan-3β,12α-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside and (25*R*)-spirost-5-en-3β,12α-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside, respectively. The stereostructure of jatropham dimer, the plain structure of which was presented previously, was confirmed by X-ray crystallographic analysis. The inhibitory activity on cyclic AMP phosphodiesterase of the steroidal saponins was evaluated.

INTRODUCTION

Lilium hansonii is a perennial plant native to the island of Dagelet off the coast of Korea and is classified on the basis of the flower shapes into the subgenus Martagon [1]. Lilies are often attacked by a considerable number of pests and diseases, and the worst disorders to which lilies are vulnerable are a number of viral diseases [2]. *Lilium hansonii* is noted for its strong resistance to viral diseases and it is one of the most adaptable lilies; on several occasions it has appeared at the head of the list of easily grown lilies [3].

Our previous chemical analysis revealed that the bulbs contain (−)-5-hydroxy-3-methyl-3-pyrrolin-2-one (jatropham) and its glucoside as the characteristic constituents [4]. We have reinvestigated the bulbs and isolated two new jatropham derivatives and three new steroidal saponins together with several known compounds. This paper reports the structural elucidation of the new compounds and inhibitory activity on cyclic AMP phosphodiesterase of the steroidal saponins.

RESULTS AND DISCUSSIONS

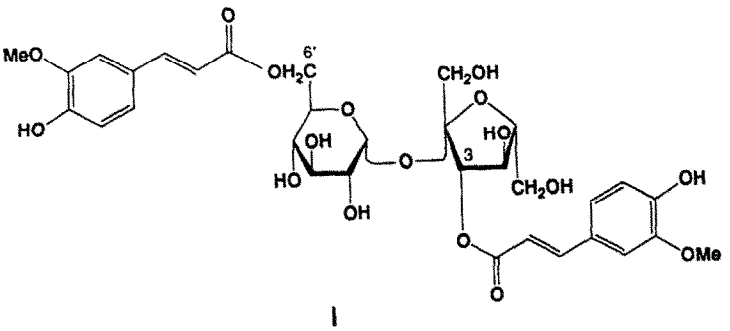
Fresh bulbs of *L. hansonii* were extracted with hot methanol and the methanolic extract was partitioned between chloroform and water, and then between 1-butanol and water. A series of chromatographic separations of the 1-butanol-soluble phase yielded compounds **1**–**12**.

Compounds **1**–**5** were identified as 3,6′-di-*O*-feruloylsucrose [5, 6], 1-*O*-*p*-coumaroyl-3-*O*-feruloylglycerol [7–9], jatropham, (±)-5-*O*-methyljatropham and jatropham 5-*O*-β-D-glucopyranoside, respectively [4].

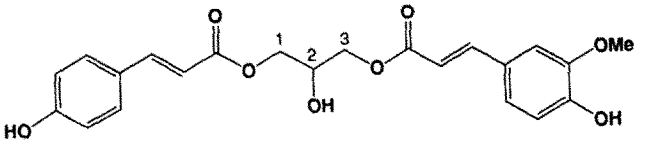
Compound **6** was obtained as a white amorphous powder, $[\alpha]_D$ −70.0° (methanol). The secondary ion (SI) mass spectrum showed a $[M+Na]^+$ peak at *m/z* 460 and a $[M+H]^+$ peak at *m/z* 438, corresponding to the molecular formula $C_{17}H_{27}NO_{12}$. The ^1H and ^{13}C NMR spectra of **6** showed signals due to 5-*O*-glycosylated jatropham, a 3-*O*-glycosylated β-D-glucopyranosyl unit and a terminal β-D-glucopyranosyl unit. The structure of **6** was determined to be jatropham 5-*O*-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside.

Compound **7** was obtained as a colourless syrup, $[\alpha]_D$ +40.9° (methanol). The high-resolution EI mass spectrum showed the molecular ion peak at *m/z* 238.0979, confirming the molecular formula to be $C_{11}H_{14}N_2O_4$ (calc. 238.0954). The IR spectrum showed broad absorption bands in the range of 3400–2400 cm^{-1}, and treatment of **7** with diazomethane gave a monomethyl ester (**7a**), indicating the presence of a carboxyl group in the molecule. The ^1H NMR spectrum of **7** (see Fig. 1) showed signals for a methyl group on a double bond at δ1.88 (*dd*, *J* = 1.7, 1.7 Hz) and a secondary methyl group at δ1.17 (*d*, *J* = 7.1 Hz). By tracing out the proton–proton coupling systems from the two methyl groups through the ^1H–^1H COSY spectrum, the two partial structures Me–CH–CH_2–CH and Me–C=CH–CH were revealed. The existence of a jatropham unit in **7** was suggested by the partial structure Me–C=CH–CH and by the prominent fragment ion peak at *m/z* 96 (base peak) in the EI mass

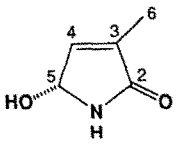
*Author to whom correspondence should be addressed.



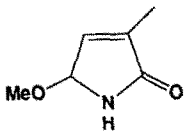
1



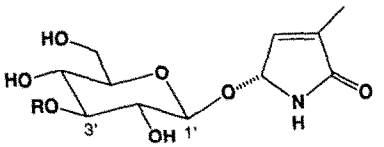
2



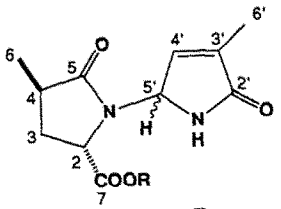
3



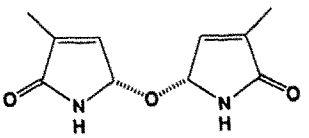
4



	R
5	H
6	β-D-Glc*p*

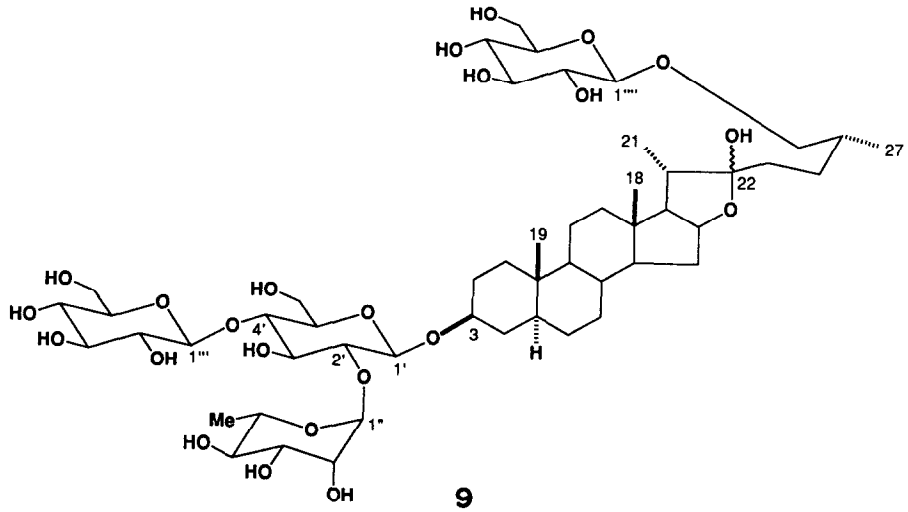


	R
7	H
7a	Me

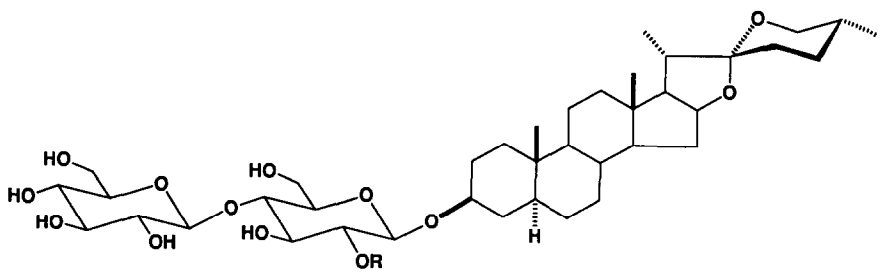


8

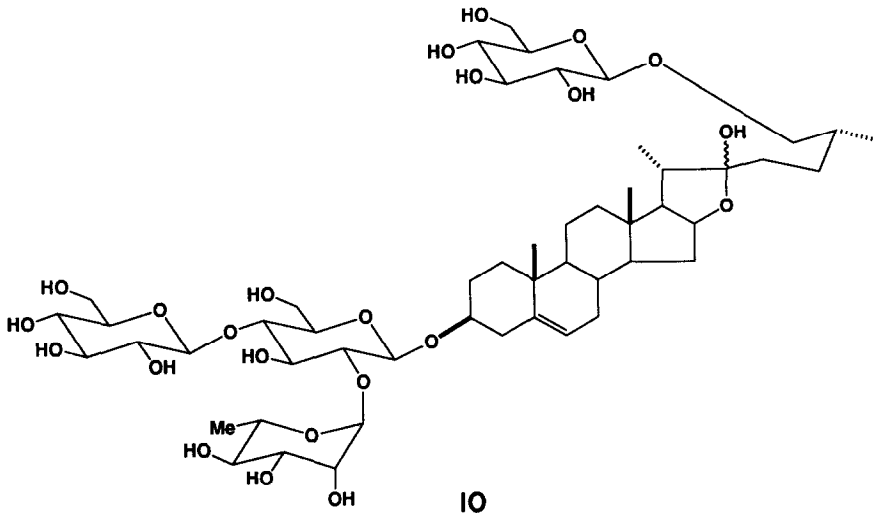
spectrum. The ^{13}C NMR spectrum of **7** showed three carbonyl carbon signals at δ180.5, 175.8 and 175.3. In the ^{1}H–^{13}C long-range COSY (COLOC) spectrum (see Fig. 2), the signal at δ175.3 showed long-range correlations with the proton signals at δ1.88 (H-6′) and 6.62 (H-4′), leading to the assignment of the signal as the carbonyl group of the jatropham moiety. Further, the signal at δ180.5, which was predicted to be due to a carbonyl group of a five-membered lactam from the chemical shift value (authentic 2-pyrrolidinone: δ179.4) [10], showed long-range correlations with the proton signals at δ1.17 (H-6), 2.29 (H-3a) and 3.88 (H-2). The remaining signal at δ175.8 was assigned as the carbonyl group of the carboxyl moiety and showed long-range correlation with the proton signal at δ1.94 (H-3b). Thus, the presence of a 4-methyl-5-oxo-2-pyrrolidinecarboxylic acid moiety in **7** was evident. The ^{1}H NMR signal due to the H-5′ proton of the jatropham moiety was shifted to lower field by 0.69 ppm as compared with that of jatropham (**3**), and the ^{13}C NMR signal due to the C-5′ carbon of the jatropham moiety showed long-range correlation with the signal due to the H-2 proton of the 4-methyl-5-oxo-2-pyrrolidinecarboxylic acid moiety. The above data revealed that the C-5′ carbon of the jatropham moiety is directly linked to the nitrogen atom of the 4-methyl-5-oxo-2-pyrrolidinecarboxylic acid moiety. Accordingly, the structure of **7** was established as 1-(3-methyl-2-oxo-3-pyrrolinyl)-4-methyl-5-oxo-2-pyrrolidinecarboxylic acid. By the NOESY spectrum, the relative stereostructure of **7** was partially determined to be (2*S**,4*R**) (Fig. 3).



9



	R
9a	α-L-Rha*p*
9b	H



10

Compound **8** was obtained as colourless prisms recrystallized from methanol, mp 213–215° (dec.). The molecular formula, $C_{10}H_{12}N_2O_3$, was determined by the high-resolution EI mass spectrum (*m/z* 208.0867 $[M]^+$; calc. 208.0849). The IR, ^1H and ^{13}C NMR spectra identified **8** as 5,5′-oxydi(3-methyl-3-pyrrolin-2-one) [11]. Compound **8** was a *meso* or a racemic compound because it showed no specific rotation and no Cotton effect in the CD spectrum. X-Ray crystallographic analysis showed that **8** was a *meso* compound (Fig. 4) and that each molecule was connected by the hydrogen bonds between the molecules to form chain-polymers or ribbon-shaped

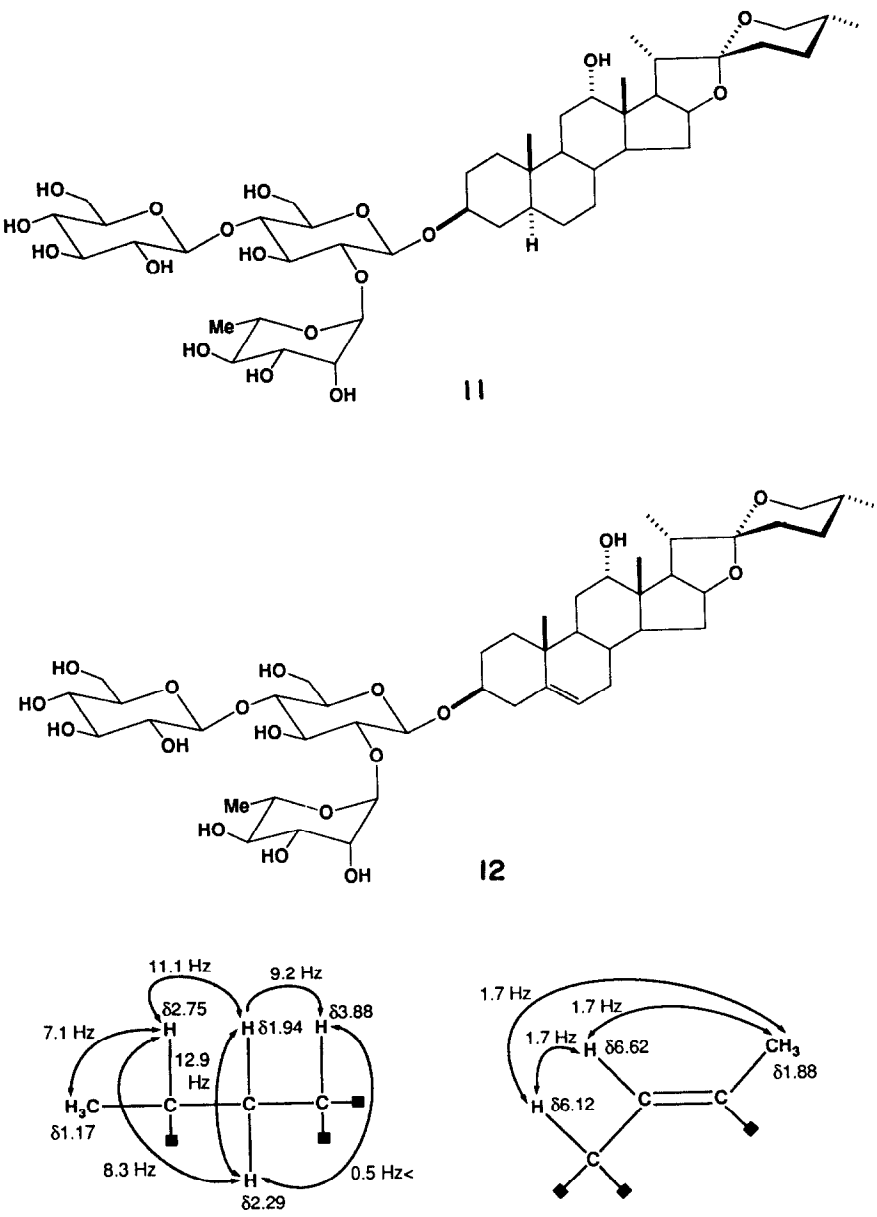


Fig. 1. ^{1}H NMR chemical shifts and J values of compound **7** (methanol-d_4).

polymers in the crystal. There is no hydrogen bond between the polymers and each polymer is thought to be linked by the van der Waals force (Fig. 5).

Compound **9** was obtained as a white amorphous powder, $[\alpha]_D -48.8°$ (MeOH) and the molecular formula, $C_{51}H_{86}O_{23}$, was confirmed by the SI mass spectrum and elemental analysis. The ^{1}H NMR spectrum contained signals for four anomeric protons at δ6.20 (1H, *br s*) 5.11 (1H, *d*, $J=7.7$ Hz), 4.98 (1H, *d*, $J=7.7$ Hz) and 4.80 (1H, *d*, $J=7.7$ Hz), three secondary methyl protons at δ1.73 (3H, *d*, $J=6.1$ Hz), 1.33 (3H, *d*, $J=7.0$ Hz) and 0.99 (3H, *d*, $J=6.5$ Hz), and two angular methyl protons at δ0.88 and 0.86 (each *s*). The signal at δ1.73 was due to the methyl group of 6-deoxyhexose. The structure of **9**, based upon a 22-hydroxyfurostanol derivative, was suggested by a quaternary carbon signal at δ110.6 in the ^{13}C NMR spectrum (Table 1) [12]. Acid hydrolysis of **9** with 1 M hydrochloric acid (dioxane–H_2O, 1:1) yielded D-glucose and L-rhamnose as the carbohydrate compounds, and an aglycone, identified as (25*R*)-5α-spirostan-3β-ol (tigogenin). On enzymatic hydrolysis with β-glucosidase, **9** afforded a tigogenin trisaccharide (**9a**) which was formed by the splitting of the terminal β-D-glucopyranose from the C-26 position. The ^{13}C NMR spectrum of **9a** showed the presence of a terminal β-D-glucopyranosyl unit, a terminal α-L-rhamnopyranosyl unit and a 2,4-diglycosylated β-D-glucopyranosyl unit in the molecule. Acid hydrolysis of **9a** in mild conditions yielded tigogenin disaccharide (**9b**), the ^{13}C NMR data of which allowed identification of the disaccharide as β-D-glucopyranosyl-

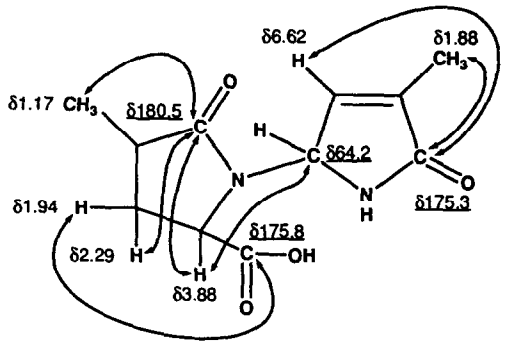


Fig. 2. ^{1}H–^{13}C long-range correlations of compound **7** (methanol-d_4). Underlined figures indicate ^{13}C NMR chemical shifts.

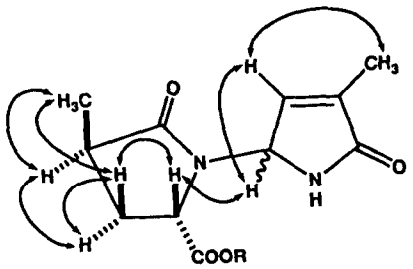


Fig. 3. NOE of compound **7** (methanol-d_4).

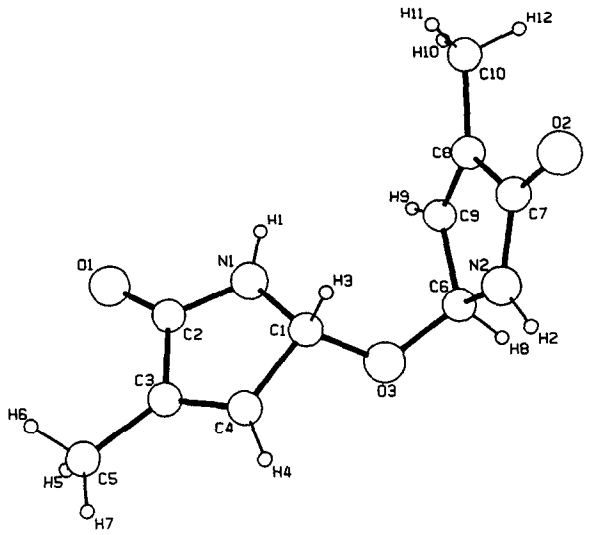


Fig. 4. Perspective drawing of compound **8**.

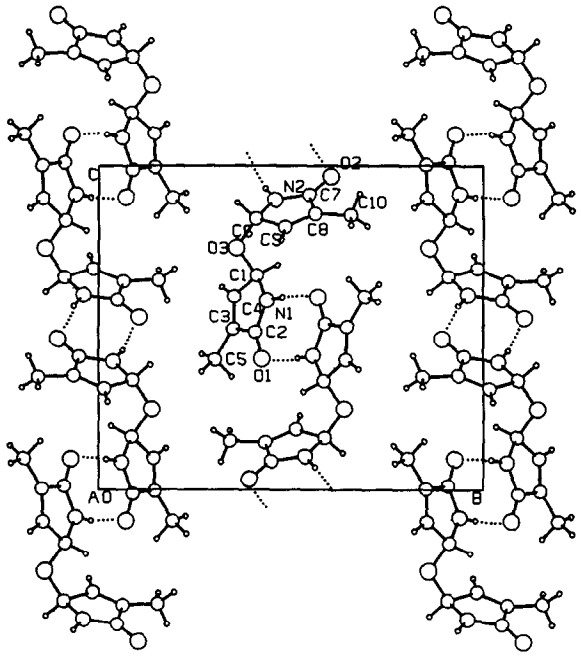


Fig. 5.

(1→4)-β-D-glucopyranose [13]. Thus, the structure of **9** was elucidated as 26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostan-3β,22ξ-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Compound **10**, $C_{51}H_{84}O_{23}$, was shown by the ^{1}H and ^{13}C NMR spectra to be the Δ^5-derivative of **9** and the structure was identified as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-en-3β,22ξ-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside [14].

Compounds **11**, $C_{45}H_{74}O_{18}$, and **12**, $C_{45}H_{72}O_{18}$, were spirostanol saponins. The ^{1}H and ^{13}C NMR signal patterns of the sugar moieties of **11** and **12** were superimposable with those of **9a**. Acid hydrolysis of **11** yielded D-glucose, L-rhamnose and an aglycone (**11a**), $C_{27}H_{44}O_4$. The ^{1}H NMR spectrum indicated that **11a** was a tigogenin derivative with an axial hydroxyl group (δ3.96, 1H, *br s*, $W_{1/2}=10.2$ Hz) in addition to an equatorial 3β-hydroxyl group (δ3.79, 1H, *br m*, $W_{1/2}=24.4$ Hz). The location of the additional hydroxyl group, and the linkage position of the trisaccharide at the aglycone, were established by the following spectral data. In the ^{13}C NMR spectrum of **11a**, the signals arising from C-9 (δ48.6), C-14 (δ48.0) and C-17 (δ53.9) were displaced upfield, accompanied by downfield shifts of the signals due to C-11 (δ29.4) and C-13 (δ45.4) as compared with those of tigogenin, indicating the presence of an axial 12α-hydroxyl group. Further, the signal due to C-3 was shifted to higher field by 7.1 ppm, whereas the signals due to C-2 and C-4 moved to lower field by 2.7 and 4.9 ppm, respectively, as compared with those of **11**, which showed the trisaccharide to be linked at the C-3 hydroxyl position. The structure of **11** was formulated as (25*R*)-5α-spirostan-3β,12α-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside.

Acid hydrolysis of **12** yielded D-glucose, L-rhamnose and an aglycone (**12a**), $C_{27}H_{42}O_4$. The ^{1}H and ^{13}C NMR spectra indicated that **12a** was a (25*R*)-spirost-5-en-3β-ol (diosgenin) with an axial 12α-hydroxyl group. The structure of **12** was characterized as (25*R*)-spirost-5-en-3β,12α-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. Compounds **6**, **7**, **9**, **11** and **12** are new naturally occurring compounds.

Table 1. ^{13}C NMR spectral data of compounds **9**, **9a**, **9b**, **10**, **11**, **11a**, tigogenin, **12** and **12a**

C	**9**	**9a**	**9b**	**10**	**11**	**11a**	Tigogenin	**12**	**12a**
1	37.3	37.3	37.2	37.5	37.2	37.4	37.5	37.4	37.7
2	29.9	29.9	30.0	30.2	29.9	32.6	32.5	30.1	22.6
3	77.6[a]	77.6[a]	77 5	78.2[a]	77.3	70.6	70.6	78.3[a]	71.3
4	34.5	34.5	34 9	39.0	34.5	39.4	39.3	39.0	43.6
5	44.7	44.7	44.7	140.8	44.9	45.4	45 2	140.1	142.2
6	29.0	29.0	29.0	121.8	29.1	29.2	29.1	122.0	121.2
7	32.4[b]	32.4	32.5	32.5[b]	32 5[a]	32.5[a]	32.5	32.4	32.3[a]
8	35.3	35.3	35.3	31.7	35.7	35.8	35.4	32.1	32.1
9	54.5	54.5	54.5	50.4	48.5	48 6	54.6	44 5	44.5
10	36.0	36.0	35.9	37.2	35.7	35.7	35.9	37.0	36.8
11	21.3	21.3	21.3	21.1	29.4	29.4	21.1	29.4	29.3
12	40.3	40.2	40.2	40.0	71.5	71.6	40.3	71.3	71.5
13	41.1	40.8	40.8	40.8	45.4	45.4	40.8	45 1	45.1
14	56.4	56.5	56.5	56.6	47.9	48.0	57.6	48.2	48.4
15	32.5[b]	32.1	32.2	32.4[b]	32.3[a]	32.3[a]	32.1	32.4	32.4[a]
16	81.1	81.2	81.2	81.1	81.1	81.1	81.1	81 1	81.1
17	64.0	63.1	63.1	63.9	53.9	53.9	63.1	53 9	53.9
18	16.7	16.6	16.6	16.4	17.3	17.4	16.7	17.3	17.3
19	12.4	12.5	12.4	19.4	12.5	12.5	12.5	19.3	19.5
20	40.7	42.0	42.0	40.7	42.3	42.3	42.0	42.3	42.3
21	16.4	15.0	15.0	16.5	14.9	14.9	15.0	14.9	14.9
22	110.6	109.2	109.2	110.7	109.3	109.3	109.2	109.3	109.3
23	37.2	31.9	31.9	37.2	32.0	31.9	31.9	32.0	31.9
24	28.4	29.3	29.3	28.4	29.6	29.7	29.3	29.4	29.5
25	34.3	30.6	30.6	34.3	30.7	30.7	30.6	30.7	30 7
26	75.3	66.9	66.9	75.3	66.9	66 9	66.9	66.9	66.9
27	17.5	17.3	17.3	17.5	17.4	17.4	17.3	17.2	17 3
1′	99.6	99.6	102.0	100.1	99.6			100.0	
2′	77.7[a]	77.8[a]	74.8[a]	77.7	77.7			77.7	
3′	76.2	76.2	76.5[b]	76.2	76.2			76.2	
4′	82.2	82.2	81.5	82.0	82.2			82.1	
5′	77.3	77.3	76.9[b]	77.4	77.3			77 4	
6′	62.2[c]	62.1	62.4[c]	62.1[c]	62.2[b]			62 2[b]	
1″	101.9	101.9		101.8	101.9			101.8	
2″	72.4	72.4		72.4	72.4			72.5	
3″	72.8	72.8		72.8	72.8			72.8	
4″	74.1	74.2		74.1	74.1			74.2	
5″	69.4	69.4		69.4	69.4			69.5	
6″	18.6	18.7		18.6	18.7			18.7	
1‴	105.2	105.3	105.0	105.2	105.2			105.2	
2‴	75.0	75.0	74.9[a]	75.0	75.0			75.0	
3‴	78.5[d]	78.5[b]	78.5[d]	78.4[a]	78.5[c]			78.5[a]	
4‴	71.3	71.3	71.6	71.3	71.3			71.4	
5‴	78.3[d]	78.3[b]	78.3[d]	78.3[a]	78.3[c]			78.2[a]	
6‴	62.1[c]	62.1	62.5[c]	62.0[c]	62.1[b]			62 0[b]	
1⁗	104.9			104.9					
2⁗	75.2			75.2					
3⁗	78.6[d]			78.6[a]					
4⁗	71.8			71.8					
5⁗	78.2[d]			78.2[a]					
6⁗	62.9			62.9					

Spectra were measured in pyridine-d_5.

[a–d]Assignments with the same superscript may be reversed in each column.

Lilium hansonii crosses well with *L. martagon* and a number of hybrids of this parentage have been introduced [3]. Both lilies contain jatropham and its derivatives as the characteristic constituents [4, 15].

The inhibitory activity on cyclic AMP phosphodiesterase [16, 17] of the steroidal saponins was examined. The results are shown in Table 2. The furostanol saponin (**9**) exhibited only a weak inhibitory activity (IC_{50} 103 $\times 10^{-5}$ M), but the corresponding spirostanol saponin (**9a**) showed considerable activity (IC_{50} 0.7×10^{-5} M).

Table 2. Inhibitory activity on cyclic AMP phosphodiesterase of the steroidal saponins

	IC_{50} ($\times 10^{-5}$ M)
9	103
9a	0.7
9b	>500
10	48.5
11	177
12	34.5

EXPERIMENTAL

General. NMR: 1D (Bruker AM-400) and 2D (Bruker AM-500). CC: silica gel (Fuji Davison), ODS (Nacalai Tesque), Sephadex LH-20 (Pharmacia) and Diaion HP-20 (Mitsubishi-kasei). TLC: precoated Kieselgel 60 F_{254} (0.25 mm or 0.5 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck). HPLC: Tosoh HPLC system (Tosoh: pump, CCPM; detector, RI-8010 or UV-8000; controller, CCP controller PX-8010) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-kasei, 10 mm i.d. × 250 mm, ODS, 5 μm) or a CIG pre-packed column (Kusano Kagakukikai, 20 mm i.d. × 100 mm, ODS, 20 μm).

Extraction and isolation. Fresh bulbs of *L. hansonii* (6.5 kg) purchased from Heiwaen, Japan, were cut into pieces and extracted with hot MeOH. After removal of the solvent by evapn, the MeOH extract was successively fractionated with $CHCl_3$ and *n*-BuOH. CC of the *n*-BuOH extract on silica gel and elution with $CHCl_3$–MeOH, with increasing proportions of MeOH (19:1, 9:1, 4:1, 2:1), gave 4 frs.

Purification of fr. 1 by a silica gel column with $CHCl_3$–EtOAc, $CHCl_3$–Me_2CO, $CHCl_3$–MeOH, $CHCl_3$–MeOH–25% NH_3 sol. and EtOAc–MeOH systems and a Sephadex LH-20 column with MeOH, and finally by HPLC with MeOH–H_2O (2:3) gave compounds **2** (35.0 mg), **3** (114 mg), **4** (1.66 g), **7** (31.2 mg) and **8** (7.5 mg).

Fr. 2 was chromatographed on silica gel with $CHCl_3$–MeOH (5:1) to give **1** (130 mg).

Fractionation of fr. 3 was carried out by passage through a Diaion HP-20 column with H_2O gradually enriched with MeOH. Chromatography of the H_2O and 25% MeOH in H_2O eluate frs on silica gel with EtOAc–MeOH (7:1) and $CHCl_3$–MeOH (5:1) and on ODS with MeOH–H_2O (1:9) yielded **5** (2.43 g) and **6** with a few impurities, which were further purified by prep. TLC with *n*-BuOH–Me_2CO–H_2O (4:5:1) and HPLC with MeOH–H_2O (1:9) to furnish **6** (2.3 mg) as a pure compound. Chromatography of the 100% MeOH fr. on silica gel with EtOAc–MeOH (8:1, 5:1) and on ODS with MeOH–H_2O (4:1) gave **11** and **12** as almost pure compounds. Final purification of **11** and **12** was achieved by HPLC with MeOH–H_2O (4:1), **11** (14.6 mg) and **12** (13.4 mg).

Fr. 4 was subjected to Diaion HP-20 CC with increasing amounts of MeOH in H_2O. Compounds **9** (73.4 mg) and **10** (50.8 mg) were isolated from the 75% MeOH in H_2O and 100% MeOH eluate frs after similar chromatographic separations as for the 100% MeOH eluate fr. of fr. 3.

Compound **6**. Amorphous powder, $[\alpha]_D^{29}$ −70.0° (MeOH; *c* 0.10). SIMS *m/z*: 460 $[M+Na]^+$, 438 $[M+H]^+$; IR ν_{max}^{film} cm^{-1}: 3354 (OH), 2923, 2860 (CH), 1697 (C=O), 1652 (C=C), 1573, 1413, 1375, 1261, 1074, 890, 870, 803; UV λ_{max}^{MeOH} nm (log ε): 240sh (3.39); CD (MeOH; *c* 1.19×10^{-4}) nm (θ): 254 (+5042); ^1H NMR (pyridine-d_5): δ6.75 (1H, *br s*, H-4), 5.88 (1H, *br s*, H-5), 5.32 (1H, *d*, *J* = 7.9 Hz, H-1''), 5.06 (1H, *d*, *J* = 7.8 Hz, H-1'), 1.85 (3H, *dd*, *J* = 1.4, 1.4 Hz, H-6); ^{13}C NMR (pyridine-d_5): δ173.3 (C-2), 139.3 (C-4), 137.0 (C-3), 105.9 (C-1''), 101.9 (C-1'), 88.6 (C-3'), 85.2 (C-5), 78.7 (C-3'')*, 78.3 (C-5'')*, 78.2 (C-5')*, 75.6 (C-2''), 73.7 (C-2'), 69.8 (C-4'), 62.6 (C-6'')**, 62.3 (C-6')**, 10.0 (C-6). *, ** Signals may be interchanged.

Compound **7**. Colourless syrup, $[\alpha]_D^{27}$ +40.9° (MeOH; *c* 0.43). EIMS *m/z* (rel. int.): 238.0979 $[M]^+$ (19), calc. for $C_{11}H_{14}N_2O_4$: 238.0954, 223 (5), 210 (20), 195 (9), 168 (13), 152 (8), 138 (15), 113 (19), 98 (88), 96 (100), 86 (18), 68 (24); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400–2400 (COOH), 3280 (NH), 2970, 2930, 2870 (CH), 1715 (C=O), 1660 (C=O), 1640 (C=C), 1450, 1400, 1380, 1300, 1260, 1235, 1180, 1155, 1100, 1080, 995, 865, 825; ^1H NMR (methanol-d_4): δ6.62 (1H, *dq*, *J* = 1.7, 1.7 Hz, H-4'), 6.12 (1H, *dq*, *J* = 1.7, 1.7 Hz, H-5'), 3.88 (1H, *br d*, *J* = 9.2 Hz, H-2), 2.75 (1H, *m*, H-4), 2.29 (1H, *br dd*, *J* = 12.9, 8.3 Hz, H-3a), 1.94 (1H, *ddd*, *J* = 12.9, 11.1, 9.2 Hz, H-3b), 1.88 (3H, *dd*, *J* = 1.7, 1.7 Hz, H-6'), 1.17 (3H, *d*, *J* = 7.1 Hz, H-6); ^{13}C NMR (methanol-d_4): δ180.5 (C-5), 175.8 (C-7), 175.3 (C-2'), 139.6 (C-3'), 139.3 (C-4'), 64.2 (C-5'), 56.2 (C-2), 36.8 (C-4), 33.9 (C-3), 15.9 (C-6), 10.6 (C-6').

Methylation of compound **7**. Compound **7** (5.0 mg) was dissolved in MeOH and cooled at 0°. CH_2N_2 in Et_2O was added to the sample soln. After being set aside for 2 hr at 0°, the reaction mixture was evapd under red. pres., and the crude residue was subjected to HPLC with MeOH–H_2O (3:2) to produce a monomethyl ester (**7a**; 1.5 mg) as a colourless syrup, $[\alpha]_D^{26}$ +40.0° (MeOH; *c* 0.30). EIMS *m/z* (rel. int.): 252 $[M]^+$ (13), 237 $[M-Me]^+$ (5), 224 (10), 193 (12), 165 (19), 138 (9), 112 (8), 98 (100), 96 (45), 85 (43), 83 (67), 70 (21); IR ν_{max}^{film} cm^{-1}: 3260 (NH), 3090, 2959, 2940, 2880 (CH), 1741, 1704 (C=O), 1655 (C=C), 1585, 1556, 1456, 1437, 1411, 1375, 1262, 1211, 1180, 1144, 1102, 1090, 1045, 1020, 870, 800, 719; ^1H NMR (methanol-d_4): δ6.62 (1H, *dq*, *J* = 1.7, 1.7 Hz, H-4'), 6.12 (1H, *dq*, *J* = 1.7, 1.7 Hz, H-5'), 3.94 (1H, *dd*, *J* = 9.1, 1.0 Hz, H-2), 3.70 (3H, *s*, COOMe), 2.75 (1H, *m*, H-4), 2.28 (1H, *ddd*, *J* = 13.2, 8.9, 1.0 Hz, H-3a), 1.92 (1H, *ddd*, *J* = 13.2, 11.8, 9.1 Hz, H-3b), 1.88 (3H, *dd*, *J* = 1.7, 1.7 Hz, H-6'), 1.18 (3H, *d*, *J* = 7.1 Hz, H-6); ^{13}C NMR (methanol-d_4): δ179.3 (C-5), 174.6 (C-2'), 171.3 (C-7), 139.2 (C-3' and C-4'), 64.1 (C-5'), 56.1 (C-2), 53.1 (COOMe), 36.8 (C-4), 33.3 (C-3), 16.0 (C-6), 10.6 (C-6').

Compound **8**. Colourless prisms (MeOH), mp 213–215° (dec.), $[\alpha]_D^{26}$ ±0° (MeOH; *c* 0.20). EIMS *m/z* (rel. int.): 208.0867 $[M]^+$ (1.1), calc. for $C_{10}H_{12}N_2O_3$: 208.0849, 112 (12), 96 (100), 69 (10); UV λ_{max}^{MeOH} nm (log ε): 235 (3.10). The CD spectrum measured in MeOH showed no Cotton effect. Other spectral data: see ref. [11].

X-Ray analysis of **8**. Crystal system: monoclinic. Crystal dimensions (mm): 0.600 × 0.200 × 0.100. No reflections used for unit cell determination (2θ range): 25 (86.9–89.9°). Omega scan peak width at half-height: 0.27. Lattice parameters: *a* = 5.5693(5) Å, *b* = 14.578(2) Å, *c* = 12.530(1) Å, β = 95.90 (1)°, *V* = 1011.9(2) $Å^3$. Space group: $P2_1/c$ (no. 14). *Z* value: 4. D_{calc}: 1.367 $g\,cm^{-3}$. F_{000}: 440. μ (CuK_α): 8.14 cm^{-1}. Diffractometer: Rigaku AFC5R. Radiation: CuK_α (λ = 1.54178 Å). Temp.: 23°. Attenuators: Ni foil (factors: 3.5, 12.7, 44.9). Take-off angle: 6.0°. Detector aperture: 6.0 mm horizontal, 6.0 mm vertical. Crystal to detector distance: 25.8 cm. Scan type: ω-2θ. Scan rate: 32.0° min^{-1} (in omega; 2 rescans). Scan width: (1.73 + 0.30 tan θ)°. 2θ max: 120.2°. No. of reflections measured: total: 1765, unique: 1585 (R_{int} = 0.032). Corrections: Lorentz polarization, absorption (trans. factors: 0.87–1.13), decay (−4.40% decline), secondary extinction (coefficient: 0.80581E-06). Structure solution: direct methods. Refinement: full-matrix least-squares. Function minimized: $\Sigma w (|Fo| - |Fc|)^2$. Least-squares weights: $4Fo^2/\sigma^2$ (Fo^2). *p*-Factor: 0.06. Anomalous dispersion: all non-hydrogen atoms. No. observations ($I > 3.00\ \sigma(I)$): 1028. No. variables: 173. Reflection/parameter ratio: 5.94. Residuals: *R*; R_w: 0.041; 0.056. Goodness of fit

indicator: 1.31. Max. shift/error in final cycle: 0.48. Max. peak in final diff. map: 0.17 e Å^{-3}. Minimum peak in final diff. map: −0.19 e Å^{-3}.

Compound **9**. Amorphous powder, $[\alpha]_D^{25}$ −48.8° (MeOH; *c* 0.40). (Found: C, 57.06; H, 8.12. Calc. for $C_{51}H_{86}O_{23}$: C, 57.40; H, 8.12%.) SIMS *m/z*: 1049 $[M-OH]^+$, $[M-glucosyl]^+$; IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2920 (CH), 1445, 1375, 1300, 1255, 1155, 1035, 980, 900, 820, 800, 695; ^{1}H NMR (pyridine-d_5): δ6.20 (1H, *br s*, H-1″), 5.11 (1H, *d*, *J* = 7.7 Hz, H-1‴), 4.98 (1H, *d*, *J* = 7.7 Hz, H-1′), 4.80 (1H, *d*, *J* = 7.7 Hz, H-1⁗), 1.73 (3H, *d*, *J* = 6.1 Hz, H-6″), 1.33 (3H, *d*, *J* = 7.0 Hz, H-21), 0.99 (3H, *d*, *J* = 6.5 Hz, H-27), 0.88, 0.86 (each 3H, *s*, H-18, H-19).

Acid hydrolysis of **9**. Hydrolysis of **9** (16.0 mg) with 1 M HCl (dioxane–H_2O, 1:1) was carried out at 100° for 3 hr under an N_2 atmosphere. The reaction mixture was neutralized with 1 M NaOH and chromatographed on silica gel with $CHCl_3$–Me_2CO (9:1) and $CHCl_3$–MeOH–H_2O (20:10:1) and on Sephadex LH-20 with MeOH to yield D-glucose (4.7 mg), L-rhamnose (2.0 mg) and an aglycone (4.7 mg), identified as tigogenin. D-Glucose: $[\alpha]_D^{26}$ +54.0° (H_2O; *c* 0.47); TLC, R_f 0.36 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). L-Rhamnose: $[\alpha]_D^{26}$ +7.8° (H_2O; *c* 0.20); TLC, R_f 0.69 (*n*-BuOH–Me_2CO–H_2O, 4:5:1).

Enzymatic hydrolysis of **9**. Compound **9** (40.0 mg) was dissolved in an AcOH–AcONa buffer (pH 5) with β-glucosidase (30 mg), and the mixture was incubated at room temp. overnight. The crude products were purified by silica gel CC with $CHCl_3$–MeOH–H_2O (20:10:1) and HPLC with EtOH–H_2O (7:3) to yield D-glucose (5.5 mg) and the corresponding spirostanol saponin (**9a**; 30.7 mg). Compound **9a**: amorphous powder, $[\alpha]_D^{26}$ −56.7° (pyridine; *c* 0.60). (Found: C, 58.31; H, 8.25. Calc. for $C_{45}H_{74}O_{17}\cdot 2H_2O$: C, 58.55; H, 8.51%.) SIMS *m/z*: 741 $[M-rhamnosyl+2H]^+$, 723 $[M-glucosyl]^+$; IR ν_{max}^{film} cm^{-1}: 3442 (OH), 2940, 2891 (CH), 1470, 1460, 1375, 1361, 1343, 1281, 1243, 1173, 1148, 1114, 1081, 1062, 1043, 994, 984, 963, 925, 900, 865, 843 [(25*R*)-spiroacetal, intensity 925 < 900]; ^{1}H NMR (pyridine-d_5): δ6.21 (1H, *br s*, H-1″), 5.11 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.96 (1H, *d*, *J* = 7.2 Hz, H-1′), 3.58 (1H, *dd*, *J* = 10.5, 3.1 Hz, H-26a), 3.50 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 1.74 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.14 (3H, *d*, *J* = 6.9 Hz, H-21), 0.86 (3H, *s*, H-19), 0.82 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.5 Hz, H-27).

Partial hydrolysis of **9a**. Compound **9a** (25.0 mg) was treated with 0.2 M HCl (dioxane–H_2O, 1:1) at 100° for 1 hr. The reaction mixture was neutralized with 1 M NaOH, and purified by silica gel CC with EtOAc–MeOH (5:1) and HPLC with MeOH–H_2O (19:1). Compound **9a** (16.0 mg) was recovered unchanged and a partial hydrolysate (**9b**; 2.2 mg) was obtained. Compound **9b**: amorphous powder, $[\alpha]_D^{26}$ −62.5° (pyridine; *c* 0.40). FABMS *m/z*: 763 $[M+Na]^+$, 741 $[M+H]^+$; IR ν_{max}^{film} cm^{-1}: 3351 (OH), 2924, 2848 (CH), 1457, 1378, 1261, 1052, 1030, 983, 922, 899, 864, 801 [(25*R*)-spiroacetal, intensity 922 < 899]; ^{1}H NMR (pyridine-d_5): δ5.22 (1H, *d*, *J* = 7.8 Hz, H-1″), 4.99 (1H, *d*, *J* = 7.7 Hz, H-1′), 3.59 (1H, *dd*, *J* = 10.5, 3.2 Hz, H-26a), 3.51 (1H, *dd*, *J* = 10.5, 10.5 Hz, H-26b), 1.14 (3H, *d*, *J* = 7.0 Hz, H-21), 0.83 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.7 Hz, H-27), 0.68 (3H, *s*, H-19).

Compound **11**. Amorphous powder, $[\alpha]_D^{26}$ −56.0° (MeOH; *c* 0.20). SIMS *m/z*: 942 $[M+K+H]^+$, 924 $[M+Na-H]^+$, 903 $[M+H]^+$; IR ν_{max}^{film} cm^{-1}: 3383 (OH), 2928, 2880 (CH), 1457, 1379, 1260, 1245, 1160, 1054, 985, 925, 900, 865, 815 [(25*R*)-spiroacetal, intensity 925 < 900]; ^{1}H NMR (pyridine-d_5): δ6.21 (1H, *br s*, H-1″), 5.11 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.93 (1H, *d*, *J* = 7.3 Hz, H-1′), 3.60–3.50 (2H, H-26), 3.09 (1H, *t*-like, *J* = 8.0 Hz, H-17), 1.74 (3H, *d*, *J* = 6.3 Hz, H-6″), 1.20 (3H, *d*, *J* = 6.9 Hz, H-21), 0.95 (3H, *s*, H-18), 0.90 (3H, *s*, H-19), 0.70 (3H, *d*, *J* = 3.6 Hz, H-27).

Acid hydrolysis of **11**. Compound **11** (12.0 mg) was subjected to acid hydrolysis as for **9** and the reaction mixture was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (20:10:1) and HPLC with MeOH–H_2O (9:1) to yield D-glucose, L-rhamnose and an aglycone (**11a**; 1.2 mg). Compound **11a**: amorphous powder, $[\alpha]_D^{26}$ −45.0° ($CHCl_3$; *c* 0.24). EIMS *m/z* (rel. int.): 432 $[M]^+$ (6), 360 (9), 345 (9), 318 (13), 300 (25), 271 (9), 248 (6), 139 (100), 115 (14), 107 (18); IR ν_{max}^{film} cm^{-1}: 3464 (OH), 2930, 2855 (CH), 1457, 1380, 1246, 1185, 1175, 1155, 1150, 1115, 1072, 1060, 1048, 1012, 985, 960, 925, 900, 870 [(25*R*)-spiroacetal, intensity 925 < 900]; ^{1}H NMR (pyridine-d_5): δ4.65 (1H, *ddd*, *J* = 7.7, 7.7, 7.0 Hz, H-16), 3.96 (1H, *br s*, $W_{1/2}$ = 10.2 Hz, H-12), 3.79 (1H, *br m*, $W_{1/2}$ = 24.4 Hz, H-3), 3.60–3.50 (2H, H-26), 3.11 (1H, *dd*, *J* = 8.0, 7.7 Hz, H-17), 1.21 (3H, *d*, *J* = 6.9 Hz, H-21), 0.98 (3H, *s*, H-18), 0.86 (3H, *s*, H-19), 0.70 (3H, *d*, *J* = 4.6 Hz, H-27).

Compound **12**. Amorphous powder, $[\alpha]_D^{26}$ −65.2° (MeOH; *c* 0.71). SIMS *m/z*: 923 $[M+Na]^+$; IR ν_{max}^{film} cm^{-1}: 3393 (OH), 2929, 2880 (CH), 1457, 1378, 1265, 1245, 1160, 1070, 1054, 985, 925, 900, 870, 840, 815 [(25*R*)-spiroacetal, intensity 925 < 900]; ^{1}H NMR (pyridine-d_5): δ6.23 (1H, *br s*, H-1″), 5.31 (1H, *br d*, *J* = 5.0 Hz, H-6), 5.11 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.92 (1H, *d*, *J* = 6.7 Hz, H-1′), 3.60–3.50 (2H, H-26), 3.08 (1H, *dd*, *J* = 8.7, 7.9 Hz, H-17), 1.76 (3H, *d*, *J* = 6.2 Hz, H-6″), 1.19 (3H, *d*, *J* = 6.9 Hz, H-21), 1.09 (3H, *s*, H-19), 0.96 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.5 Hz, H-27).

Acid hydrolysis of **12**. Compound **12** (12.0 mg) was subjected to acid hydrolysis as for **9** to yield D-glucose, L-rhamnose and an aglycone (**12a**; 1.9 mg). Compound **12a**: amorphous powder, $[\alpha]_D^{26}$ −63.2° ($CHCl_3$; *c* 0.38). EIMS *m/z* (rel. int.): 430 $[M]^+$ (5), 358 (10), 316 (13), 298 (35), 139 (100), 115 (14); IR ν_{max}^{film} cm^{-1}. 3489 (OH), 2951, 2929, 2864 (CH), 1452, 1379, 1244, 1172, 1055, 1009, 982, 960, 921, 900, 870, 839, 807 [(25*R*)-spiroacetal, intensity 921 < 900]; ^{1}H NMR (pyridine-d_5): δ5.41 (1H, *br d*, *J* = 4.6 Hz, H-6), 4.65 (1H, *ddd*, *J* = 7.7, 7.7, 6.8 Hz, H-16), 4.02 (1H, *br d*, *J* = 3.6 Hz, H-12), 3.80 (1H, *br m*, $W_{1/2}$ = 22.0 Hz, H-3), 3.60–3.50 (2H, H-26), 3.09 (1H, *dd*, *J* = 8.7, 7.7 Hz, H-17), 1.21 (3H, *d*, *J* = 7.0 Hz, H-21), 1.09 (3H, *s*, H-19), 0.99 (3H, *s*, H-18), 0.70 (3H, *d*, *J* = 5.4 Hz, H-27).

Assay of phosphodiesterase. Phosphodiesterase activity was assayed by the method described in a previous paper [16].

Acknowledgements—We are grateful to Mrs C. Sakuma for the measurements of the 2D NMR spectra, and Dr Y. Shida and Mrs Y. Katoh for the measurements of the mass spectra.

REFERENCES

1. Shimizu, M. (1976) in *Nippon No Yuri*, pp. 112–116. Seibundo-Shinkosya, Tokyo.
2. Haw, S. G. (1986) in *The Lilies of China*, pp. 37–39. B. T. Batsford, London.
3. Bryan, J. E. (1989) in *Bulbs*, Vol. II, p. 251. Timber Press, Portland, OR.
4. Shimomura, H., Sashida, Y., Mimaki, Y. and Minegishi, Y. (1987) *Phytochemistry* **26**, 582.
5. Strack, D., Sachs, G., Römer, A. and Wiermann, R. (1981) *Z. Naturforsch.* **36c**, 721.
6. Shimomura, H., Sashida, Y. and Mimaki, Y. (1986) *Phytochemistry* **25**, 2897.
7. Cooper, R., Gottlieb, H. E. and Lavie, D. (1978) *Phytochemistry* **17**, 1673.
8. Shimomura, H., Sashida, Y. and Mimaki, Y. (1987) *Phytochemistry* **26**, 844.
9. Shimomura, H., Sashida, Y., Mimaki, Y. and Iitaka, Y. (1988) *Chem. Pharm. Bull.* **36**, 2430.
10. Breitmaier, E. and Voelter, W. (1987) in *Carbon-13 NMR Spectroscopy*, 3rd edn, p. 279. VCH, Weinheim.

11. Haladová, M., Eisenreichová, E., Bučková, A., Tomko, J., Uhrín, D. and Ubik, K. (1991) *Collect. Czech. Chem. Commun.* **56**, 436.
12. Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur, R. S. (1985) *Phytochemistry* **24**, 2479.
13. Malinow, M. R., Gardner, J. O., Nelson, J. T., McLaughlin, P., Upson, B. and Aigner-Held, R. (1986) *Steroids* **48**, 197.
14. Watanabe, Y., Sanada, S., Ida, Y. and Shoji, J. (1983) *Chem. Pharm. Bull.* **31**, 3486.
15. Abdullaev, N. D., Samikov, K., Antsupova, T. P., Yagudaev, M. R. and Yunusov, S. Yu. (1987) *Khim. Prir. Soedin.* 692.
16. Nikaido, T., Ohmoto, T., Noguchi, H., Kinoshita, T., Saitoh, H. and Sankawa, U. (1981) *Planta Med.* **43**, 18.
17. Nikaido, T., Ohmoto, T., Kinoshita, T., Sankawa, U., Nishibe, S. and Hisada, S. (1981) *Chem. Pharm. Bull.* **29**, 3586.