Three New Furostanol Saponins from the Bulbs of *Ipheion uniflorum*

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Phytochemical screening of the bulbs of *Ipheion uniflorum* (Liliaceae) has led to the isolation of three new furostanol saponins (2—4) along with a known phytoecdysteroid, ecdysterone (1). The structures of the new compounds were determined by two-dimensional NMR techniques, ^{1}H–^{1}H correlation spectroscopy (COSY), homonuclear Hartmann–Hahn (HOHAHA), phase-sensitive nuclear Overhauser effect spectroscopy (NOESY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectra, and hydrolysis to be 3β-hydroxy-22α-methoxy-26-*O*-β-D-glucopyranosyloxy-5α-furost-25(27)-en-2-one 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-α-L-arabinopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside} (2) and its 25(27)-dihydro derivatives (3: 25*S*; 4: 25*R*). The inhibitory activity exhibited by compounds 1—4 and the corresponding spirostanol saponins (2a and 3a) of 2 and 3 on cAMP phosphodiesterase was assayed as a primary screening test to identify new compounds with medicinal potential.

Keywords *Ipheion uniflorum*; Allioideae; Liliaceae; furostanol saponin; two-dimensional NMR spectroscopy; cAMP phosphodiesterase inhibition

We have already examined the underground parts of *Allium giganteum*,[1] *A. aflatunense*,[1b] *A. schubertii*,[2] *A. albopilosum*,[3] *A. ostrowskianum*,[3] and *Agapanthus inapertus*,[4] which are members of the subfamily Allioideae in Liliaceae, and isolated a considerable number of new steroidal saponins and cholestane glycosides. As a part of our contribution to the study of this subfamily, we have now carried out phytochemical screening of the bulbs of *Ipheion uniflorum*, which is native to South America and has a characteristic smell like garlic.[5] As a result of this, three new furostanol saponins with up to six monosaccharides and a known phytoecdysteroid were isolated. This paper reports the structural characterization of the new saponins, based on extensive two-dimensional (2D) NMR analysis and hydrolysis. The inhibitory activity exhibited by the isolated compounds and their derivatives on cAMP phosphodiesterase was also investigated.

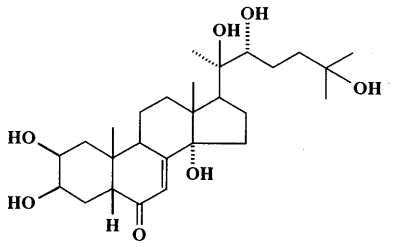
The 1-butanol-soluble phase of the methanolic extract of *I. uniflorum* bulbs gave compounds **1**—**4** after a series of chromatographic separations.

Compound **1**, which was obtained in a yield of 0.012%, was identified as ecdysterone by its IR, ^{1}H-NMR and ^{13}C-NMR spectra.[6] This is the first isolation of a phytoecdysteroid from plants of the subfamily Allioideae.

Compound **2** was obtained as a white amorphous powder, $[\alpha]_D$ −72.0° (chloroform–methanol, 1 : 1) with a molecular formula, $C_{62}H_{100}O_{32}$, deduced from the negative-ion FAB-MS (*m/z* 1356 $[M]^-$), ^{13}C-NMR spectrum and elemental analysis. A positive color reaction with Ehrlich's reagent suggested that **2** was a furostanol saponin.[7] Acid hydrolysis of **2** with 1 N hydrochloric acid in dioxane–H_2O (1 : 1) gave several unidentified artifactual sapogenols; no genuine aglycon could be obtained, and D-glucose, D-galactose, D-xylose, L-rhamnose and L-arabinose were identified by HPLC following their conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives.[8]

The ^{1}H–^{1}H correlation spectroscopy (COSY) combined with 2D homonuclear Hartmann–Hahn (HOHAHA) spectra, which were measured in pyridine-d_5–methanol-d_4 (10 : 1) to minimize signal overlap, clarified the ^{1}H–^{1}H spin-networks of the aglycon moiety of **2**, giving rise to the structural fragments as shown in Fig. 1. Furthermore, signals for a carbonyl carbon, an acetal carbon and two quaternary carbons were observed in the ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **2**. The connectivities of the partial structures A and B, two tertiary methyl groups and a methoxyl group through quaternary carbons were established by interpretation of the ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) spectrum, leading to a structure with the A—E rings of a 22-methoxyfurostanol skeleton (Fig. 2). The quaternary carbon signal observed at δ 41.3 showed $^2J_{C,H}$ and $^3J_{C,H}$ correlation peaks with the ^{1}H signals at δ 2.22 (m, H-20) and 0.75 (3H, s, H-18), and was assigned to C-13. Another quaternary carbon signal at δ 41.1 was assignable to C-10, which was correlated to the ^{1}H signals at δ 2.41 and 1.92 (ABq, J = 12.9 Hz, H-1 equatorial and H-1 axial), and 0.82 (3H, s, H-19). The correlation peaks between δ 205.7 (C=O) and each of the ^{1}H signals at δ 2.41 and 1.92, and 4.75 (dd, J = 11.2, 2.5 Hz, H-3) allowed the location of the carbonyl group to be assigned to C-2. The remaining free bonds were C-22 and C-23, and the connection from C-22 to C-23 was supported by a nuclear Overhauser effect (NOE) correlation between the OMe (δ 3.26) and H-23b (δ 2.01) protons in the phase-sensitive NOE correlation spectroscopy (PHNOESY) spectrum.

The NOE correlations, H-19/H-1 equatorial, H-4 axial and H-8, H-18/H-20, and H-17/H-14 and H-16 provided evidence for the A/B *trans*, B/C *trans*, C/D *trans* and D/E *cis* ring fusions. An intense NOE between the H-16α proton and C-22 methoxy protons led to the assignment of the C-22α configuration (Fig. 3).



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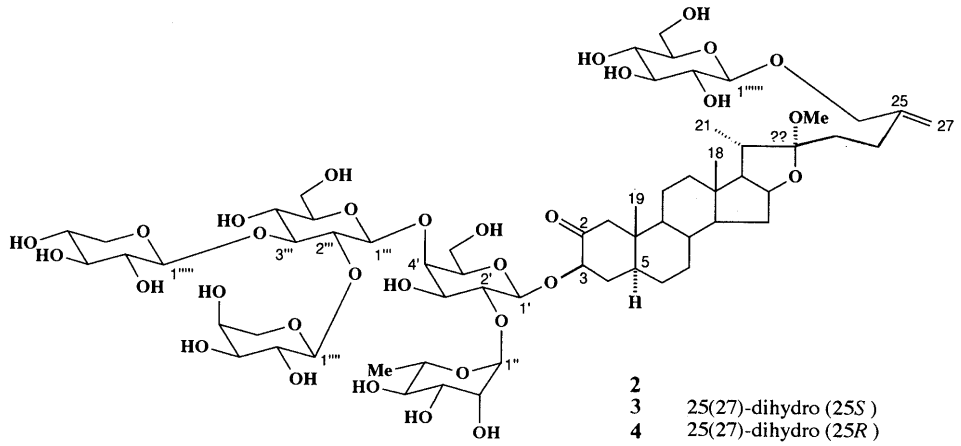


2
3 25(27)-dihydro (25*S*)
4 25(27)-dihydro (25*R*)

Chart 1

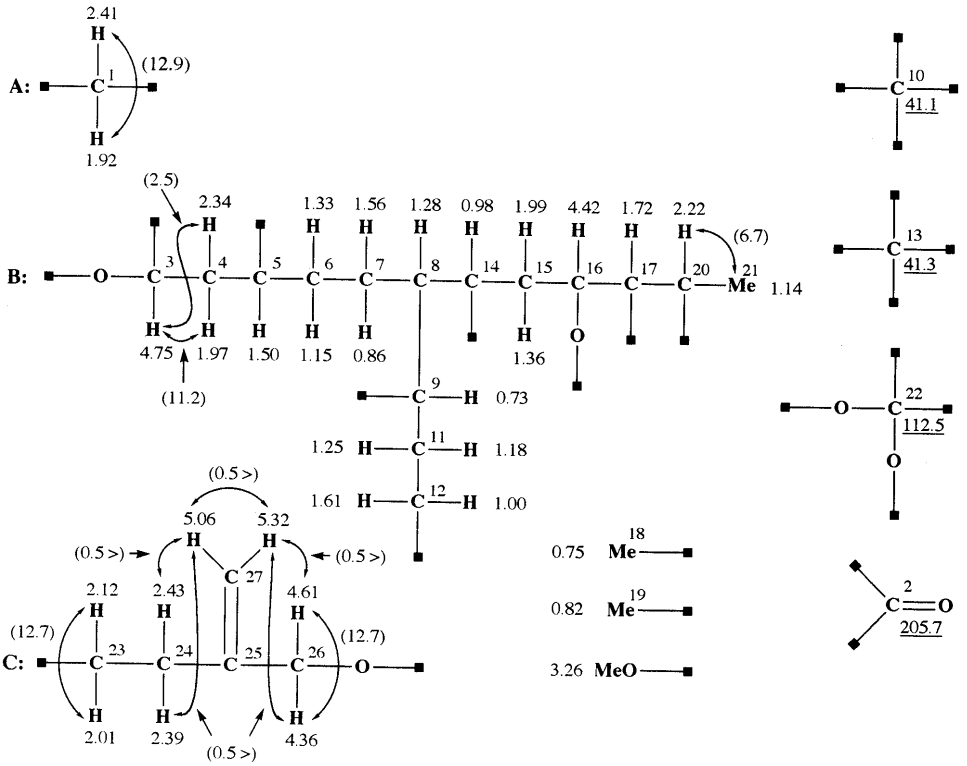


Fig. 1. Partial Structures of the Aglycon Moiety of **2** Shown by the ^{1}H–^{1}H COSY, HOHAHA, ^{13}C-NMR and DEPT Spectra

Recorded in pyridine-d_5–methanol-d_4 (10 : 1). *J* values (Hz) in the ^{1}H-NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C-NMR chemical shifts.

The above data demonstrated that the aglycon of **2** was 3,26-dihydroxy-22α-methoxy-5α-furost-25(27)-en-2-one.

The complex structures of the saccharide moieties were solved by the concerted use of the 2D NMR spectra. Detailed inspection of the ^{1}H–^{1}H COSY and HOHAHA spectra allowed the sequential assignments of the ^{1}H

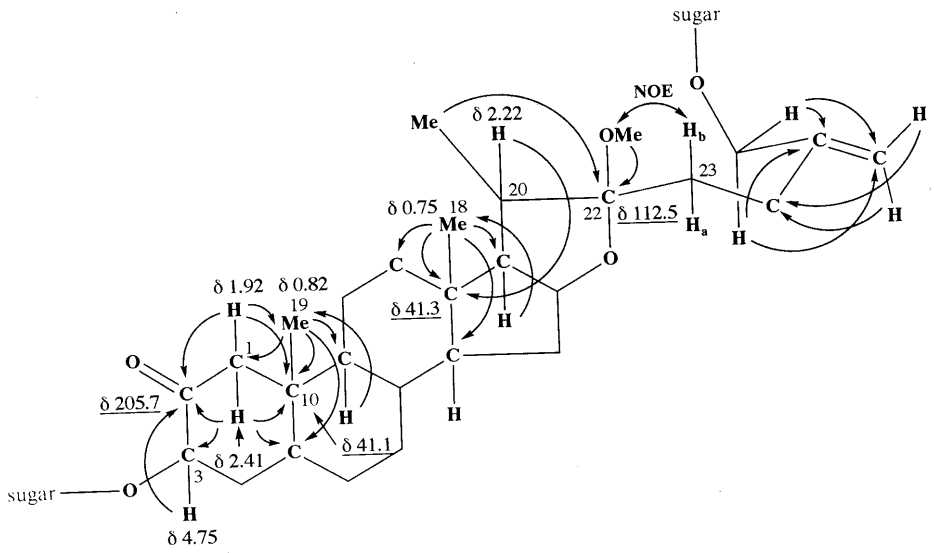


Fig. 2. ^1H–^{13}C Long-Range Correlations of the Aglycon Moiety of **2** Observed in the HMBC Spectrum

Recorded in pyridine-d_5–methanol-d_4 (10 : 1). Underlined fugures indicate ^{13}C-NMR chemical shifts.

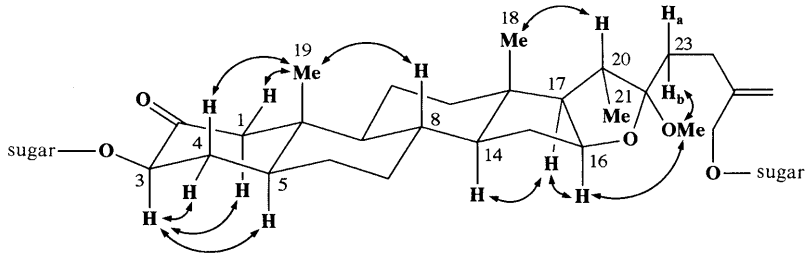


Fig. 3. NOE Correlations of **2**

Recorded in pyridine-d_5–methanol-d_4 (10 : 1).

resonances for each monosaccharide to be made, starting from the easily distinguished anomeric protons. The ^1H-detected heteronuclear multiple quantum coherence (HMQC) spectrum correlated all the ^1H resonances with those of the corresponding carbons, leading to unambiguous assignments of the ^{13}C resonances (Table II). Comparison of the ^{13}C-NMR chemical shifts thus assigned with those of the reference methyl glycosides,[9] taking into account the known effects of *O*-glycosylation and the result of acid hydrolysis, indicated that **2** contained a terminal α-L-rhamnopyranosyl (δ 101.6, 72.2, 72.5, 73.8, 69.9 and 18.4), a terminal α-L-arabinopyranosyl (δ 105.9, 73.2, 74.7, 69.7 and 67.4), a terminal β-D-xylopyranosyl (δ 104.9, 75.0, 78.5, 70.6 and 67.3), a terminal β-D-glucopyranosyl (δ 103.7, 75.0, 78.4, 71.6, 78.4 and 62.7), a 2,4-disubstituted β-D-galactopyranosyl (δ 99.7, 77.0, 76.1, 81.5, 75.5 and 60.5) and a 2,3-disubstituted β-D-glucopyranosyl (δ 105.5, 81.7, 88.0, 70.2, 77.6 and 62.7) units. The β-configurations of the anomeric centers of the glucose, galactose, arabinose and xylose moieties were supported by the large *J* values of the anomeric protons (7.7—8.1 Hz). The $^3J_{C-H}$ correlation from each anomeric proton, across the glycosidic bond to the carbon of another substituted monosaccharide or the aglycon, revealed the sugar sequences. In the HMBC spectrum, the anomeric proton signals at δ 6.17 (br s, rhamnose), 5.24 (d, $J=7.7$ Hz, arabinose), 5.15 (d, $J=7.8$ Hz, xylose), 4.90 (d, $J=8.1$ Hz, 2,3-disubstituted glucose), 4.88 (d, $J=8.1$ Hz, 2,4-disubstituted galactose) and 4.84 (d, $J=7.9$ Hz, terminal glucose) exhibited correlations with the ^{13}C signals at δ 77.0 (C-2 of 2,4-disubstituted galactose), 81.7 (C-2 of 2,3-disubstituted glucose), 88.0 (C-3 of 2,3-disubstituted glucose), 81.5 (C-4 of 2,4-disubstituted galactose), 80.1 (C-3 of aglycon) and 72.1 (C-26 of aglycon) (Fig. 4). The configuration of the C-3 hydroxyl group of the aglycon bearing the pentasaccharide moiety was determined to be β from the multiplicity of the H-3 proton ($J_{H\text{-}3,H\text{-}4\,axial}=11.2$ Hz, $J_{H\text{-}3,H\text{-}4\,equatorial}=2.5$ Hz), and by the NOE correlations of H-3/H-1 axial, H-4 equatorial and H-5 axial. From the data presented above, the structure of **2** was established as 3β-hydroxy-22α-methoxy-26-*O*-β-D-glucopyranosyloxy-5α-furost-25(27)-en-2-one 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-α-L-arabinopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}.

Enzymatic hydrolysis of **2** with β-glucosidase gave D-glucose and the corresponding spirostanol saponin (**2a**), which was then tested for cAMP phosphodiesterase inhibitory activity. The spectral data of **2a** are shown in Table I and the Experimental section.

The spectral data of **3** and **4** were very similar to those of **2**. The negative-ion FAB-MS of **3** and **4** gave the same molecular ion peak at *m/z* 1358, which was only 2 mass units greater than that of **2**. The ^1H-NMR spectra of **3**

Table I. ^{13}C-NMR Spectral Data for Compounds **1**, **2**, **2**[a], **3**, **3a**, and **4**

C	1	2	2[a]	2a	3	3a	4
1	38.0	53.2	53.3	53.3	53.3	53.2	53.3
2	68.1	205.1	205.7	205.2	205.1	205.2	205.1
3	68.1	80.0	80.1	80.1	80.1	80.1	80.1
4	32.4	35.8	35.9	35.8	35.8	35.8	35.8
5	51.4	44.0	44.1	44.0	44.0	44.0	44.0
6	203.4	27.8	27.8	27.8	27.8	27.8	27.8
7	121.7	32.1	32.1	32.1	32.1	32.1	32.1
8	166.1	34.5	34.6	34.5	34.5	34.5	34.5
9	34.5	53.7	53.7	53.6	53.7	53.6	53.7
10	38.7	41.1	41.1	41.1	41.1	41.1	41.1
11	21.1	21.2	21.3	21.3	21.3	21.3	21.3
12	31.8	39.7	39.8	39.8	39.7	39.8	39.7
13	48.1	41.1	41.3	40.7	41.1	40.7	41.1
14	84.2	56.1	56.2	56.2	56.1	56.2	56.1
15	32.0	32.0	32.1	32.0	32.0	32.0	32.1
16	21.5	81.4	81.4	81.4	81.3	81.2	81.3
17	50.1	64.2	64.3	63.0	64.3	62.8	64.3
18	17.9	16.4	16.4	16.4	16.4	16.4	16.4
19	24.5	12.7	12.7	12.7	12.7	12.7	12.7
20	76.9	40.5	40.5	41.9	40.5	42.5	40.6
21	21.7	16.2	16.2	15.0	16.3	14.8	16.3
22	77.6	112.4	112.5	109.4	112.7	109.7	112.7
23	27.5	31.7	31.7	33.2	31.0	26.2[b]	30.9
24	42.6	28.2	28.2	29.0	28.2	26.4[b]	28.3
25	69.6	146.9	146.9	144.5	34.5	27.6	34.3
26	30.0	72.1	72.1	65.1	74.9	65.1	75.2
27	30.1	111.2	111.3	108.7	17.5	16.3	17.2
OMe		47.4	47.4		47.4		47.3
1′		99.8	99.7	99.8	99.8	99.8	99.8
2′		77.1	77.0	77.1	77.1	77.1	77.1
3′		76.2	76.1	76.2	76.2	76.2	76.2
4′		81.4	81.5	81.4	81.4	81.4	81.4
5′		75.5	75.5	75.6	75.6	75.5	75.6
6′		60.4	60.5	60.5	60.5	60.5	60.5
1″		101.7	101.6	101.7	101.7	101.7	101.7
2″		72.3	72.2	72.4	72.3	72.3	72.3
3″		72.7	72.5	72.7	72.7	72.7	72.7
4″		74.0	73.8	74.1	74.1	74.0	74.1
5″		69.9	69.9	69.9	69.9	69.9	69.9
6″		18.5	18.4	18.5	18.5	18.5	18.5
1‴		105.5	105.5	105.5	105.5	105.5	105.5
2‴		81.6	81.7	81.6	81.6	81.6	81.6
3‴		88.0	88.0	88.0	88.0	88.0	88.0
4‴		70.4	70.2	70.4	70.4	70.4	70.4
5‴		77.7	77.6	77.7	77.7	77.7	77.7
6‴		62.9	62.7	62.9	63.0	62.9	62.9
1⁗		105.9	105.9	105.9	105.9	105.9	105.9
2⁗		73.3	73.2	73.3	73.4	73.3	73.4
3⁗		74.8	74.7	74.8	74.9	74.8	74.9
4⁗		69.8	69.7	69.8	69.8	69.8	69.8
5⁗		67.3	67.4	67.4	67.4	67.4	67.4
1⁗′		105.0	104.9	105.0	105.1	105.0	105.0
2⁗′		75.1	75.0	75.1	75.1	75.1	75.1
3⁗′		78.7	78.5	78.8	78.8	78.7	78.8
4⁗′		70.7	70.6	70.7	70.7	70.7	70.7
5⁗′		67.3	67.3	67.4	67.4	67.3	67.4
1⁗″		103.9	103.7		105.0		105.0
2⁗″		75.2	75.0		75.3		75.2
3⁗″		78.6	78.4		78.7		78.7
4⁗″		71.8	71.6		71.9		71.9
5⁗″		78.5	78.4		78.5		78.5
6⁗″		62.9	62.7		63.0		63.0

Spectra were recorded in pyridine-d_5 (100 MHz) except for **2**.[a] *a*) Spectrum was recorded in pyridine-d_5–methanol-d_4 (10 : 1) (125 MHz). *b*) Assignments may be interchangeable.

Table II. ^{1}H- and ^{13}C-NMR Chemical Shifts for Saccharide Moieties of Compound **2**[a]

		^{1}H-NMR	^{13}C-NMR
Gal	1′	4.88 d (8.1)	99.7
	2′	4.42	77.0
	3′	4.10	76.1
	4′	4.42	81.5
	5′	3.97	75.5
	6′	4.58	60.5
		4.13	
Rha	1″	6.17 br s	101.6
	2″	4.67 br d (2.9)	72.2
	3″	4.38 dd (9.4, 2.9)	72.5
	4″	4.17 dd (9.4, 9.4)	73.8
	5″	4.72 dq (9.4, 6.1)	69.9
	6″	1.71 d (6.1)	18.4
Glc	1‴	4.90 d (8.1)	105.5
	2‴	4.17 dd (9.0, 8.1)	81.7
	3‴	4.03 dd (9.0, 9.0)	88.0
	4‴	3.79 dd (9.0, 9.0)	70.2
	5‴	3.75	77.6
	6‴	4.43	62.7
		3.97	
Ara	1⁗	5.24 d (7.7)	105.9
	2⁗	4.29 dd (8.7, 7.7)	73.2
	3⁗	3.94 dd (8.7, 3.7)	74.7
	4⁗	4.10	69.7
	5⁗	4.57 dd (12.5, 3.5)	67.4
		3.54 br d (12.5)	
Xyl	1⁗′	5.15 d (7.8)	104.9
	2⁗′	3.86 dd (8.3, 7.8)	75.0
	3⁗′	3.96 dd (8.3, 8.3)	78.5
	4⁗′	4.03 ddd (10.8, 8.3, 3.1)	70.6
	5⁗′	4.17 dd (10.8, 3.1)	67.3
		3.60 dd (10.8, 10.8)	
Glc	1⁗″	4.84 d (7.9)	103.7
	2⁗″	3.97	75.0
	3⁗″	4.13	78.4
	4⁗″	4.10	71.6
	5⁗″	3.86	78.4
	6⁗″	4.47 br d (10.8)	62.7
		4.28 dd (10.8, 5.8)	

a) Spectra were recorded in pyridine-d_5–methanol-d_4 (10 : 1) (500 MHz for ^{1}H-NMR, 125 MHz for ^{13}C-NMR). *J* values in parentheses are expressed in Hz.

and **4** displayed two three-proton doublet signals at δ 1.16 (d, J=6.8 Hz) and 1.05 (d, J=6.6 Hz) in **3**, and at δ 1.19 (d, J=6.8 Hz) and 1.01 (d, J=6.7 Hz) in **4** in addition to the signal due to the methyl group of rhamnose. No signal arising from an exomethylene group was seen in the ^{1}H- and ^{13}C-NMR spectra of **3** and **4**. The above data led us to believe that **3** and **4** are 25(27)-dihydro derivatives of **2**. Hydrogenation of **2** over platinum oxide gave **3** and **4** in an approximate ratio of 2 : 1. Treatment of **3** with β-glucosidase gave D-glucose and the corresponding spirostanol saponin (**3a**). The IR (ν_{max} 915>890),[10] ^{1}H-NMR [δ 1.08 (d, J=6.8 Hz, H-27)] and ^{13}C-NMR (δ 109.7, 26.2, 26.4, 27.6, 65.1 and 16.3 (C-22—C-27)][9a] of **3a** indicated that it was a 25*S*-spirostanol derivative. The structure of **3** was therefore 3β-hydroxy-22α-methoxy-26-*O*-β-D-glucopyranosyloxy-(25*S*)-5α-furostan-2-one 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-α-L-arabinopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside}, while that of **4** was the C-25 isomer (25*R*) of **3**.

The inhibition of cAMP phosphodiesterase activity

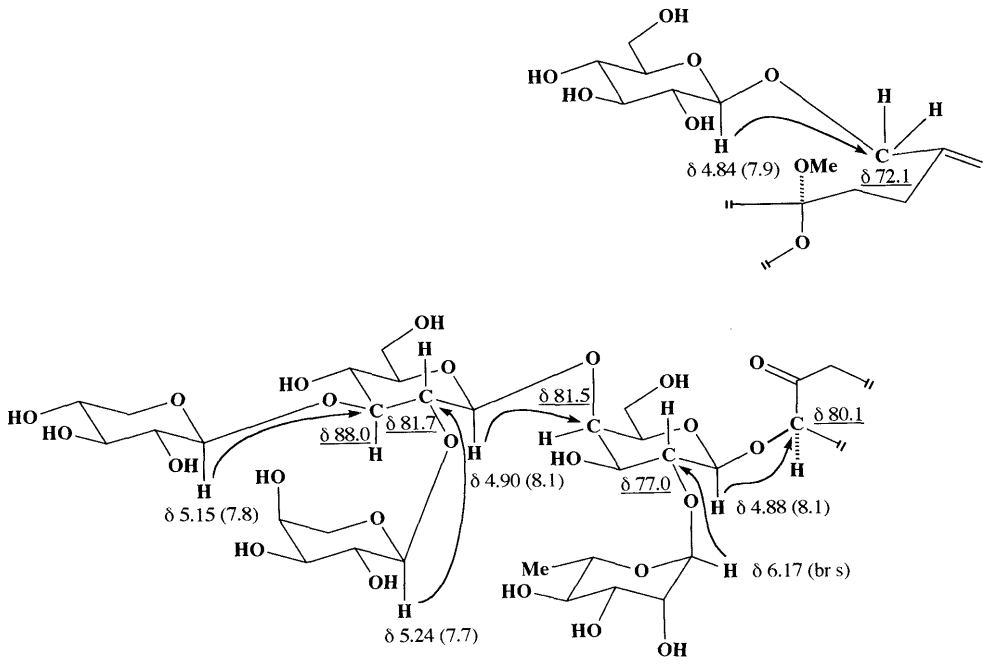
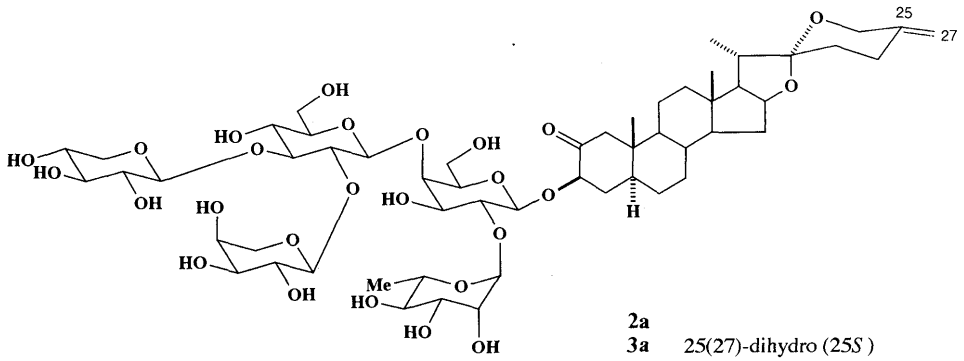


Fig. 4. ^{1}H–^{13}C Long-Range Correlations of the Saccharide Moieties of **2**

Recorded in pyridine-d_5–methanol-d_4 (10:1). J values (Hz) in the ^{1}H-NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C-NMR chemical shifts.



2a
3a 25(27)-dihydro (25*S*)

Chart 2

Table III. Inhibition of cAMP Phosphodiesterase Activity

Compound	IC_{50} ($\times 10^{-5}$ M)
1	18.3
2	89.3
2a	29.9
3	41.2
3a	20.0
4	145
Papaverine (positive control)	3.0

produced by compounds **1**—**4**, **2a** and **3a** was used as a primary screening test to identify new medicinal agents.[11] The IC_{50} values are listed in Table III. The phytoecdysteroid (**1**) and spirostanol saponins (**2a** and **3a**) derived from the furostanol saponins (**2** and **3**) by enzymatic hydrolysis, exhibited moderate inhibitory activity compared with papaverine, the positive control.

Experimental

Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 spectrophotometer and MS on a VG AutoSpec E instrument. Elemental analysis was carried out using Perkin-Elmer 240B elemental analyzer. 1D NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for ^{1}H-NMR) and 2D NMR on a Bruker AM-500 instrument (500 MHz for ^{1}H-NMR). Chemical shifts are given as δ-values with reference to tetramethylsilane (TMS), the internal standard. Assignments of the ^{13}C-NMR spectra of **2a**, **3**, **3a** and **4** were made on the basis of the various DEPT spectra and by correlation with data from **2**. Silica-gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-Kasei) and octadecylsilanized (ODS) silica-gel (Nacalai Tesque) were used for column chromatographies. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a Tosoh HPLC system (Tosoh: 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 or 4.6 mm i.d. × 250 mm, ODS, 5 μm) or a TSK-gel Silica-60 column (Tosoh, 4.6 mm i.d. × 250 mm, silica-gel, 5 μm). The liquid scintillation counter used was an Aloka LSC-903 instrument. Beef heart phosphodiesterase was purchased from Boehringer (Germany). Snake venom nucleotidase and cyclic AMP were obtained from Sigma (U.S.A.), and [^{3}H]cAMP from the Radiochemical Center (UK).

Isolation Fresh bulbs of *I. uniflorum* (2.9 kg), purchased from

Heiwaen, Japan, were cut into pieces and extracted with MeOH under reflux. The extract was concentrated almost to dryness under reduced pressure and the crude residue, after dilution with H_2O, was extracted with *n*-BuOH. The *n*-BuOH-soluble phase was fractionated on a silica-gel column, eluting with a gradient mixture of CH_2Cl_2–MeOH (6:1; 4:1; 2:1), and finally with MeOH to give four fractions (I—IV). Fraction II was chromatographed on silica-gel using $CHCl_3$–MeOH–H_2O (50:10:1) as the solvent system to give compound **1** (345 mg; 0.012%). Fraction III contained a considerable amount of saccharides, the removal of which was performed by column chromatography on Diaion HP-20 with an increasing amount of MeOH in H_2O. The fractions eluted with 80% MeOH and 100% MeOH were combined and purified by silica-gel column chromatography with a mobile phase consisting of $CHCl_3$–MeOH–H_2O (7:4:1). Finally, preparative HPLC with MeOH–H_2O (13:7) yielded compounds **2** (44 mg; 0.0015%), **3** (49 mg; 0.0017%) and **4** (18 mg; 0.00062%).

Compound 1 A white amorphous powder, $[\alpha]_D^{27}$ +58.5° (*c*=0.40, MeOH). Negative-ion FAB-MS *m/z*: 479 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2950 and 2880 (CH), 1640 (C=O), 1445, 1380, 1315, 1265, 1225, 1210, 1145, 1055, 1025, 955, 930, 905, 880. ^{1}H-NMR (methanol-d_4) δ: 5.81 (1H, d, *J*=2.6 Hz, H-7), 3.95 (1H, br d, *J*=2.8 Hz, H-3), 3.83 (1H, ddd, *J*=12.0, 3.6, 3.6 Hz, H-2), 3.32 (1H, dd, *J*=10.1, 1.6 Hz, H-22), 1.20 (3H×2, s, H-26, H-27), 1.19 (3H, s, H-21), 0.96 (3H, s, H-18), 0.89 (3H, s, H-19).

Compound 2 A white amorphous powder, $[\alpha]_D^{28}$ −72.0° (*c*=0.10, $CHCl_3$–MeOH (1:1)). *Anal.* Calcd for $C_{62}H_{100}O_{32}\cdot 4H_2O$: C, 52.09; H, 7.61. Found: C, 52.07; H, 7.50. Negative-ion FAB-MS *m/z*: 1356 $[M]^-$, 1223 [M−arabinosyl (or xylosyl)]$^-$, 1210 [M−rhamnosyl]$^-$, 929 [M−arabinosyl−xylosyl−glucosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2930 (CH), 1720 (C=O), 1450, 1370, 1165, 1070, 1040, 900, 775. ^{1}H-NMR (pyridine-d_5) δ: 6.28 (1H, br s, H-1″), 5.31 (1H, d, *J*=7.8 Hz, H-1‴), 5.21 (1H, d, *J*=7.7 Hz, H-1⁗), 4.96 (1H, d, *J*=7.8 Hz, H-1‴), 4.91 (1H, d, *J*=7.7 Hz, H-1′), 4.90 (1H, d, *J*=8.1 Hz, H-1⁗⁗), 3.27 (3H, s, OMe), 1.77 (3H, d, *J*=6.1 Hz, H-6″), 1.16 (3H, d, *J*=6.9 Hz, H-21), 0.83 (3H, s, H-19), 0.76 (3H, s, H-18).

Acid Hydrolysis of 2 A solution of **1** (8 mg) in 1 N HCl (dioxane–H_2O, 1:1) was refluxed for 3 h under an Ar atmosphere. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column, and then transferred to a silica-gel column, eluting with hexane–Me_2CO (5:1) and then $CHCl_3$–MeOH (1:1) to give several unidentified artifactual sapogenols and a mixture of monosaccharides (3.4 mg). Glucose, galactose, rhamnose, arabinose and xylose were identified as being present in the mixture by direct TLC comparison with authentic samples: glucose, *Rf* 0.40; galactose, *Rf* 0.34; xylose, *Rf* 0.58; rhamnose, *Rf* 0.65; arabinose, *Rf* 0.48 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). The sugar mixture was applied to a Sep-Pak C_{18} cartridge (Waters), then eluted with MeOH–H_2O (1:4) followed by MeOH. To the MeOH–H_2O (1:4) eluate (2.5 mg) in H_2O (1 ml), (−)-α-methylbenzylamine (5 mg) was added followed by Na[BH_3CN] (8 mg) in EtOH (1 ml). The mixture was left standing for 4 h at 40 °C, then acetylated with Ac_2O (0.3 ml) in pyridine (0.3 ml). The reaction mixture was passed through a Sep-Pak C_{18} cartridge, initially eluting with H_2O–MeCN (4:1, 10 ml), and then with MeCN (10 ml). The MeCN fraction was then passed through a Toyopak IC-SP M cartridge (Tosoh), eluting with EtOH (10 ml), to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which was then analyzed by HPLC.[8] Derivatives of D-glucose, D-galactose, D-xylose, L-rhamnose and L-arabinose were detected.

Enzymatic Hydrolysis of 2 Compound **2** (20 mg) was dissolved in an AcOH/AcONa buffer (pH 5) with β-glucosidase (20 mg), and the mixture incubated at room temperature overnight. The crude products were chromatographed on silica-gel with $CHCl_3$–MeOH–H_2O (25:10:1) as mobile phase and ODS silica-gel with MeOH–H_2O (4:1) to yield the corresponding spirostanol saponin (**2a**) (11.7 mg) and D-glucose. Compound **2a**: a white amorphous powder, $[\alpha]_D^{28}$ −46.0° (*c*=0.10, pyridine). Negative-ion FAB-MS *m/z*: 1162 $[M]^-$, 1032 [M−arabinosyl (or xylosyl)]$^-$, 734 [M−arabinosyl−xylosyl−glucosyl]$^-$, 591 [M−arabinosyl−xylosyl−glucosyl−rhamnosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2930 (CH), 1720 (C=O), 1445, 1370, 1225, 1080, 1040, 975, 920. ^{1}H-NMR (pyridine-d_5) δ: 6.30 (1H, br s, H-1″), 5.32 (1H, d, *J*=7.6 Hz, H-1⁗), 5.23 (1H, d, *J*=7.8 Hz, H-1⁗⁗), 4.97 (1H, d, *J*=7.9 Hz, H-1‴), 4.92 (overlapping with H_2O signal, H-1′), 4.83 and 4.79 (each 1H, br s, H-27a, H-27b), 1.78 (3H, d, *J*=6.1 Hz, H-6″), 1.09 (3H, d, *J*=6.9 Hz, H-21), 0.85 (3H, s, H-19), 0.79 (3H, s, H-18).

Compound 3 A white amorphous powder, $[\alpha]_D^{28}$ −62.0° (*c*=0.10, $CHCl_3$–MeOH (1:1)). Negative-ion FAB-MS *m/z*: 1358 $[M]^-$, 1225 [M−arabinosyl (or xylosyl)]$^-$, 1094 [M−arabinosyl−xylosyl]$^-$, 934 [M−arabinosyl−xylosyl−glucosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2925 (CH), 1720 (C=O), 1440, 1370, 1155, 1070, 1040. ^{1}H-NMR (pyridine-d_5) δ: 6.29 (1H, br s, H-1″), 5.31 (1H, d, *J*=7.7 Hz, H-1⁗), 5.21 (1H, d, *J*=7.7 Hz, H-1⁗⁗), 4.96 (1H, d, *J*=7.8 Hz, H-1‴), 4.90 (1H, d, *J*=7.6 Hz, H-1′), 4.84 (1H, d, *J*=7.8 Hz, H-1⁗⁗⁗), 3.26 (3H, s, OMe), 1.77 (3H, d, *J*=6.1 Hz, H-6″), 1.16 (3H, d, *J*=6.8 Hz, H-21), 1.05 (3H, d, *J*=6.6 Hz, H-27), 0.83 (3H, s, H-19), 0.77 (3H, s, H-18).

Enzymatic Hydrolysis of 3 Compound **3** (30 mg) was treated with β-glucosidase (25 mg) as described for **2** to yield the corresponding spirostanol saponin **3a** (9.2 mg) and D-glucose. Compound **3a**: a white amorphous powder, $[\alpha]_D^{28}$ −34.0° (*c*=0.10, pyridine). Negative-ion FAB-MS *m/z*: 1164 $[M]^-$, 1032 [M−arabinosyl (or xylosyl)]$^-$, 899 [M−arabinosyl−xylosyl]$^-$, 737 [M−arabinosyl−xylosyl−glucosyl]$^-$, 593 [M−arabinosyl−xylosyl−glucosyl−rhamnosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2925 (CH), 1710 (C=O), 1445, 1360, 1210, 1160, 1080, 1055, 980, 915, 890 (intensity 915>890, 25*S*-spiroacetal). ^{1}H-NMR (pyridine-d_5) δ: 6.29 (1H, br s, H-1″), 5.32 (1H, d, *J*=7.6 Hz, H-1⁗), 5.22 (1H, d, *J*=7.7 Hz, H-1⁗⁗), 4.95 (overlapping with H_2O signal, H-1‴), 4.91 (1H, d, *J*=7.8 Hz, H-1′), 1.77 (3H, d, *J*=6.1 Hz, H-6″), 1.14 (3H, d, *J*=6.8 Hz, H-21), 1.08 (3H, d, *J*=6.8 Hz, H-27), 0.84 (3H, s, H-19), 0.78 (3H, s, H-18).

Compound 4 A white amorphous powder, $[\alpha]_D^{28}$ −40.0° (*c*=0.10, $CHCl_3$–MeOH (1:1)). Negative-ion FAB-MS *m/z*: 1358 $[M]^-$, 1225 [M−arabinosyl (or xylosyl)]$^-$, 1094 [M−arabinosyl−xylosyl]$^-$, 1080 [M−arabinosyl (or xylosyl)−rhamnosyl]$^-$, 932 [M−arabinosyl−xylosyl−glucosyl]$^-$, 785 [M−arabinosyl−xylosyl−glucosyl−rhamnosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2925 (CH), 1720 (C=O), 1445, 1370, 1255, 1155, 1065, 1045, 910, 890, 780. ^{1}H-NMR (pyridine-d_5) δ: 6.29 (1H, br s, H-1″), 5.32 (1H, d, *J*=7.7 Hz, H-1⁗), 5.22 (1H, d, *J*=7.7 Hz, H-1⁗⁗), 4.96 (1H, d, *J*=7.8 Hz, H-1‴), 4.91 (1H, d, *J*=7.7 Hz, H-1′), 4.84 (1H, d, *J*=7.8 Hz, H-1⁗⁗⁗), 3.27 (3H, s, OMe), 1.77 (3H, d, *J*=6.1 Hz, H-6″), 1.19 (3H, d, *J*=6.8 Hz, H-21), 1.01 (3H, d, *J*=6.7 Hz, H-27), 0.84 (3H, s, H-19), 0.77 (3H, s, H-18).

Catalytic Hydrogenation of 2 A mixture of **2** (8 mg) and PtO_2 in MeOH–THF (1:1) was stirred under an atmosphere of H_2 at room temperature for 12 h. The reaction mixture, after removal of PtO_2 by filtration, was subjected to preparative HPLC with MeOH–H_2O (1:1) as mobile phase to yield **3** (3.8 mg) and **4** (1.5 mg).

Assay of cAMP Phosphodiesterase Activity The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously.[11b,c] The assay was a two-step isotopic procedure. Tritium-labelled cAMP was hydrolyzed to 5′-AMP by phosphodiesterase, and the 5′-AMP was then further hydrolyzed to adenosine by snake venom nucleotidase. The hydrolysate was treated with an anion-exchange resin (Dowex AG1-X8; BIO-RAD) to adsorb all charged nucleotides, leaving [^{3}H]adenosine as the only labelled compound to be counted.

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