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Steroidal saponins from the aerial parts of *Dracaena draco* and their cytostatic activity on HL-60 cells

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Abstract

Chemical examination of the aerial parts of *Dracaena draco* has led to the isolation of a total of nine steroidal saponins, including five new ones. The structures of the new saponins were determined by spectral data and a few chemical transformations to be (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranosyl} 24-*O*-β-D-fucopyranoside, (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}, (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranoside}, (23*S*)-spirosta-5,25(27)-diene-1β,3β,23-triol 1-*O*-{*O*-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranoside} and (23*S*)-spirosta-5,25(27)-diene-1β,3β,23-triol 1-*O*-{*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranoside}. The isolated saponins were evaluated for their cytostatic activity on leukemia HL-60 cells.

Keywords: *Dracaena draco*; Liliaceae; Aerial parts; Steroidal saponins; Spirostanol saponins; Cytostatic activity; HL-60 cells

1. Introduction

Dracaena draco (Agavaceae) is native to the Canary Islands. Its trunk and leaves excrete a dark red secretion, so-called 'Dragon's Blood', which has an astringent effect and has been used as an antidiarrheic and a hemostatic drug. As part of our contribution to the study of plants of the family Agavaceae (Mimaki, Kuroda, Takaashi, & Sashida, 1997; Mimaki, Kuroda, Takaashi, & Sashida, 1998), we have carried out a phytochemical screening of the aerial parts of *D. draco*, on whose constituents nothing thus far has been reported. This resulted in the isolation of a total of nine steroidal saponins, five of which are considered to be new compounds. This paper reports the identification and structural determination of the isolated saponins based on spectroscopic data, including two-dimensional NMR spectroscopic techniques and the results of hydrolytic cleavage. The cytostatic activity of the isolated saponins on leukemia HL-60 cells is also discussed.

2. Results and discussion

The aerial parts of *D. draco* (fresh weight of 4.8 kg) were extracted with hot methanol. After removal of saccharides contained in the extract by passage through a porous ion-exchange resin (Diaion HP-20) column, it was repeatedly chromatographed on silica gel, octadecylsilanized (ODS) silica gel and Sephadex LH-20 to give compounds **1** (3.8 mg), **2** (77.1 mg), **3** (25.1 mg), **4** (119 mg), **5** (103 mg), **6** (114 mg), **7** (124 mg), **8** (45.6 mg) and **9** (28.6 mg).

Compounds **1–4** were known constituents and identified as (25*R*)-spirost-5-en-3β-ol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside} (**1**) (Nohara, Miyahara, & Kawasaki, 1975; Espejo, Llavot, Jung, & Giral, 1982), spirosta-5,25(27)-diene-1β,3β-diol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside} (**2**) (Bombardelli, Bonati, Gabetta, & Mustich, 1971; Pourrat, Lamaison, Gramain, & Remuson, 1982; Korkashvili, Dzhikiya, Vugalter, Pkheidze, & Kemertelidze, 1985; Mimaki, Kuroda, Kameyama, Yokosuka, & Sashida, 1988), (23*S*)-spirosta-5,25(27)-diene-1β,3β,23-triol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside} (**3**) (Takaashi et al., 1995) and 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-

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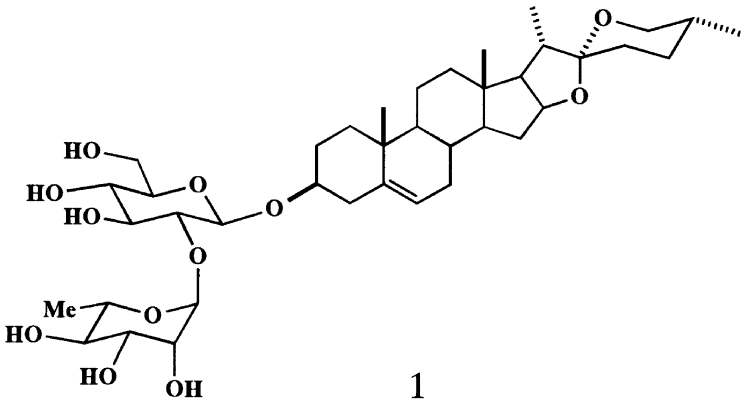

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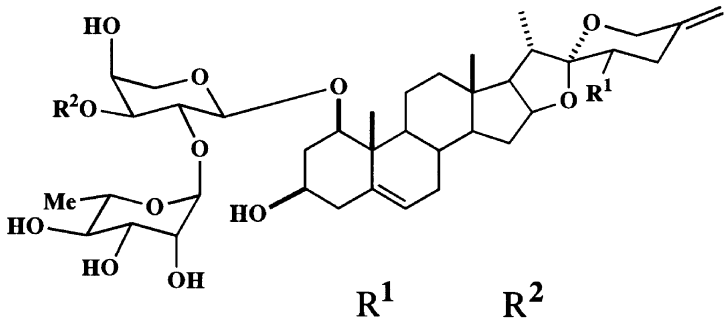
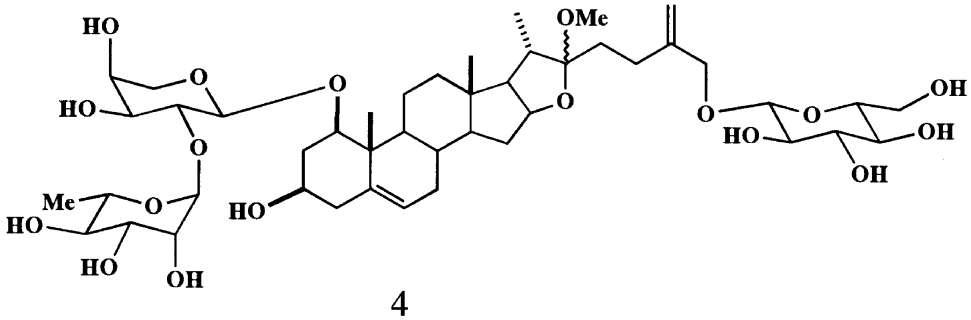


	R^1	R^2
2	H	H
3	OH	β-D-Xyl*p*



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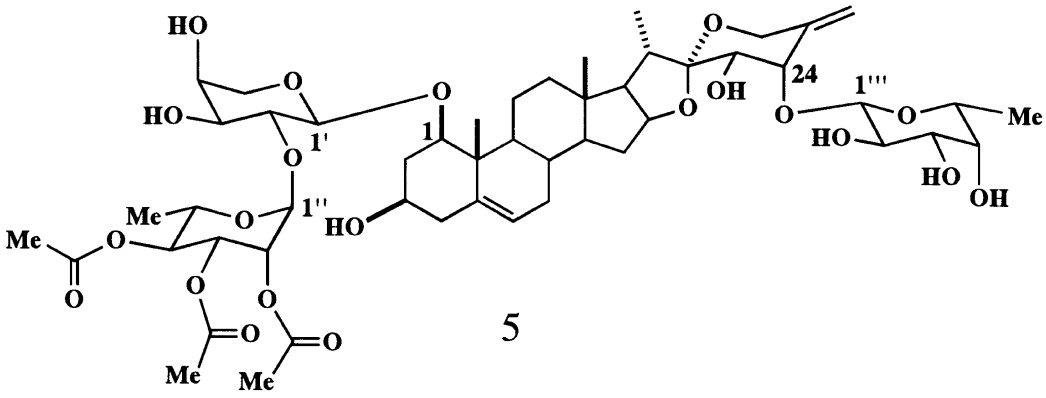
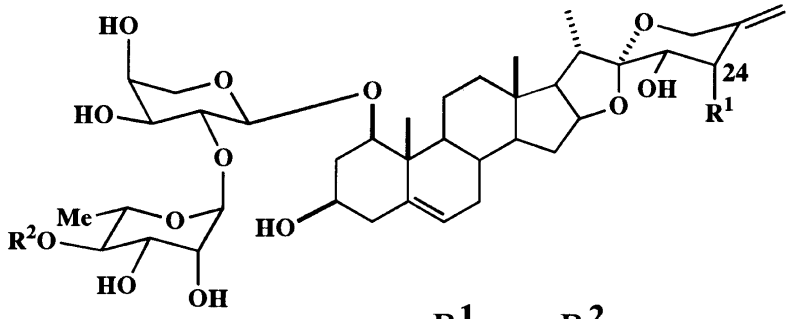
Structure 1.

furosta-5,25(27)-diene-1β,3β,22ξ,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside} (**4**) (Bombardelli, Bonati, Gabetta, & Mustich, 1972; Korkashvili et al., 1985; Mimaki et al., 1998), respectively. The physical and spectral data were consistent with the indicated literature values. Copies of the original spectra are obtainable from the authors.

Compound **5** was obtained as an amorphous solid, $[\alpha]_D$ −62.0° in methanol. It was assigned the molecular formula $C_{50}H_{74}O_{21}$ by the ^{13}C NMR data, positive-ion FABMS (*m/z* 1033 $[M+Na]^+$), negative-ion FABMS (*m/z* 1009 $[M-H]^-$) and elemental analysis. The glycosidic nature of **5** was shown by strong IR absorptions at 3480 and 1050 cm^{-1}. Preliminary inspection of the ^1H NMR spectrum (pyridine-d_5) of **5** led to assigning the signals due to two tertiary methyl groups at δ 1.36 and 0.98 (each 3H, s), three secondary methyl groups at δ 1.48 (3H, d, *J*=6.4 Hz), 1.42 (3H, d, *J*=6.2 Hz) and 1.09



5



	R^1	R^2
6	OH	H
7	OH	Ac
8	H	H
9	H	Ac

Structure 2.

(3H, d, $J=7.0$ Hz), an exomethylene group at δ 5.25 and 5.09 (each 1H, br s), an olefinic proton at δ 5.61 (1H, br d, $J=5.7$ Hz) and three anomeric protons at δ 6.22 (1H, d, $J=1.4$ Hz), 5.15 (1H, d, $J=7.9$ Hz) and 4.62 (1H, d, $J=7.7$ Hz). The presence of three acetyl groups in **5** was confirmed by the IR (1755 cm^{-1}), ^1H NMR [δ 2.15, 2.01 and 1.95 (each 3H, s)] and ^{13}C NMR [δ 170.4, 170.3 and 170.2 (C=O) and 20.7 × 2 and 20.5 (Me)] spectra. When **5** was submitted to acid hydrolysis with 1 M hydrochloric acid in dioxane–H_2O (1:1), it was hydrolysed to yield L-arabinose, L-rhamnose and D-fucose as the carbohydrate compounds, together with several unidentified artifactual sapogenols; no genuine aglycone could be obtained. The monosaccharides were identified by HPLC analysis following their conversion to the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives (Oshima & Kumanotani, 1981; Oshima, Yamauchi, & Kumanotani, 1982). The above data and one distinctive quaternary carbon signal at δ 111.7 (Agrawal, Jain, Gupta, & Thakur, 1985) led to the hypothesis that **5** was a spirostanol saponin with three monosaccharides and three acetyl groups. Analysis of the ^1H–^1H COSY, HOHAHA and HMQC spectra, which were measured in a mixed solvent of pyridine-d_5 and methanol-d_4 (11:1) to eliminate the signals due to exchangeable protons and optimize the spectral dispersion, allowed the identification of the ^1H–^1H spin-networks and assignment of the corresponding one-bond coupled carbon signals arising from the aglycone moiety as shown in Table 1. The ^{13}C NMR spectral shifts thus assigned featured a strong similarity to those of recurvoside C, a polyhydroxylated spirostanol bisdesmoside isolated by us from *Nolina recurvata* stems (Takaashi et al., 1995), implying that the structure of the aglycone was (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol. The ^1H NMR shifts and their multiplicities and NOE correlations observed in the phase-

Table 1
^{1}H and ^{13}C NMR chemical shift assignments for **5**[a]

Position	^{1}H	J (Hz)	^{13}C
1	3.76 dd	11.9, 3.9	83.5
2eq	2.63 br dd	11.9, 3.9	37.4
ax	2.17 q-like	11.9	
3	3.80 m	25.0[b]	68.0
4eq	2.57 dd	12.2, 4.3	43.7
ax	2.65 dd	12.2, 12.2	
5	–		139.3
6	5.60 br d	5.6	125.1
7α	1.48		32.0
β	1.79 br dd	12.4, 5.6	
8	1.48		33.0
9	1.44		50.3
10	–		42.9
11eq	2.80 br d	10.8	24.0
ax	1.55		
12eq	1.57 br dd	11.6, 3.2	40.5
ax	1.26 ddd	11.6, 11.6, 3.4	
13	–		40.8
14	1.07		56.7
15α	1.80		32.3
β	1.38		
16	4.61 q-like	7.5	83.1
17	1.68 dd	8.6, 6.7	61.6
18	0.95 s		16.8
19	1.32 s		14.9
20	2.84		37.5
21	1.04 d	7.0	14.8
22	–		111.8
23	3.90 d	3.8	70.3
24	4.74 d	3.8	82.3
25	–		144.0
26a	4.79 br d	11.6	61.6
b	3.97 d	11.6	
27a	5.22 d	1.3	113.8
b	5.09 br s		
1′	4.57 d	7.7	99.9
2′	4.38 dd	9.2, 7.7	74.6
3′	4.03 dd	9.2, 3.3	75.7
4′	4.08 dd	3.3, 1.7	70.3
5′	4.20 dd	11.6, 1.7	67.7
	3.62 d	11.6	
1″	6.12 d	1.5	97.7
2″	5.89 dd	3.4, 1.5	70.5
3″	5.84 dd	10.1, 3.4	70.4
4″	5.55 dd	10.1, 10.1	71.9
5″	4.90 dq	10.1, 6.2	66.5
6″	1.39 d	6.2	18.0
1‴	5.05 d	7.9	106.2
2‴	4.32 dd	9.4, 7.9	72.9
3‴	4.00 dd	9.4, 3.2	75.2
4‴	3.93 br d	3.2	72.8
5‴	3.72 br q	6.4	71.6
6‴	1.43 d	6.4	17.2
Ac	2.16 s		170.6
	2.01 s		170.6
	1.96 s		170.4
			20.7
			20.7
			20.7

[a]Spectra were measured in pyridine-d_5–methanol-d_4 (11:1).
[b]$W_{1/2}$.

Table 2
^{13}C NMR spectral data for compounds **5**–**9**[a]

C	**5**	**6**	**7**	**8**	**9**
1	83.4	83.8	83.7	83.8	83.6
2	37.4	37.3	37.5	37.3	37.4
3	67.9	68.1	67.9	68.1	67.9
4	43.8	43.6	43.9	43.6	43.8
5	139.3	139.3	139.3	139.3	139.3
6	124.9	124.6	124.9	124.6	124.6
7	31.9	31.8	31.9	31.9	32.0
8	32.9	32.8	32.9	32.9	32.9
9	50.2	50.3	50.3	50.3	50.3
10	42.8	42.7	42.8	42.8	42.8
11	23.9	23.9	23.9	23.9	23.8
12	40.4	40.4	40.4	40.5	40.5
13	40.6	40.5	40.6	40.6	40.7
14	56.6	56.7	56.7	56.7	56.7
15	32.3	32.1	32.2	32.2	32.2
16	82.9	83.1	83.2	81.9	81.9
17	61.4	61.2	61.3	62.3	62.4
18	16.7	16.7	16.7	16.7	16.8
19	14.8	14.9	14.8	14.8	14.8
20	37.4	36.9	37.0	35.6	35.7
21	14.7	14.5	14.6	14.5	14.6
22	111.7	112.5	112.6	111.7	111.7
23	70.2	69.5	69.6	68.4	68.4
24	82.1	74.0	74.0	38.6	38.7
25	143.9	146.1	146.2	144.1	144.2
26	61.4	60.6	60.7	64.1	64.2
27	113.7	112.4	112.4	109.3	109.3
1′	99.8	100.5	100.4	100.5	100.4
2′	74.6	75.0	74.0	75.1	74.1
3′	75.7	75.8	76.2	75.8	76.1
4′	70.2	70.0	70.2	70.0	70.2
5′	67.7	67.3	67.7	67.2	67.7
1″	97.5	101.5	100.8	101.5	100.8
2″	70.4	72.3	72.2	72.3	72.2
3″	70.2	72.3	69.9	72.3	69.9
4″	71.8	74.1	76.3	74.1	76.3
5″	66.4	69.3	66.5	69.3	66.5
6″	17.9	18.8	18.3	18.8	18.3
1‴	106.2				
2‴	73.0				
3‴	75.3				
4‴	72.8				
5‴	71.5				
6‴	17.2				
Ac	170.4		170.8		170.8
	170.3		21.0		21.0
	170.2				
	20.7				
	20.7				
	20.5				

[a]Spectra were measured in pyridine-d_5.

sensitive NOESY spectrum well supported the proposed structures including the ring junction patterns of the steroid nucleus and the configurations of the oxygen atoms on it. The ^{1}H–^{1}H COSY and HOHAHA experiments also allowed the sequential assignment of the resonances for

the three monosaccharides, starting from the easily distinguished anomeric protons. Multiplet patterns and measurements of coupling constants confirmed the presence of an α-L-arabinopyranosyl ($^4C_1$), α-L-rhamnopyranosyl ($^1C_4$) and β-D-fucopyranosyl ($^4C_1$) units in **5** (Table 1). In the HMBC spectrum, the anomeric proton signals of the rhamnose (δ 6.12) and arabinose (δ 4.57) showed $^3J_{C,H}$ correlations with C-2 of the arabinose (δ 74.6) and C-1 of the aglycone (δ 83.5), respectively, leading to an *O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl structure attached to C-1 of the aglycone. The remaining anomeric proton signal at δ 5.05 (fucose) showed a $^3J_{C,H}$ correlation with the δ 82.3 (C-24 of the aglycone) resonance, giving evidence that the fucose was directly attached to C-24 of the aglycone. The C-2, C-3 and C-4 hydroxyl groups of the rhamnose were presumed to be esterified with acetic acid since the proton signals due to H-2, H-3 and H-4 resonated in unexpected lower fields at δ 5.89 (dd, J=3.4, 1.5 Hz), 5.84 (dd, J=10.1, 3.4 Hz) and 5.55 (dd, J=10.1, 10.1 Hz), respectively. This was confirmed by the observation of $^3J_{C,H}$ correlations from the H-2, H-3 and H-4 protons to the carbonyl carbons of the acetyl groups (Fig.1). Accordingly, the structure of **5** was determined to be (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranosyl} 24-*O*-β-D-fucopyranoside.

The ^{1}H NMR spectrum of **6** ($C_{38}H_{58}O_{14}$) showed signals for three steroid methyl groups at δ 1.41 (3H, s), 1.11 (3H, d, J=7.0 Hz) and 1.02 (3H, s), an exomethylene group at δ 5.11 (1H, d, J=1.3 Hz) and 5.02 (1H, br s), and an olefinic proton at δ 5.55 (1H, br d, J=5.3 Hz), which were essentially analogous to those of **5**. However, the signals due to the three acetyl groups and fucopyranosyl moiety identified in the ^{1}H and ^{13}C NMR (Table 2) spectra of **5** were absent from those of **6**, whose carbon signal of the aglycone C-24 shifted upfield by 8.1 ppm and was observed at δ 74.0. The six signals at δ 101.5, 72.3×2, 74.0, 69.3 and 18.8 in the ^{13}C NMR spectrum of **6** (Table 2) were attributable to a terminal α-L-rhamnopyranosyl unit. Acetylation of **6** with acetic anhydride in pyridine gave the corresponding octaacetate (**6a**). The above data indicated that **6** was related to **5** without the three acetyl groups and fucopyranosyl moiety. This was

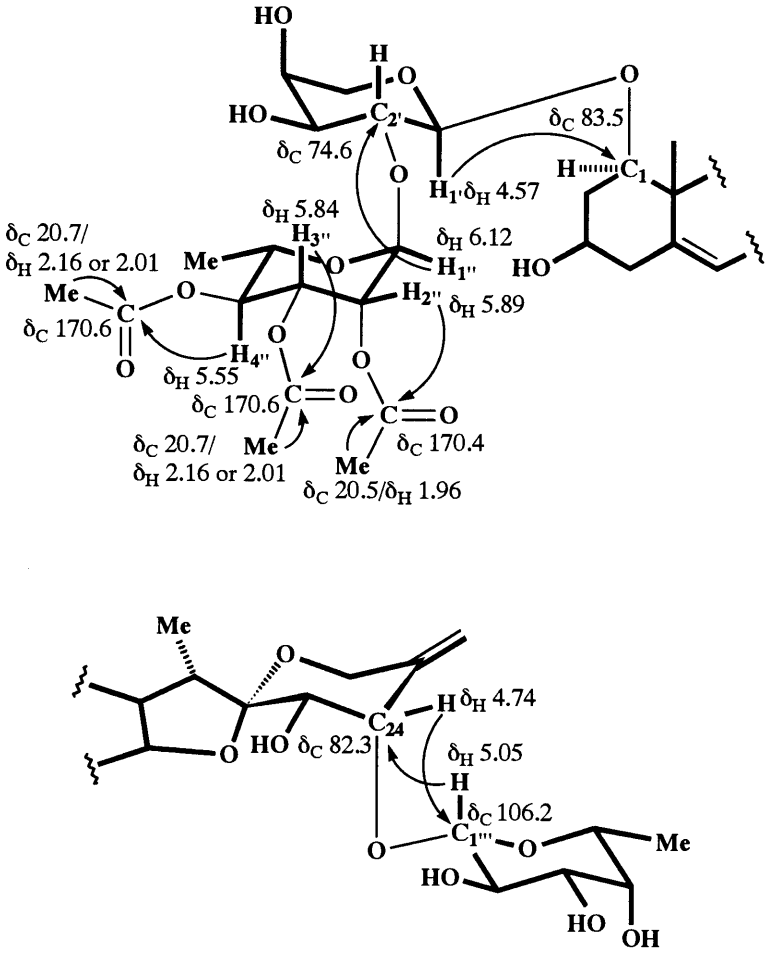


Fig. 1. HMBC correlations of the saccharide moieties of **5**. The spectrum was measured in pyridine - d_5 - methanol - d_4 and $J_{C,H}$ parameter was optimized for 8HZ.

confirmed by preparation of **6** from **5** by alkaline hydrolysis of **5** with 3% sodium methoxide in methanol followed by partial acid hydrolysis with 0.2 M hydrochloric acid in dioxane–H_2O (1:1) at 100°C for 30 min. Thus, the structure of **6** was formulated as (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}.

The negative-ion FABMS of **7** ($C_{40}H_{60}O_{15}$) exhibited an $[M–H]^-$ ion at *m/z* 779, which exceeded that of **6** by 42 mass units. The presence of an acetyl group in the molecule was shown by the IR (1725 cm^{-1}), ^1H NMR [δ 2.04 (3H, s)] and ^{13}C NMR [δ 170.8 (C=O) and 21.0 (Me)] spectra. When treated with 3% sodium methoxide, **7** was hydrolysed to yield **6**. Therefore, **7** was found to be a monoacetate of **6**. In the ^{13}C NMR spectrum of **7**, the signal of the rhamnose C-4 carbon was shifted to a lower field by 2.2 ppm, whereas the signals due to C-3 and C-5 occurred at higher shifts by 2.4 and 2.8 ppm, respectively, as compared with those of **6**. Furthermore, the downfield-shifted proton signal at δ 5.82 was assigned to H-4 of the rhamnose by its multiplicity (dd) with the *J* values of 9.6 and 9.6 Hz. Thus, the acetyl group was shown to be linked to the rhamnose C-4 hydroxy position, and the structure of **7** was characterized as (23*S*,24*S*)-spirosta-5,25(27)-diene-1β,3β,23,24-tetrol 1-*O*-{*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranoside}.

The spectral features of **8** ($C_{38}H_{58}O_{13}$) were quite similar to those of **6** with exceptions of the ^{13}C NMR signals due to C-24 of the aglycone and its neighboring carbons; the signal assignable to C-24, which was observed as a hydroxymethine signal at δ 74.0 in **6**, was displaced by a methylene signal at δ 38.6 in **8**. Acetylation of **8** with acetic anhydride in pyridine introduced seven acetyl groups into the molecule (**8a**). The above data indicated that **8** was related to **6** without the C-24 hydroxyl group and this was confirmed by the ^{13}C NMR assignment of the aglycone moiety of **8**, which was superimposable with that of **3**. Thus, the structure of **8** was assigned as (23*S*)-spirosta-5,25(27)-diene-1β,3β,23-triol 1-*O*-{*O*-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranoside}.

The presence of an acetyl group in compound **9** ($C_{40}H_{60}O_{14}$) was revealed by the IR, ^1H and ^{13}C NMR spectral data. Alkaline hydrolysis of **9** afforded **8**. The acylation shifts of an acetyl group linkage were recognized at C-4 (+2.2 ppm) and its adjoining carbons at C-3 (−2.4 ppm) and C-5 (−2.8 ppm) by comparison of the ^{13}C NMR spectrum of **9** with that of **8**. In addition, the downfield-shifted proton signal at δ 5.80 (dd, J=9.6, 9.6 Hz) was assigned to H-4 of the rhamnose moiety. The structure of **9** was shown to be (23*S*)-spirosta-5,25(27)-diene-1β,3β,23-triol 1-*O*-{*O*-(4-*O*-acetyl-α-L-rhamnopyranosyl)-(1→2)-α-L-arabinopyranoside}.

Compounds **5**–**9** are new steroidal saponins.

Cytostatic activity of the isolated saponins on human promyelocytic leukemia HL-60 cells was evaluated. The cells were continuously treated with each sample for 72 h and the cell growth was measured by an MTT assay procedure. Compound **1** and **5** showed relatively potent cytostatic activity (**1**: IC_{50} 1.3 μg ml^{-1}; **5**: 2.6 μg ml^{-1}) compared with etoposide used as a positive control (0.3 μg ml^{-1}).

3. Experimental

3.1. General

FABMS: VG AutoSpec E (matrix: Magic Bullet, a mixture of dithiothreitol and dithioerythritol, 3:1; Tokyo-Kasei-Kogyo). NMR (ppm, *J* Hz): 1D (Bruker AM-400, 400 MHz for ^1H NMR) and 2D (Bruker AM-500, 500 MHz for ^1H NMR). CC: silica gel (Fuji-Silysia Chemical), ODS silica gel (Nacalai Tesque), Sephadex LH-20 (Pharmacia) 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 mm thick, Merck). HPLC: a Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, UV-8000) equipped with a Capcell Pak C_{18} column (Shiseido, 4.6 mm i.d. × 250 mm, ODS, 5 μm). Microplate reader: Immuno-Mini NJ-2300 (Inter Med, Japan). HL-60 cells: ICN Biomedicals, USA. RPMI 1640 medium: GIBCO BRL, USA. All other chemicals used were of biochemical reagent grade.

3.2. Plant material

D. draco used in this study was purchased from Exotic Plants, Chiba Prefecture in Japan and a voucher plant specimen is on file in the school of Pharmacy, Tokyo University of Pharmacy and Life Science.

3.3. Extraction and isolation

The plant material (fresh weight, 4.8 kg) was extracted with hot MeOH. The MeOH extract was concentrated under red. pres. and the viscous concentrate was suspended in H_2O. The suspension was passed through a Diaion HP-20 column using gradients of MeOH in H_2O. The MeOH eluate fr. was chromatographed on silica gel eluting with a stepwise gradient mixture of $CHCl_3$–MeOH system (19:1; 9:1; 6:1; 4:1; 2:1; 1:1) and finally with MeOH, gave six frs (I–VI). Fr. II was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (90:10:1), ODS silica gel with MeOH–H_2O (16:7) and MeCN–H_2O (2:3) and on Sephadex LH-20 with MeOH to give **5** (103 mg). Fr. III was also subjected to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (80:10:1; 70:10:1) and ODS silica gel CC with MeOH–H_2O (2:1) and MeCN–H_2O (5:8) to result in the isolation of **7** (124 mg) and **9** (28.6 mg). CC of fr. IV on silica gel eluting with $CHCl_3$–MeOH–H_2O (60:10:1) and ODS silica gel

CC with MeCN–H_2O (5:9) yielded **1** (3.8 mg), **2** (77.1 mg), **6** (114 mg) and **8** (45.6 mg). Fr. V was further fractionated by a silica gel column eluting with $CHCl_3$–MeOH–H_2O (30:10:1) into two frs (Va and Vb). Fr. Va was subjected to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (40:10:1) and ODS silica gel CC with MeOH–H_2O (4:3) and MeCN–H_2O (1:2) to give **3** (25.1 mg). Fr. Vb was purified by CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (40:10:1), ODS silica gel with MeCN–H_2O (5:14) and on Sephadex LH-20 with MeOH to furnish **4** (119 mg).

3.4. Compound 5

Amorphous solid. $[\alpha]^{24}_D$ −62.0° (MeOH; *c* 0.10). (Found: C, 58.22; H, 7.61. Calc. for $C_{50}H_{74}O_{21}\cdot H_2O$: C, 58.35; H, 7.44%). Positive-ion FABMS *m/z* 1033 $[M+Na]^+$. Negative-ion FABMS *m/z* 1009 $[M-H]^-$, 967 $[M-Ac]^-$, 925 $[M-Ac\times 2]^-$, 883 $[M-Ac\times 3]^-$, 737 [M–Ac × 3–rhamnosyl]$^-$, 605 [M–Ac × 3–rhamnosyl–arabinosyl]$^-$, 591 [M–Ac × 3–rhamnosyl–fucosyl]$^-$. IR ν^{KBr}_{max} cm^{-1}: 3480 (OH), 2940 (CH), 1755 (C═O), 1050. ^1H NMR (pyridine-d_5): δ 6.22 (1H, d, *J* = 1.4 Hz, H-1″), 5.96 (1H, dd, *J* = 3.4, 1.4 Hz, H-2″), 5.93 (1H, dd, *J* = 10.0, 3.4 Hz, H-3″), 5.63 (1H, dd, *J* = 10.0, 10.0 Hz, H-4″), 5.61 (1H, br d, *J* = 5.7 Hz, H-6), 5.25 and 5.09 (each 1H, br s, H_2-27), 5.15 (1H, d, *J* = 7.9 Hz, H-1‴), 4.62 (1H, d, *J* = 7.7 Hz, H-1′), 3.86 (1H, m, H-3), 3.81 (1H, dd, *J* = 12.1, 3.8 Hz, H-1), 2.15, 2.01 and 1.95 (each 3H, s, Ac × 3), 1.48 (3H, d, *J* = 6.4 Hz, Me-6‴), 1.42 (3H, d, *J* = 6.2 Hz, Me-6″), 1.36 (3H, s, Me-19), 1.09 (3H, d, *J* = 7.0 Hz, Me-21), 0.98 (3H, s, Me-18).

3.5. Acid hydrolysis of 5

A soln of **5** (8.1 mg) in 1 M HCl (dioxane–H_2O, 1:1, 5 ml) was heated at 100°C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column and chromatographed on silica gel eluting with $CHCl_3$–MeOH (9:1; 1:1) to give an aglycone fraction (3.5 mg) and a sugar fraction (1.9 mg). TLC analysis of the aglycone fraction showed that it contained several unidentified artifactual sapogenols. The sugar fraction was dissolved in H_2O (1 ml), to which (−)-α-methylbenzylamine (5 mg) and Na[BH_3CN] (8 mg) in EtOH (1 ml) were added. After being set aside at 40°C for 4 h followed by addition of AcOH (0.2 ml) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temperature for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge (Waters) with H_2O–MeCN (4:1; 1:1, each 5 ml) mixtures as solvents. The H_2O–MeCN (1:1) eluate was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides (Oshima & Kumanotani, 1981; Oshima et al., 1982), which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2:3); flow rate, 0.8 ml min^{-1}; detection, UV 230 nm. The derivatives of D-fucose, L-arabinose and L-rhamnose were detected. R_t (min): 13.11 (L-arabinose derivative), 15.26 (D-fucose derivative) and 19.54 (L-rhamnose derivative).

3.6. Compound 6

Amorphous solid. $[\alpha]^{26}_D$ −88.0° (MeOH; *c* 0.10). (Found: C, 58.75; H, 8.25. Calc. for $C_{38}H_{58}O_{14}\cdot 2H_2O$: C, 58.90; H, 8.06%). Negative-ion FABMS *m/z* 737 $[M-H]^-$. IR ν^{KBr}_{max} cm^{-1}: 3420 (OH), 2920 (CH), 1040. ^1H NMR (pyridine-d_5): δ 6.33 (1H, br s, H-1″), 5.55 (1H, br d, *J* = 5.3 Hz, H-6), 5.11 (1H, d, *J* = 1.3 Hz, H-27a), 5.02 (1H, br s, H-27b), 4.68 (1H, d, *J* = 7.5 Hz, H-1′), 3.87 (1H, m, H-3), 3.78 (1H, dd, *J* = 11.8, 3.8 Hz, H-1), 1.73 (3H, d, *J* = 6.1 Hz, Me-6″), 1.41 (3H, s, Me-19), 1.11 (3H, d, *J* = 7.0 Hz, Me-21), 1.02 (3H, s, Me-18).

3.7. Acetylation of 6

Compound **6** (12 mg) was acetylated with Ac_2O in pyridine and the crude acetate was chromatographed on silica gel eluting with hexane–Me_2CO (3:1) to afford the corresponding octaacetate (**6a**) (10.8 mg). IR ν^{KBr}_{max} cm^{-1}: 2960 (CH), 1745 (C═O), 1240, 1040. ^1H NMR (pyridine-d_5): δ 6.14 (1H, d, *J* = 4.2 Hz, H-24), 5.77 (1H, dd, *J* = 10.1, 3.4 Hz, H-3″), 5.65 (1H, br d, *J* = 5.9 Hz, H-6), 5.62 (1H, dd, *J* = 3.5, 1.9 Hz, H-4′), 5.60 (1H, dd, *J* = 10.1, 10.1 Hz, H-4″), 5.59 (1H, d, *J* = 1.9 Hz, H-1″), 5.52 (1H, dd, *J* = 3.4, 1.9 Hz, H-2″), 5.41 (1H, dd, *J* = 9.9, 3.5 Hz, H-3′), 5.32 (1H, d, *J* = 4.2 Hz, H-23), 5.21 and 5.14 (each 1H, br s, H_2-27), 4.90 (1H, m, H-3), 4.81 (1H, br d, *J* = 12.4 Hz, H-26a), 4.74 (1H, dq, *J* = 10.1, 6.2 Hz, H-5″), 4.65 (1H, q-like, *J* = 6.9 Hz, H-16), 4.64 (1H, d, *J* = 7.6 Hz, H-1′), 4.32 (1H, dd, *J* = 9.9, 7.6 Hz, H-2′), 4.19 (1H, dd, *J* = 12.7, 1.9 Hz, H-5′a), 4.05 (1H, d, *J* = 12.4 Hz, H-26b), 3.78 (1H, d, *J* = 12.7 Hz, H-5′b), 3.70 (1H, dd, *J* = 12.1, 4.2 Hz, H-1), 2.20, 2.19, 2.18, 2.14, 2.10, 2.09, 2.02 and 2.01 (each 3H, s, Ac × 8), 1.49 (3H, d, *J* = 6.2 Hz, Me-6″), 1.32 (3H, s, Me-19), 1.10 (3H, d, *J* = 7.0 Hz, Me-21), 0.98 (3H, s, Me-18).

3.8. Preparation of 6 by alkaline hydrolysis of 5 followed by partial acid hydrolysis

Compound **5** (40.5 mg) was treated with 3% NaOMe in MeOH (3 ml) at room temperature for 30 min. After neutralization of the reaction mixture by passage through an Amberlite IR-120B (Organo) column, it was subjected to acid hydrolysis with 0.2 M HCl in dioxane–H_2O (1:1, 4 ml) at 100°C for 30 min. The reaction mixture was neutralized by passing it through an Amberlite IRA-

93ZU column and chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (40:10:1) and ODS silica gel with MeCN–H_2O (1:2) to furnish **6** (6.1 mg).

3.9. Compound 7

Amorphous solid. $[\alpha]^{26}_D$ −78.0° (MeOH; *c* 0.10). (Found: C, 58.59; H, 7.84. Calc. for $C_{40}H_{60}O_{15}\cdot 2H_2O$: C, 58.80; H, 7.90%). Positive-ion FABMS *m/z* 803 $[M+Na]^+$. Negative-ion FABMS *m/z* 779 $[M-H]^-$, 737 $[M-Ac]^-$, 591 [M–Ac–rhamnosyl]$^-$. IR ν^{KBr}_{max} cm^{-1}: 3435 (OH), 2900 (CH), 1725 (C=O), 1235, 1025. ^{1}H NMR (pyridine-d_5): δ 6.41 (1H, br s, H-1″), 5.82 (1H, dd, *J*=9.6, 9.6 Hz, H-4″), 5.62 (1H, br d, *J*=5.4 Hz, H-6), 5.11 (1H, d, *J*=1.3 Hz, H-27a), 5.01 (1H, br s, H-27b), 4.69 (1H, d, *J*=7.7 Hz, H-1′), 3.91 (1H, m, H-3), 3.84 (1H, dd, *J*=11.9, 3.9 Hz, H-1), 2.04 (3H, s, Ac), 1.44 (3H, d, *J*=6.2 Hz, Me-6″), 1.37 (3H, s, Me-19), 1.12 (3H, d, *J*=7.0 Hz, Me-21), 1.05 (3H, s, Me-18).

3.10. Alkaline hydrolysis of 7

Compound **7** (5.6 mg) was treated with 3% NaOMe in MeOH (3 ml) at room temperature for 30 min. After neutralization of the reaction mixture by passage through an Amberlite IR-120B column, it was subjected to CC on ODS silica gel eluting with MeCN–H_2O (1:2) to afford **6** (2.8 mg).

3.11. Compound 8

Amorphous solid. $[\alpha]^{24}_D$ −90.0° (MeOH; *c* 0.10). (Found: C, 60.79; H, 8.23. Calc. for $C_{38}H_{58}O_{13}\cdot 3/2H_2O$: C, 60.86; H, 8.20%). Negative-ion FABMS *m/z* 721 $[M-H]^-$. IR ν^{KBr}_{max} cm^{-1}: 3420 (OH), 2920 (CH), 1045. ^{1}H NMR (pyridine-d_5): δ 6.32 (1H, br s, H-1″), 5.55 (1H, br d, *J*=5.3 Hz, H-6), 4.85 and 4.82 (each 1H, br s, H_2-27), 4.67 (1H, d, *J*=7.4 Hz, H-1′), 3.87 (1H, m, H-3), 3.77 (1H, dd, *J*=11.8, 3.8 Hz, H-1), 1.72 (3H, d, *J*=6.2 Hz, Me-6″), 1.40 (3H, s, Me-19), 1.12 (3H, d, *J*=7.0 Hz, Me-21), 1.04 (3H, s, Me-18).

3.12. Acetylation of 8

Compound **8** (19.7 mg) was acetylated with Ac_2O in pyridine and the crude acetate was chromatographed on silica gel eluting with hexane–Me_2CO (3:1) to afford the corresponding heptaacetate (**8a**) (24.8 mg). IR ν^{KBr}_{max} cm^{-1}: 2965 (CH), 1745 (C=O), 1235, 1035. ^{1}H NMR (pyridine-d_5): δ 5.77 (1H, dd, *J*=10.1, 3.4 Hz, H-3″), 5.64 (1H, br d, *J*=5.9 Hz, H-6), 5.62 (1H, dd, *J*=3.5, 1.8 Hz, H-4′), 5.60 (1H, dd, *J*=10.1, 10.1 Hz, H-4″), 5.59 (1H, d, *J*=1.9 Hz, H-1″), 5.53 (1H, dd, *J*=3.4, 1.9 Hz, H-2″), 5.42 (1H, dd, *J*=9.9, 3.5 Hz, H-3′), 5.17 (1H, dd, *J*=11.8, 5.5 Hz, H-23), 4.90 (1H, m, H-3), 4.87 and 4.83 (each 1H, br s, H_2-27), 4.74 (1H, dq, *J*=10.1, 6.2 Hz, H-5″), 4.64 (1H, d, *J*=7.6 Hz, H-1′), 4.58 (1H, q-like, *J*=7.3 Hz, H-16), 4.40 (1H, br d, *J*=12.2 Hz, H-26a), 4.32 (1H, dd, *J*=9.9, 7.6 Hz, H-2′), 4.19 (1H, dd, *J*=12.9, 1.8 Hz, H-5′a), 3.99 (1H, d, *J*=12.2 Hz, H-26b), 3.78 (1H, d, *J*=12.9 Hz, H-5′b), 3.70 (1H, dd, *J*=12.0, 4.0 Hz, H-1), 2.20, 2.18, 2.15×2, 2.08, 2.03 and 2.02 (each 3H, s, Ac ×7), 1.50 (3H, d, *J*=6.2 Hz, Me-6″), 1.32 (3H, s, Me-19), 1.11 (3H, d, *J*=7.0 Hz, Me-21), 1.02 (3H, s, Me-18).

3.13. Compound 9

Amorphous solid. $[\alpha]^{26}_D$ −62.0° (MeOH; *c* 0.10). (Found: C, 59.75; H, 8.44. Calc. for $C_{40}H_{60}O_{14}\cdot 2H_2O$: C, 59.98; H, 8.05%). Positive-ion FABMS *m/z* 787 $[M+Na]^+$. Negative-ion FABMS *m/z* 763 $[M-H]^-$, 721 $[M-Ac]^-$, 575 [M–Ac–rhamnosyl]$^-$. IR ν^{KBr}_{max} cm^{-1}: 3430 (OH), 2920 (CH), 1730 (C=O), 1240, 1030. ^{1}H NMR (pyridine-d_5): δ 6.39 (1H, br s, H-1″), 5.80 (1H, dd, *J*=9.6, 9.6 Hz, H-4″), 5.62 (1H, br d, *J*=5.4 Hz, H-6), 4.85 and 4.82 (each 1H, br s, H_2-27), 4.68 (1H, d, *J*=7.7 Hz, H-1′), 3.92 (1H, m, H-3), 3.84 (1H, dd, *J*=11.9, 3.9 Hz, H-1), 2.05 (each 3H, s, Ac), 1.43 (3H, d, *J*=6.2 Hz, Me-6″), 1.36 (3H, s, Me-19), 1.13 (3H, d, *J*=7.0 Hz, Me-21), 1.07 (3H, s, Me-18).

3.14. Alkaline hydrolysis of 9

Compound **9** (5.6 mg) was treated with 3% NaOMe in MeOH (3 ml) at room temperature for 30 min. After neutralization of the reaction mixture by passage through an Amberlite IR-120B column, it was subjected to CC on ODS silica gel eluting with MeCN–H_2O (1:2) to afford **8** (2.6 mg).

3.15. Cell culture and assay for cytostatic activity

HL-60 cells were maintained in the RPMI 1640 medium containing 10% fetal bovine serum supplemented with L-glutamine, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. The leukemia cells were washed and resuspended in the above medium to 3×10^4 cells ml^{-1} and 196 μl of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO_2/air for 24 h at 37°C. After incubation, 4 μl of EtOH–H_2O (1:1) solution containing the sample was added to give the final concentrations of 0.01–10 μg ml^{-1}; 4 μl of EtOH–H_2O (1:1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent and then cell growth was evaluated by an MTT assay procedure (Sargent & Tayler, 1989). The MTT assay was carried out according to a modified method of Sargent and Tayler as follows. After termination of the cell culture, 10 μl of 5 mg ml^{-1} MTT in phosphate buffered saline was added to every well and the plate was further reincubated in 5% CO_2/air for 4 h at 37°C. The plate was then centrifuged at 1500*g* for 5

min to precipitate cells and formazan. An aliquot of 150 μl of the supernatant was removed from every well and 175 μl of DMSO was added to dissolve the formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader at 550 nm. A dose–response curve was plotted for each sample of **1** and **5**, which showed more than 50% of cell growth inhibition at the sample concentration of 10 μg ml^{-1}, and the concentration giving 50% inhibition (IC_{50}) was calculated.

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