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NOROLEANANE SAPONINS FROM *CELMISIA PETRIEI*

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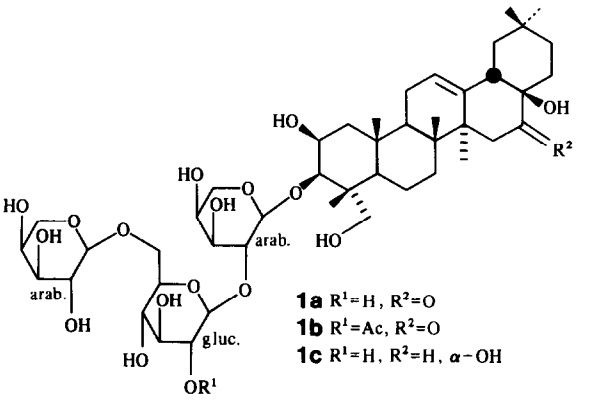
Key Word Index—*Celmisia petriei*; Compositae; noroleanane saponins; antinutrients; ^{13}C NMR; spin-lattice relaxation times; camellenodiol.

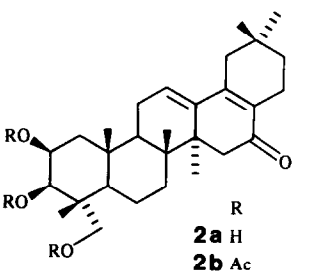
Abstract—Two biologically active noroleanane saponins from *Celmisia petriei* are identified as 3-*O*-(α-L-arabinopyranosyl (1 → 6)-β-D-glucopyranosyl (1 → 2)-α-L-arabinopyranosyl), 2β,17,23-trihydroxy-28-norolean-12-en-16-one and its 2″-*O*-acetyl derivative. ^{13}C NMR and T_1 measurements allowed the determination of the sugar sequence and the majority of the linkage positions, but gave ambiguous results for the inner arabinose sugar. The structure of camellenodiol is revised to 3β,17-dihydroxy-28-norolean-12-en-16-one.

INTRODUCTION

The *Celmisia* form a genus of some 60 species of mountain daisy indigenous largely to New Zealand where they are among the commoner members of the alpine flora. The basal part of the young leaves of *Celmisia petriei* (Cheesem) form a major item in the spring/summer diet of the endangered takahe bird (*Notornis mantelli*). Since the takahe is believed to be under severe nutritional stress in its remaining territories and in view of its restricted diet and known selective grazing behaviour [1] a study for possible antinutrients in its diet was undertaken.

We wish to report the structure of two 28-noroleanane saponins, **1a** and **1b**, isolated from the basal part of *C. petriei* which show the antifungal, haemolytic and cholesterol precipitating behaviour associated with other known antinutritional saponins [2, 3].





RESULTS AND DISCUSSION

Saponins **1a** and **1b** were detected using a TLC antifungal bioassay system [4] and were isolated using column (silica gel and Sephadex LH-20) and droplet counter-current chromatography.

Saponin **1a** (mp 207–209°), at lower R_f, gave a molecular formula $C_{45}H_{72}O_{18}$ from an analysis of its field desorption mass spectrum and ^{13}C NMR data (Table 1). The ^{13}C NMR data indicated the presence of three sugar residues, a trisubstituted double bond, an isolated ketone, six methyls and four primary and one tertiary carbinol carbons.

Saponin **1b** (mp 202–205°C) analysed as $C_{47}H_{74}O_{19}$ and was formulated as an acetate derivative of **1a** (μ_{max} 1730 cm^{-1}; ^{13}C NMR: δ 21.2, 170.0; ^{1}H NMR: δ 2.16, *s*). This was confirmed by the conversion of **1b** to **1a** upon basic hydrolysis.

Acid hydrolysis of both **1a** and **1b** led to the identification of the sugar components as arabinose and glucose in a ratio of 2:1 respectively. The aglycone is therefore a nortriterpene, $C_{29}H_{46}O_5$, containing one primary, two secondary and one tertiary carbinol carbons in addition to the functions listed above. Attempts to prepare this compound under a variety of conditions proved unsuccessful with only the dehydrated aglycone, dienone **2a**, $C_{29}H_{44}O_4$ ([M]$^+$ 456.3237), being isolated in variable yield (20–43%). The dienone partial structure –CH=CR–CR=CR–RCO was established from the UV (λ_{max} 298 nm) and ^{13}C NMR spectra. The UV maximum of **2a**, while significantly different from that predicted (306 nm), is similar to that of maragenin II (λ_{max} 299 nm) whose X-ray structure (**3**) has recently been reported [5].

Acetylation of **2a** gave the triacetate **2b** ([M]$^+$ 582) which established a pentacyclic ring structure. The mass spectra of both the dienone **2a** and its triacetate **2b**

Table 1. ^{13}C Chemical shifts ($\delta c \pm 0.1$) of saponins **1a–1c**, dehydrosapogenin (**2a**), maragenin (II) (**3**) and the 3-acetate of camellenodiol (**7**)

C	**1a** (NT_1) (C_5D_5N)	**1b** (C_5D_5N)	**1c** (C_5D_5N)	**2a** (C_5D_5N)	**3** ($CDCl_3$)	**7** ($CDCl_3$)
1	43.8 (0.19)*	43.7	44.0	45.1	38.7	38.1
2	70.6 (0.16)	70.4	70.5	71.6	27.1	23.7
3	83.1 (0.16)	82.7	83.3	72.8	78.7	80.8 (*d*)†
4	42.8	42.6	42.8	42.5	38.7	37.7
5	47.6	47.1	47.8	48.2	55.3	55.4
6	18.0	17.8	18.0	18.1	18.2	18.2
7	33.1 (0.20)	32.9	33.5	33.5	33.4	32.6
8	40.4	40.3	40.2	39.1	38.7	39.9
9	47.6	47.5	47.8	46.8	46.1	47.2
10	36.9	36.8	37.1	37.0	36.8	37.0
11	24.2 (0.24)	24.0	24.2	24.6	24.1	23.7
12	124.3 (0.20)	124.2	122.8	127.5	126.8 (*d*)	125.4 (*d*)
13	142.7	142.5	145.8	139.3§	139.1§ (*s*)	140.4 (*s*)
14	48.5	48.4	42.2	45.2	44.9	47.2
15	43.5 (0.16)	43.3	38.0‡	40.3‡	40.4‡	43.0
16	215.4	215.1	77.9	199.2	200.4 (*s*)	213.3 (*s*)
17	76.5 (> 1)	76.4	71.4	129.1§	128.9§ (*s*)	76.5
18	52.9 (0.19)	52.8	48.2	146.2§	146.8§ (*s*)	52.5
19	48.2	48.1	48.5	44.4‡	44.1	47.2
20	31.0	30.9	31.2	29.2	29.2	30.8
21	31.7 (0.20)	31.6	35.1	34.6	33.4	32.3
22	37.3 (0.15)	37.2	38.7‡	21.2	20.6	37.7
23	66.9 (0.22)	66.6	66.8	67.5	28.1	28.1
24	15.1	14.9	15.0	14.6	15.6	15.4
25	17.3	17.1	17.4	17.4	15.6	16.7
26	17.9	17.8	18.0	18.0	17.9	17.3
27	27.3	27.3	27.2	28.1	28.1	27.0
28	—	—	—	—	—	—
29	32.8	32.7	33.0	28.6	28.6	32.6
30	23.8	23.7	24.8	23.3	23.1	23.7
OAc	—	170.0	—	—	—	170.8
OAc	—	21.2	—	—	—	21.2
1′	101.9 (0.20)	102.0	101.8			
2′	78.8 (0.22)	78.9	78.6			
3′	72.5‖ (0.19)	72.6	72.4			
4′	67.4 (0.16)	67.4	67.3			
5′	65.4 (0.15)	64.3	65.6			
1″	105.8 (0.20)	103.2	105.7			
2″	75.5 (0.16)	75.6	75.5			
3″	78.1 (0.15)	76.2	78.0			
4″	71.5 (0.16)	71.7	71.4			
5″	76.1 (0.13)	76.2	76.0			
6″	68.5 (0.15)	68.4	68.3			
1‴	105.3 (0.27)	105.3	105.2			
2‴	72.5‖ (0.25)	72.5	72.4			
3‴	74.3 (0.26)	74.2	74.2			
4‴	69.0 (0.24)	68.9	69.0			
5‴	64.2 (0.22)	64.3	64.0			

*Relaxation times (NT_1) in parentheses.
†Published multiplicities in parentheses.
‡, §Assignments in vertical columns may be reversed.
‖Resolved in mixed solvent (CD_3OD:C_5D_5N).

contained intense ions at m/z 216 ($C_{15}H_{20}O$) and 203 ($C_{14}H_{19}O$). M/z 216 was assigned as the retro Diels–Alder fragment typical of Δ^{12}-pentacyclic triterpenes [6]. Rings D and E therefore contained the dienone function; the alcohols being confined to the A/B ring system. The ion m/z 203 was assigned as **4** arising via fission of the 8,14 bond [6] and its high intensity (100%) suggested that the ketone group was located at C-16.

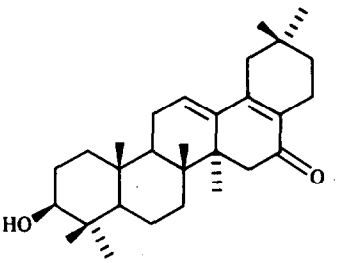
The ^{13}C NMR multiplicities of the dienone **2a** suggested a noroleanane skeleton. Two high field methylenes (δ 18.1 and 21.2) were readily assigned to C-6 and C-11 respectively thus locating the alcohol substituents in ring A. Three high field methyls (δ 14.6, 17.4, 18.0; C's 24, 25 and 26 respectively) and a shielded C-5 doublet [7] located the primary alcohol group at C-23. Dienone **2a** formed two acetonide derivatives on reaction with phosphomolybdic acid–acetone consistent with a 2,3,23-trihydroxy substitution pattern. The ^{1}H NMR spectra of the triacetate **2b** gave a complete separation of the carbinol protons and allowed assignment of the stereochemistry of the ring A hydroxyls as 2β,3β. In particular the 3α proton doublet at δ 4.93 showed axial-equatorial coupling (J_{ea} = 4.1 Hz) as required for a 2β-hydroxyl group [8]. A coupling of J = 10 Hz has been reported [9] for the alternative 2α,3β configuration. Comparison of the ^{13}C NMR of **2a** with that reported for maragenin II (**3**) [5], methyl-polygalacic acid (**5a**) [10] and phytolacagenin [11] confirmed structure **2a**. The complete ^{13}C NMR assignments are given in Table 1.

Saponin **1a** is related to 3-*O*-β-D-glucopyranosyl-polygalacic acid (**5b**) [10]. The ^{13}C NMR of **5b** shows δ 70.3, 83.8 and 66.5 for C-2, C-3 and C-23 respectively compared with signals at δ 70.6, 83.1 and 66.9 available for assignment to the corresponding positions in **1a**. The glycosidic linkage is therefore at C-3. The alternative glycosidic linkage at C-23 would result in a downfield shift of the C-23 signal by several ppm [12], to a region where no CH_2 signal was found. A glycoside bound to the tertiary hydroxyl group would give a high field anomeric carbon signal.

^{13}C NMR relaxation times (T_1 measurements) have been used to determine the sugar sequences of a number of saponins [13–16]. The T_1 value for a particular carbon atom, when multiplied by the number (N) of directly bonded hydrogen atoms, gives a measure (NT_1) of the flexibility of the molecule at that point. In the case of a saponin the values of NT_1 are shortest for the triterpenoid and the inner sugar, and longest for the terminal sugar. Values of NT_1 are shown in Table 1 for 11 CH and CH_2 groups in the triterpenoid moiety of **1a**. The average of these values was 0.19 *s*, with a standard deviation of 0.03 *s* which is consistent with experimental uncertainty. Values of NT_1 for the sugar carbons were spread from 0.13 *s* to 0.27 *s* indicating a varying flexibility along the sugar chain.

The FDMS of **1a** showed a prominent ion at $[M-133]^+$ indicative of a terminal arabinose moiety. The five longest NT_1 values correspond to chemical shifts consistent with values reported for terminal α-arabinopyranoside units linked to glucose units [14, 17]. Chemical shift data for β-arabinopyranoside and arabinofuranoside structures are sufficiently different for these alternatives to be eliminated [18]. An abrupt change in molecular flexibility was indicated by the average value of NT_1 (0.25 *s*) for the terminal sugar, compared with an average value of 0.17 *s* for the other two sugar units. Such a change is characteristic of a linkage involving a methylene group, rather than a direct link to the ring of the middle sugar [13]. The glucose unit must be linked at C-6, since the ^{13}C NMR of **1a** showed no signal at δ 62 which would correspond to the $-CH_2OH$ group of an unsubstituted glucopyranoside [18]. The middle sugar is therefore a 1,6-linked glucopyranoside. Chemical shifts are consistent with those reported for 1,6-linked β-glucopyranose [14, 17, 19]. The chemical shift for C-1 of a β-glucoside is sensitive to the environment of the glycosidic linkage [20], and the assignment of C-1″ is therefore dependent on identification of the linkage position on the inner sugar. The alternative α-form of the middle sugar can be eliminated by comparison with shifts reported for α-glucopyranosides [21].

The inner sugar must, by elimination, be arabinose. This was supported by the inertness of **1a** to hydrolysis by emulsin (EC 3.2.1.21). The distribution of chemical shifts was not consistent with a furanoside structure [18]. Although chemical shifts have been reported for 1,2-linked [22] and 1,4-linked [14] α-arabinopyranose structures neither set of shifts gave a convincing fit for the remaining resonances. No chemical shifts have been reported for a 1,3-linked α-arabinopyranose structure, but the effects of 3-*O*-glycosylation could be estimated from data for β-galactopyranose and β-fucose, since these



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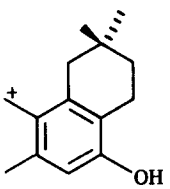

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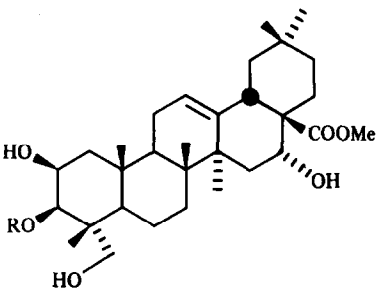


5a R=H
5b R=β-D-Glucopyranosyl

sugars are conformationally similar [9; 18, 19, 23–25]. While the observed chemical shifts were within the ranges predicted, the chemical shift predictions were so variable that the NMR evidence remained ambiguous. The linkage point was therefore determined by chemical means.

When periodate oxidation of **1a**, followed by acid hydrolysis failed to release any trace of intact arabinose (or glucose) a classical permethylation analysis was undertaken. Analysis of the $NaBH_4$ and $NaBD_4$ derived partially methylated alditol acetates by GC and GC/MS showed that the inner arabinose was in fact 1,2-linked. The complete ^{13}C NMR assignments are given in Table 1. For the inner sugar only one signal was found within 1 ppm of the ranges reported for other 1,2-linked arabinopyranoses [22]. This poor fit could be the result of a conformational change of the pyranose ring as substitution at C-2′ is found to move the 2′-oxygen function from an equatorial and towards an axial orientation [16]. Such a conformational change is associated with an increase of J_{CH} for C-1′ together with a high field shift of the C-4′ signal [16]. We observed $J_{CH} = 164$ Hz for C-1′ of **1a**, compared with $J_{CH} = 160$ Hz for α-arabinopyranose and methyl-α-arabinopyranoside [26]. This together with a −1.6 ppm shift of C-4′ relative to C-4″ provided evidence for a conformational change.

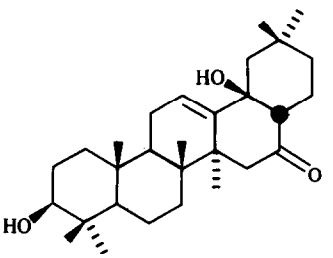
Comparison of the ^{13}C NMR spectra of **1a** and **1b** indicated that the acetate was linked to C-2″ of **1b**. Shielding of C-1″ and C-3″, with little effect on C-2″, is consistent with this conclusion [27]. The shift of the C-5′ signal of **1b** is ascribed to a consequent change in the conformation of the arabinose ring, as discussed above.

Structure of the aglycone and camellenodiol

On biogenic grounds a 28-nor-17β hydroxyl system as in **1a** appeared most likely. The ^{13}C NMR of **1a** was assigned using that of derivative **1c**, prepared as a single isomer on sodium borohydride reduction. Predicted shifts for **1c** were obtained using data for methyl 3-*O*-β-D-glucopyranosyl polygalacic acid (**5b**) [10] and for rings B–E of methyl quillaic acid [7]. The D ring of polygalacic acid differs from **1c** in having a –COOMe group rather than a hydroxyl group at C-17. Data for 1-methylcyclohexane carboxylic acid and 1-methyl cyclohexanol [28] suggested that the C-22 and C-18 signals of **1c** should appear about 5 ppm downfield of the corresponding signals for methyl polygalacic acid. The ^{13}C NMR of **1c** showed signals within 3 ppm of each predicted position, with most signals being within 1 ppm (Table 1). On comparison with **1a**, signals assigned to ring D should shift by only a few ppm and in each case these signals can be identified without ambiguity.

The alternative structure for **1a**, having a tertiary hydroxyl at C-18 rather than C-17, is also that proposed (**6**) for rings B–E of the triterpene camellenodiol isolated from *Camellia japonica* [5].

Camellenodiol and saponin **1a** have similar CD curves, with both showing a negative Cotton effect consistent with octant behaviour of an alpha axial hydroxyl group [29]. Comparison of the ^{13}C NMR data for camellenodiol [5] with that for **1a** (Table 1) suggested they possessed identical structures for rings B–E. However a C-18 hydroxyl as in structure **6** would result in significant downfield shifts for C's 13,19 and 30 relative to hederagenin [7] or methyl polygalacic acid (**5a**) [10]. In particular, the lowest field methylene (C-19, δ 46.4) should be



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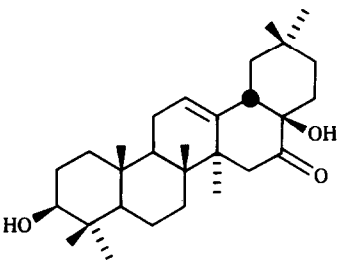

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further deshielded by about 8 ppm to a region where no methylene is observed. The 28-nor-17β-hydroxy structure **7** is therefore proposed for camellenodiol and leads to the ^{13}C NMR assignments for maragenin (II) and the 3-acetate of camellenodiol shown in Table 1.

In conclusion, the saponins **1a** and **1b** contain a novel 2β,3β,17β,23-tetrahydroxy-28-norolean-12-en-16-one aglycone and appear to be the first saponins isolated from the Astereae tribe of the Compositae. The role of these saponins in determining the selective feeding of takahe upon *C. petriei* is being investigated.

EXPERIMENTAL

Mps: uncorr; ^1H (79.5 MHz) and ^{13}C (20 MHz) NMR: Varian FT-80A spectrometer at 30°, TMS as int. standard. ^{13}C multiplicities were determined using the gated-decoupling spin-echo sequence GASPE [30]. Pulse spacings of $J_{CH}^{-1} = 8$ ms gave 4° and CH_2 carbons as positive signals while CH and Me resonances were inverted. For **1a**, 4° and CH_2 signals were further distinguished using a 3.5 ms pulse spacing in the GASPE sequence to suppress all non-4° signals. For **2a** rapid pulsing of the normal GASPE sequence was used to suppress 4° signals. Spectra of **1a** and **1b** were run in both C_5D_5N and CD_3OD. A 1:1 mixture of the two solvents was used to resolve all the carbinol signals for the inversion-recovery T_1 experiment. Each 180° pulse was preceded by a 1 sec pulse delay, and 2×10^4 transients were accumulated for each of five recovery intervals. A fully relaxed spectrum was also acquired. Poor *S/N* ratios gave an uncertainty of about ± 15% in each T_1 value. EIMS: 70 eV, double beam AEI MS30 spectrometer coupled with a SGE Jet Separator for GCMS.

Bioassay system. Samples were subjected to TLC (silica gel, glass backed) in a suitable solvent then sprayed with a nutrient spore suspension of *Cladosporium cladosporoides* and incubated at 100% humidity for 3 days (RT, dark) [4]. Active compounds appeared as white spots on a dark background. Comparison with duplicate vanillin sprayed plates identified **1a** and **1b** as distinctive orange bands.

Extraction and separation. Whole plants of *C. petriei* were collected from the Murchison Mountains, Fiordland, New Zealand in March 1978 and November 1981. A voucher specimen (No. 403299) has been deposited with Botany Division, DSIR, Lincoln. The basal 3 cm of the leaf blades (fresh or frozen) (2 kg) was blended in 95% EtOH, filtered and the filtrate adjusted to a nominal 80% EtOH concn by the addition of H_2O. After washing with petrol (× 2), the saponins were extracted into the organic phase of a $CHCl_3$–EtOH–H_2O (1:1:1) partition. The solvent was removed under red. pres. using *n*-BuOH to reduce foaming. The residue, adsorbed onto silica gel and sand, was eluted from a short silica gel column with MeOH–EtOAc (1:4) Filtration through Sephadex LH-20 with MeOH–$CHCl_3$ (1:9) gave 6.3 g extract from which the saponins were obtained by DCCC (Tokyo Rikakikai Co. Ltd., DCCC-A; $CHCl_3$–MeOH–H_2O (7:13:8) ascending mode). Silica gel chromatography (MeOH–$CHCl_3$, 1:9) gave analytical material.

Saponin **1a**. Powder, mp 207–209°; $[\alpha]_D^{21}$ − 3.6° (MeOH, *c* 4.0), CD (*c* 0.00164, MeOH) $[\theta]_{303}$ − 3050°; IR ν_{max}^{KBr} cm^{-1}: 3280, 1700, 1055; ^{1}H NMR (C_5D_5N): δ 0.86, 0.96, 1.29, 1.33, 1.33, 1.58 (methyl singlets); ^{13}C NMR: see Table 1; FDMS *m/z*: 923 $[M + Na]^+$, 790 $[M + Na^+ - 133]^+$.

Saponin **1b**. Needles from $CHCl_3$–MeOH–H_2O (7:13:8, upper phase) or amorphous power mp 202–205°; $[\alpha]_D^{21}$ + 2.9° (MeOH; *c* 3.0); CD (*c* 0.00173, MeOH) $[\theta]_{303}$ − 3720°; IR ν_{max}^{KBr} cm^{-1}: 3250, 1710, 1730 (shoulder) 1245, 1060; (20% MeOH–$CHCl_3$) 1710, 1735; ^{1}H NMR (C_5D_5N): δ 0.85, 0.95, 1.23, 1.23, 1.32, 1.55, 2.16 (methyl singlets); ^{13}C NMR: see Table 1; FDMS *m/z*: 965 $[M + Na]^+$.

Hydrolysis of the saponins. (i) *Sugar analysis.* Saponin **1a** or **1b** (25 mg) in 2 N CF_3COOH (3 ml) was refluxed (90 min), cooled and washed with Et_2O (× 2) and CH_2Cl_2 (× 2). Filtration and evaporation with added H_2O under N_2 gave sugars (11 mg). TLC identified only glucose and arabinose by R_f and distinctive colours on slow heating with 10% H_2SO_4–95% EtOH. Solvents used: EtOAc–*iso*-PrOH–*n*-BuOH–H_2O (4:2:1:1); Me_2CO–*n*-BuOH–H_2O (5:4:1); Me_2CO–$CHCl_3$–MeOH–H_2O (15:2:2:1); EtOAc–*iso*-PrOH–H_2O (65:25:12) GC (3% ECNSS-M on Chromosorb W, 220°) of the alditol acetate derivatives [31] or of the TMS-methoxime derivatives [32] (3% OV-17 capillary, 140 to 250° at 10°/min) gave arabinose: glucose (2:1). (ii) *Dienone* **2a**. **1a** (225 mg) in MeOH (10 ml) and 7% HCl (10 ml) were refluxed (4 hr). Cooling and filtration gave a precipitate, which was recrystallized (MeOH–H_2O) and chromatographed on silica gel (EtOAc) to give **2a** (46 mg, 43%); needles (MeOH–CH_2Cl_2) mp 272–275°, $[\alpha]_D^{21}$ + 59.4° (40% MeOH–$CHCl_3$, *c* 0.51); CD (*c* 0.00015, MeOH): $[\theta]_{328}$ − 8500° $[\theta]_{288}$ + 11 430°; UV λ_{max}^{EtOH} nm (log ε): 298 (4.17); IR ν_{max}^{KBr} cm^{-1}: 3350, 1630, 1595, and 1050; ^{1}H NMR (C_5D_5N): δ 0.87, 0.87, 0.89, 0.98, 1.12, 1.37 (18H, methyl singlets); 3.68, 4.14 (2H, *dd* J_{AB} = 13 Hz, H-23); 4.24 (1H, *d*, *J* = 5 Hz, H-3α); 4.52 (1H, *m*, H-2α); 6.09 (1H, *m*, H-12); ^{13}C NMR in Table 1; EIMS *m/z* (rel. int.): 456.3237 (19) $[M]^+$, $C_{29}H_{42}O_4$ requires 456.3238) 216.1503 (27); 203.1423 (100); 201 (10).

Dienone acetonides. Phosphomolybdic acid (1 mg) was added to a stirred suspension of **2a** (38.4 mg) in Me_2CO (15 ml). After 20 min, the soln was concd, quenched with satd $NaHCO_3$ (5 ml) and diluted with Et_2O (60 ml). After washing with brine and evaporation, the Et_2O residue was chromatographed on silica gel. Elution with $CHCl_3$ and crystallization (95% EtOH–CH_2Cl_2) gave firstly the less polar 2β,3β-acetonide of **2a** (21.3 mg): needles, mp 211–213°; ^{1}H NMR (C_5D_5N): δ 0.88, 0.90, 0.95, 1.19, 1.50, 1.56, 1.61, 1.71 (methyl singlets), 3.66 (1H, *br s*, H-23), 3.73 (1H, *d*, *J* = 3 Hz, H-3α), 4.35 (1H, *m*, H-2α), 6.03 (1H, *m*, H-12); EIMS *m/z* (rel. int.): 496 (6), 216 (21), 203 (100) and 201 (18). Then the 3β,23-acetonide of **2a** (4.9 mg): plates, mp 254.5–256°; ^{1}H NMR (C_5D_5N): δ 0.87, 0.90, 0.92, 1.00, 1.00, 1.34, 1.40, 1.59 (methyl singlets), 3.66 (2H, *br s*, H-23), H-2α and 3β under H_2O peak, 6.05 (1H, *m*, H-12); EIMS *m/z* (rel. int.): 496 (3), 216 (25), 203 (100), 201 (28).

Dienone triacetate **2b**. **2a** (21 mg) was treated with Ac_2O (200 μl) and pyridine (200 μl) at room temp (5 hr). The reaction mixture was then diluted with Et_2O (30 ml), washed successively with 5% H_2SO_4 (× 2), 5% $NaHCO_3$ (× 2) and brine (× 2), evaporated and eluted from silica gel (EtOAc–petrol, 1:1) to give **3b** (16 mg, 60%). Prep. TLC (EtOAc–petrol, 3:7) gave analytical material $[\alpha]_D^{24}$ + 35° (MeOH, *c* 0.19); UV λ_{max}^{MeOH} nm: 298; IR ν_{max}^{KBr} cm^{-1}: 1740, 1650, 1240 and 1040; ^{1}H NMR ($CDCl_3$): δ 0.92, 0.92, 0.97, 1.05, 1.10, 1.28, 2.00, 2.05, 2.06 (27H, methyl singlets), 3.78 (2H, *s*, H-23), 4.93 (1H, *d*, *J* = 4.1 Hz, H-3α), 5.44 (1H, *m*, H-2α), 6.10 (1H, *t*, *J* = 4 Hz, H-12); EIMS *m/z* (rel. int.): 582 (12), 216 (40), 203 (100).

Base hydrolysis of **1b**. **1b** (55.6 mg) in MeOH (2 ml), Et_3N (1 ml) and H_2O (1 ml) was kept at room temp. for 3.5 hr. Evaporation and elution from silica gel (MeOH–EtOAc, 1:4) gave pure **1a** (50 mg, 90%) by α_D, TLC and ^{13}C NMR.

Reduction of **1a**. Excess $NaBH_4$ in MeOH (30 min, room temp.). Repeated evaporation of MeOH then filtration through silica gel (MeOH–$CHCl_3$, 1:9) gave **1c**: $[\alpha]_D^{27}$ + 13.5° (MeOH, *c* 3.3).

Methylation analysis of **1c**. To a stirred suspension of NaH (40 mg) in DMF (1 ml) at 0° was added **1c** (67 mg) in further DMF (1 ml). MeI (3 × 40 μl) was added over 3 hr while warming to room temp. After stirring overnight, excess NaH was destroyed by dropwise addition of EtOH and the reaction quenched with satd NH_4Cl. Extraction with $CHCl_3$ (× 3) and evaporation with toluene gave the permethylated saponin. To ensure complete reaction the above sequence was repeated. Elution from silica gel ($CHCl_3$) gave 'one spot' permethylate (62 mg) $[\alpha]_D^{20}$ + 18° ($CHCl_3$, *c* 1.0). A sample (15 mg) was hydrolysed in refluxing EtOH–2 N CF_3COOH and portions reduced with $NaBH_4$ and $NaBD_4$ [31]. Acetylation [31] gave the partially methylated alditol acetates. Standards (*RR*$_t$ 1.0 and 0.54 respectively) were prepared from 2,3,4,6-tetra-*O*-methyl-D-glucose and after acid hydrolysis from 1-*O*-methyl-2,3,4-tri-*O*-methyl-β-L-arabinopyranoside. GC (3% OV-225 on Chromosorb W, 170°) and GC/MS (210°) identified the three components in the hydrolysate as 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylarabinitol (*RR*$_t$ 0.54); 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol (*RR*$_t$ 2.15) and 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-arabinitol (*RR*$_t$ 1.08) [33]. This latter showed EIMS *m/z*: 189, 161, 129, 117, 101, 99, 87. The 1-deutero derivative gave *m/z* 190, 161, 130, 117, 101, 100, 87.

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