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Stephen J. Bloor, and Lu Qi

J. Nat. Prod., **1994**, 57 (10), 1354-1360• DOI:
10.1021/np50112a004 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 10, pp. 1354-1360, October 1994

CYTOTOXIC SAPONINS FROM NEW ZEALAND *MYRSINE* SPECIES

STEPHEN J. BLOOR*

Industrial Research Limited, P.O. Box 31 310, Lower Hutt, New Zealand

and LU QI[1]

Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

ABSTRACT.—The observed biological activity in two New Zealand *Myrsine* species has been shown to be due to the presence of triterpene saponins. From *Myrsine australis* a series of eight oleanane-type saponins was obtained, with compounds **1–4** and **7** and **8** being novel. Also isolated were ardisiacrispin A [**5**] and ardisiacrispin B [**6**]. The structures of the new compounds were determined by chemical and spectroscopic techniques. Extracts of *Myrsine salicina* yielded only one saponin, **5**. Saponins **1–8** were shown to be combinations of four oleanane triterpenes bonded to β-D-xyl*p*(1→2)-β-D-glc*p*(1→4)-[β-D-glc*p*(1→2)]-α-L-ara*p* (compounds **1**, **3**, **5**, **7**) and this same tetrasaccharide with α-L-rha*p* replacing the β-D-xyl*p* unit (compounds **2**, **4**, **6**, **8**).

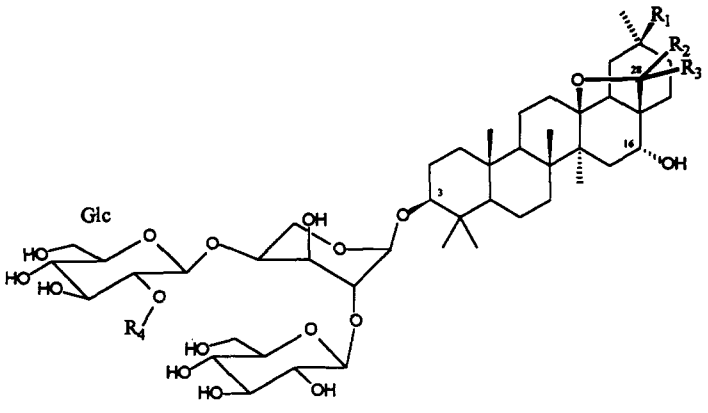
The genus *Myrsine* (family Myrsinaceae) is represented in New Zealand by at least eight species. However, only three of these are commonly encountered on the main islands, namely, *Myrsine australis* (A. Rich.) Allan, *M. salicina* Hew ex Hook., and *M. divaricata* A. Cunn. Significant in vitro antiviral activity observed in extracts of the first two species prompted us to examine these species further.

The observed antiviral activity for the crude extract of *M. australis* was traced to a series of triterpene saponins. A previous study of *M. australis* indicated the presence of a number of saponins and identified the major sapogenin as primulagenin A [**9**] (1). In our studies, the saponin fraction was found to comprise eight major compounds, 3β-*O*-{β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α-hydroxy-13β, 28-epoxy oleanane [**1**], 3β-*O*-{α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α-hydroxy-13β,28-epoxy oleanane [**2**], 3β-*O*-{β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α-hydroxyolean-28,13β-olide [**3**], 3β-*O*-{α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α-hydroxyolean-28, 13β-olide [**4**], ardisiacrispin A [**5**], ardisiacrispin B [**6**], 3β-*O*-{β-D-xylopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α, 28-dihydroxy-13β, 28-epoxy oleanane [**7**], and 3β-*O*-{α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→4)-[*O*-β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-16α,28-dihydroxy-13β, 28-epoxy oleanane [**8**], which, after examination of preliminary spectral data (fabms, ^{13}C-nmr), were shown to be combinations of four related triterpene sapogenins with each of two tetrasaccharide units.

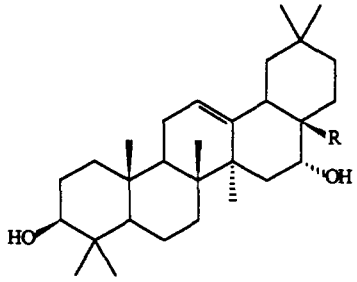
RESULTS AND DISCUSSION

Acid hydrolysis of the first pair of saponins **1** and **2**, which are the major components of the saponin mixture in *M. australis*, produced primulagenin A [**9**]. However, as observed in other similar saponins, the absence of signals for a double bond in the ^{13}C-

[1]Permanent address: College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun 130118, People's Republic of China.



1 $R_1=CH_3$, $R_2=R_3=H$, $R_4=$rha
2 $R_1=CH_3$, $R_2=R_3=H$, $R_4=$xyl
3 $R_1=CH_3$, $R_2,R_3==O$, $R_4=$rha
4 $R_1=CH_3$, $R_2,R_3==O$, $R_4=$xyl
5 $R_1=CHO$, $R_2=R_3=H$, $R_4=$rha
6 $R_1=CHO$, $R_2=R_3=H$, $R_4=$xyl
7 $R_1=CH_3$, $R_2=OH$, $R_3=H$, $R_4=$rha
8 $R_1=CH_3$, $R_2=OH$, $R_3=H$, $R_4=$xyl



9 $R=CH_2OH$
10 $R=COOH$
11 $R=CHO$

nmr spectra of **1** or **2** and the presence of a diagnostic signal for C-13 in the same spectrum at 86 ppm suggested that the true aglycone in **1** and **2** is actually protoprimulagenin A (C-13,28 ether).

Analysis of the alditol acetates derived following hydrolysis of **1** showed the presence of glucose (two), arabinose (one), and rhamnose (one) sugars, while in **2** the rhamnose was replaced by xylose. The negative-ionization fabms of **1** showed a strong molecular peak $[M-H]^-$ at *m/z* 1059 and independent losses of rhamnose or glucose indicating a branched tetrasaccharide unit with terminal rhamnose and glucose units. In addition, the negative-ionization fabms of **2**, with a molecular ion peak at *m/z* 1045, indicated a similar arrangement with a terminal pentose sugar, presumably xylose from the evidence above, replacing the rhamnose of **1**. Analysis of partially *O*-acetylated/methylated alditols (obtained by methylation, hydrolysis, acetylation) by gc and gc-ms revealed that both **1** and **2** contained a terminal glucose, a 2-substituted glucose, and a 2,4-disubstituted arabinose. As expected, **1** also contained a terminal rhamnopyranose and **2** a terminal xylopyranose. Further evidence of the nature of the linkages within the

tetrasaccharide unit was forthcoming from nmr experiments, especially using the HMBC and HMQC methods. Some of the more important connections were as follows (detailed for **2** but similar connectivities were observed for **1**): the proton at C-3 of the triterpene portion of **2** showed a long-range correlation with the carbon resonating at 104.5 ppm which must be the C-1 of the arabinose (pyranose form), since this carbon was also coupled to a CH_2 at 64.0 ppm. The ara-H-1 was then coupled to the ara-H-2 (^{1}H-^{1}H COSY) enabling the ara-C-2 to be identified (HMBC). A HMQC correlation of one of the glucose anomeric protons to this ara-C-2 established the first intersugar linkage. Similarly, the anomeric proton of the terminal xylose of **2** was coupled to the C-2 of the second glucose unit. The anomeric proton of this glucose was then linked to the ara-C-4 (HMBC). Thus, the tetrasaccharide unit in **1** is α-L-rha*p*(1→2)-β-D-glc*p*(1→4)-[β-D-glc*p*(1→2)]-α-L-ara*p*(1→)- and in **2** has the rha replaced with β-D-xyl*p*. The anomeric configurations were assigned from $J_{H1,H2}$ coupling constants. The values observed for the arabinosyl unit are similar to those observed in pyridine-d_5 for saxifragifolin A (2), indicating an α-L-arabinopyranoside in a conformational equilibrium, with the $^4C_1$ conformer dominant over the $^1C_4$ conformer.

Comparison of the ^{13}C-nmr data of **1** and **2** with saponins **3–8** revealed that the tetrasaccharides of **1**, **3**, **5**, **7** were identical; likewise those of **2**, **4**, **6**, **8**. Thus, since the structures of the two tetrasaccharide units that differentiate each pair of saponins have been defined, the remaining structural problem was the identity of the three remaining aglycones.

As described above, the aglycone of saponins **1** and **2** is protoprimulagenin A, which on hydrolysis yields primulagenin A [**9**]. Comparison of the ^{13}C-nmr data of the **3**, **4** and **7**, **8** pairs with those of **1** or **2** showed the aglycones of these saponins to be closely related to protoprimulagenin A and to differ only in the oxidation state at C-28. In **3** and **4**, C-28 is fully oxidized and part of a γ-lactone group (δ 179 ppm) and in **7** and **8**, C-28 is in a hemiacetal form (δ 99 ppm). Thus, on hydrolysis, **3** and **4** yielded echinocystic acid [**10**] and **7** and **8** yielded 3β,16α-dihydroxyolean-12-en-28-al [**11**]. Saponins **1–4**, and **7** and **8** are new compounds, and while protoprimulagenin A is a relatively common sapogenin, the lactone and hemiacetal sapogenins are unusual. The lactone sapogenin of **3** and **4** has been reported previously as leucolactone (3); however the hemiacetal sapogenin of **7** and **8** has not appeared in the literature, although 3β,16α-dihydroxy-olean-12-en-28-al [**11**] has appeared as primulagenin D (4).

The aglycone of the remaining saponin pair **5** and **6** differed slightly from that of **1** and **2** in that one of the methyl groups was replaced by a formyl group. Comparison of nmr data with known compounds established that the aglycone of **5** and **6** is cycloamiretin A (-CHO at C-30, -CH_2O at C-28). Thus **5** and **6** are β-D-xyl*p*(1→2)-β-D-glc*p*(1→4)-[β-D-glc*p*(1→2)]-α-L-ara*p*(1→3β)-cycloamiretin A and α-L-rha*p*(1→2)-β-D-glc*p*(1→4)-[β-D-glc*p*(1→2)]-α-L-ara*p*(1→3β)-cycloamiretin A, respectively. These structures are the same as those previously reported for ardisiacrispin A and B, saponins with utero-contractive properties from *Ardisia crispa* (5). Structure **6** has also been reported as saxifragifolin B from *Androsace saxifragifolia* (2) and earlier from *Cyclamen europaeum* (6).

Examination of the *Myrsine salicina* saponin fraction revealed that only one major saponin, **5**, was present in significant quantity. There was no evidence of the presence of biologically active saponins in a crude extract of the third *Myrsine* species, *M. divaricata*.

The isolation of compounds **1–8** was guided by an in vitro antiviral assay system vs. *Polio* Type 1 and *Herpes simplex* Type 1 viruses. All eight compounds showed similar levels of activity causing whole-well inhibition of cytopathic effects at 40 μg/disk. However, differences in activity were observed in the in vitro P388 lymphocytic leukemia assay

where **1** and **2** (ID_{50}s 0.85 μg/ml in each case) were considerably more cytotoxic than **3**–**8** (ID_{50}s>6 μg/ml).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were measured on Bruker AC 300 or Varian Unity 500 MHz instruments using manufacturer-supplied software. Eims and fabms were recorded using a VG70-250S spectrometer. Mps were uncorrected. Gc and gc-ms were performed with a HP5890 gas chromatograph and a HP 5950B MSD instrument.

PLANT MATERIAL.—Voucher specimens of plant materials have been deposited at the Landcare NZ Herbarium, Christchurch (*M. australis* [CHR 455342], *M. salicina* [CHR 461098], *M. divaricata* [CHR 467763]). Identities of plants were confirmed by R.P. Buxton (*M. australis*) and A.P. Druce (*M. salicina, M. divaricata*).

EXTRACTION AND ISOLATION.—Extraction of *Myrsine australis*. Plant material (1100 g dry wt, leaves and twigs) collected from Taita, Wellington, New Zealand, in June 1990, was air-dried, shredded, and extracted by steeping in MeOH (3×2 liters). MeOH was removed from the combined extracts and the residue, in H_2O (500 ml), was extracted with Et_2O (3×200 ml). The crude saponin fraction (24 g) was obtained by the addition of $CHCl_3$ to the 1-BuOH extract of the remaining aqueous solution. A portion (5.6 g) of the crude saponin fraction was chromatographed using reversed-phase flash chromatography (H_2O-MeOH, 4:6→1:9) yielding three major fractions. The first of these was a fraction composed of a mixture of

TABLE 1. ^{13}C-Nmr Data (ppm) for the Aglycone Portions of Compounds **1–8**.[a]

Carbon	Compound			
	1,2	**3,4**	**5,6**	**7,8**
1	39.1	39.0	39.2	39.2
2	26.4	26.4	26.5	26.6
3	88.9	89.9	89.0	89.1
4	39.5	39.5	39.5	39.6
5	55.5	55.4	55.6	55.6
6	17.9	17.8	17.9	18.0
7	32.8	34.0	33.3	34.4
8	42.3	42.1	42.5	42.5
9	50.4	49.8	50.4	50.4
10	36.8	36.7	36.8	36.8
11	19.1	18.6	19.1	19.3
12	36.8	34.0	34.3	39.2
13	86.3	92.4	86.3	87.3
14	44.5	42.2	44.0	43.4
15	34.4	37.1	36.8	37.0
16	77.0	72.3	77.8	76.6
17	44.5	47.8	44.5	48.3
18	51.4	51.7	53.2	47.1
19	38.9	38.4	30.5	39.2
20	31.7	31.5	48.2	32.1
21	36.8	36.0	32.5	37.2
22	31.7	28.6	32.3	33.2
23	16.4	16.4	16.4	16.3
24	28.0	27.9	28.0	28.0
25	16.4	16.2	16.3	16.5
26	19.5	17.9	18.5	19.0
27	18.5	19.3	19.7	19.6
28	77.9	179.3	77.6	99.2
29	33.7	33.3	23.1	33.9
30	24.7	24.5	207.5	24.9

[a]Spectra run in pyridine-d_5.

TABLE 2. ^{13}C-Nmr Data (ppm) for the Glycose Portions of Compounds **1–8**.[a]

Carbon	Compound	
	1, **3**, **5**, and **7**	**2**, **4**, **6**, and **8**
Ara-1	104.2	104.5
2	80.5	79.8
3	72.1	73.1
4	74.6	78.3
5	63.4	64.0
Glc-1	102.9	104.0
2	77.2	85.3
3	79.3	77.7
4	71.5	71.0
5	74.6	78.4
6	62.6	62.3
Glc′-1	103.1	104.9
2	76.2	76.1
3	77.8	78.2
4	71.7	71.7
5	78.1	78.1
6	62.4	62.9
Rha-1	101.3	
2	72.1	
3	72.4	
4	74.5	
5	69.3	
6	18.7	
Xyl-1		107.5
2		76.0
3		77.5
4		70.6
5		67.3

[a]Spectra run in pyridine-d_5.

flavonoid glycosides and was not examined further. The latter two fractions were determined to be sets of well-separated saponin mixtures (^{13}C nmr, tlc).

The third fraction (1.58 g) was readily purified by reversed-phase hplc (H_2O-MeOH, 1:4) to give saponins **1** and **2** (ca. 400 mg each). The second fraction was more complex, requiring an initial Si gel chromatographic step ($CHCl_3$-MeOH-H_2O, 65:35:10, lower layer) followed by reversed-phase hplc (H_2O-MeOH, 3:7), to give six saponins (order of reversed-phase elution) **3** (40 mg), **4** (40 mg), **5** (80 mg), **6** (120 mg), **7** (60 mg), and **8** (40 mg). On the basis of their chromatographic behavior these compounds could be considered to be four sets of two. The four sets could be separated by cc (SiO_2 or reversed-phase). However, each pair could only be separated by reversed-phase hplc.

Extraction of Myrsine salicina.—Plant material (825 g dry wt, leaves and twigs) collected at the same time and from trees adjacent to *M. australis* was treated in a similar manner to that described above. A portion (5.0 g) of the total 1-BuOH fraction (18.6 g) was chromatographed on reversed-phase SiO_2 and reversed-phase hplc to yield only saponin **5** (230 mg).

Extraction of Myrsine divaricata.—Plant material (leaves and twigs) collected from N.W. Nelson, New Zealand, was treated in a similar manner to that described above. No saponins were found.

3β-O-{β-D-Xylopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-{O-β-D-glucopyranosyl-(1→2)}-α-L-arabinopyranosyl}-16α-hydroxy-13β, 28-epoxy oleanane [**1**].—Colorless powder: mp 268–275°; $[\alpha]^{20}_D$ −10° (c=0.2, MeOH); ir (KBr) ν max 3400, 1640 cm^{-1}; negative fabms *m/z* $[M-H]^-$ 1059 (100), $[M-H-146]^-$ 914 (20), $[M-H-162]^-$ 899 (12), $[M-H-162-146]^-$ 753 (20); ^{1}H nmr (300 MHz, pyridine-d_5) δ 3.10 (1H, dd, J=11 and 4 Hz, H-3α), 3.24 (1H, d, J=7.3 Hz, H-28$_a$), 3.54 (1H, d, J=7.3 Hz, H-28$_b$), 4.84 [1H, m, H-1 (ara)], 5.07 [1H, d, J=7 Hz, H-1 (glc)], 5.28 [1H, d, J=7 Hz, H-1 (glc′)], 6.15 [1H, s, H-1 (rha)]; ^{13}C nmr, see Tables 1 and 2.

3β-O-{α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-{O-β-D-glucopyranosyl-(1→2)}-α-L-arabinopyranosyl}-16α-hydroxy-13β, 28-epoxy oleanane [**2**].—Colorless powder: mp 272–275°; $[\alpha]^{20}D$ −7.5° (*c*=0.2, MeOH); ir (KBr) ν max 3400, 1640 cm^{-1}; negative fabms *m/z* $[M-H]^-$ 1045 (100), $[M-H-132]^-$ 913 (20), $[M-H-162]^-$ 883 (10), $[M-H-162-132]^-$ 751 (13); ^1H nmr (300 MHz, pyridine-d_5) δ 3.16 (1H, dd, *J*=11 and 4 Hz, H-3α), 3.30 (1H, d, *J*=7.3 Hz, H-28_a), 3.60 [1H, d, *J*=7.3 Hz, H-28_b], 4.78 [1H, d, *J*=5.8 Hz, H-1 (ara)], 4.91 [1H, d, *J*=7 Hz, H-1(xyl)], 4.98 [1H, d, *J*=7.7 Hz, H-1 (glc)], 5.43 [1H, d, *J*=7.5 Hz, H-1 (glc′)]; ^{13}C nmr, see Tables 1 and 2.

3β-O-{β-D-Xylopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-{O-β-D-glucopyranosyl-(1→2)}-α-L-arabinopyranosyl}-16α-hydroxyolean-28, 13β-olide [**3**].—Colorless powder: mp 223–225°; $[\alpha]^{20}D$ −37.5° (*c*=0.6, MeOH); ir (KBr) ν max 3400, 1744, 1640 cm^{-1}; negative fabms *m/z* $[M-H]^-$ 1073 (100), $[M-H-146]^-$ 927 (20), $[M-H-162]^-$ 911 (10), $[M-H-146-162]^-$ 765 (23); ^1H nmr (300 MHz, pyridine-d_5) δ 3.15 (1H, dd, *J*=11 and 4 Hz, H-3α), 4.93 [1H, d, *J*=4.8 Hz, H-1 (ara)], 5.20 [1H, d, *J*=7.5 Hz, H-1 (glc)], 5.34 [1H, d, *J*=7.5 Hz, H-1 (glc′)], 6.35 [1H, s, H-1 (rha)]; ^{13}C nmr, see Tables 1 and 2.

3β-O-{α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-{O-β-D-glucopyranosyl-(1→2)}-α-L-arabinopyranosyl}-16α-hydroxyolean-28, 13β-olide [**4**].—Colorless powder: mp 229–231°; $[\alpha]^{25}D$ −35.6° (*c*=0.6, MeOH); ir (KBr) ν max 3400, 1744, 1640 cm^{-1}; negative fabms *m/z* $[M-H]^-$ 1059 (100), $[M-H-146]^-$ 927 (20), $[M-H-162]^-$ 897 (8), $[M-H-162-132]^-$ 765 (15); ^1H nmr (500 MHz, pyridine-d_5) 3.16 (1H, dd, *J*=11 and 4 Hz, H-3α), 4.79 [1H, d, *J*=5.8 Hz, H-1 (ara)], 4.92 [1H, d, *J*=7 Hz, H-1 (xyl)], 5.01 [1H, part obsc. by H_2O, H-1 (glc)], 5.49 [1H, d, *J*=7.7 Hz, H-1 (glc′)]; ^{13}C nmr, see Tables 1 and 2.

Ardisiacrispin A [**5**].—Colorless powder: mp 230–234°; $[\alpha]^{20}D$ −12.9° (*c*=0.2, MeOH); ir (KBr) ν max 3400, 1720, 1640, cm^{-1}; negative fabms *m/z* $[M-H]^-$ 1073 (100), $[M-H-146]^-$ 927 (20), $[M-H-162]^-$ 911 (10), $[M-H-146-162]^-$ 765 (15); ^1H nmr (300 MHz, pyridine-d_5) δ 3.13 (2H, m, H-3α, H-28_a), 3.52 (1H, d, *J*=7.4 Hz, H-28_b), 4.89 [1H, br s, H-1 (ara)], 5.14 [1H, d, *J*=7Hz, H-1 (glc)], 5.37 [1H, d, *J*=7 Hz, H-1 (glc′)], 6.26 [1H, s, H-1 (rha)], 9.60 (1H, s, H-30); ^{13}C nmr, see Tables 1 and 2.

Ardisiacrispin B [**6**].—Colorless powder: mp 240–245°; $[\alpha]^{20}D$ −2.4° (*c*=0.2, MeOH); ir (KBr) ν max 3400, 1720, 1640 cm^{-1}; negative fabms *m/z* 1059 (55); ^1H nmr (300 MHz, pyridine-d_5) δ 3.13 (2H, m, H-3α, H-28_a), 3.53 (1H, d, *J*=7.4 Hz, H-28_b), 4.77 [1H, d, *J*=7Hz, H-1 (ara)], 4.89 [1H, d, *J*=7 Hz, H-1 (xyl)], 4.97 [1H, d, *J*=7 Hz, H-1 (glc)], 5.42 [1H, d, *J*=7 Hz, H-1 (glc′)], 9.60 (1H, s, H-30); ^{13}C nmr, see Tables 1 and 2.

3β-O-{β-D-Xylopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-{O-β-D-glucopyranosyl-(1→2)}-α-L-arabinopyranosyl}-16α, 28-dihydroxy-13β, 28-epoxy oleanane [**7**].—Colorless powder: mp 236–9°; $[\alpha]^{25}D$ −13.1° (*c*=0.2, MeOH); ir (KBr) ν max 3400, 1640, 1560 cm^{-1}; negative fabms *m/z* $[M-H]^-$ 1075 (100), $[M-H-146]^-$ 929 (25), $[M-H-162]^-$ 913 (20); ^1H nmr (500 MHz, pyridine-d_5) δ 3.15 (1H, dd, *J*=11 and 4 Hz, H-3α), 4.95 [1H, br s, H-1 (ara)], 5.16 (1H, s, H-28), 5.24 [1H, d, *J*=7 Hz, H-1 (glc)], 5.37 [1H, d, *J*=7 Hz, H-1 (glc′)], 6.40 [1H, s, H-1 (rha)]; ^{13}C nmr, see Tables 1 and 2.

3β-O-{α-L-Rhamnopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-{O-β-D-glucopyranosyl-(1→2)}-α-L-arabinopyranosyl}-16α, 28-dihydroxy-13β, 28-epoxy oleanane [**8**].—Colorless powder: slowly softens above 250°; $[\alpha]^{25}D$ −4.7° (*c*=0.2, MeOH); ir (KBr) ν max 3400, 1640, 1560 cm^{-1}; negative fabms *m/z* $[M-H]^-$ 1061 (100), $[M-H-132]^-$ 929 (22), $[M-H-162]^-$ 899 (18), $[M-H-162-132]^-$ 767 (20); ^1H nmr (300 MHz, pyridine-d_5) δ 3.17 (1H, dd, *J*=11 and 4 Hz, H-3α), 4.79 [1H, d, *J*=5.9 Hz, H-1 (ara)], 4.92 [1H, d, *J*=6.5 Hz, H-1 (xyl)], 4.99 (d, *J*=7.6 Hz, H-1 (glc)], 5.15 (1H, s, H-28), 5.46 [1H, d, *J*=7.6 Hz, H-1 (glc′)]; ^{13}C nmr, see Tables 1 and 2.

Hydrolysis of saponins **7** *and* **8**.—A fraction containing **7** and **8** in 2 N HCl in 50% MeOH was refluxed for 2 h. The usual workup gave 3β, 16α-dihydroxyolean-12-en-28-al [**11**]. ^1H nmr (pyridine-d_5) δ 9.54 (1H, s, H-28), 5.53 (1H, t, H-12), 4.77 (1H, br s, H-16), 3.46 (1H, dd, *J*=8.5 and 7.6 Hz, H-3), 1.72 (1H, s, H-27), 1.22, 1.04, 1.04, 1.00, 0.94, 0.85 (all 3H, s); ^{13}C nmr (pyridine-d_5) δ 205.9 (C-28), 143.6 (C-13), 123.5 (C-12), 78.1 (C-3), 73.1 (C-16), 55.8 (C-5), 51.5 (C-17), 47.1 (C-9), 46.8 (C-19), 42.0 (C-14), 40.9 (C-8), 40.0 (C-18), 39.3 (C-1), 39.1 (C-4), 37.3 (C-10), 35.6 (C-21), 35.0 (C-15), 33.4 (C-7), 33.2 (C-29), 30.7 (C-20), 28.7 (C-23), 27.9 (C-2), 27.2 (C-22), 27.0 (C-27), 24.2 (C-30), 23.8 (C-11), 18.7 (C-6), 17.5 (C-26), 16.5 (C-24), 15.7 (C-25); eims *m/z* 456 (5), 438 (10), 248 (22), 207 (50), 201 (100).

Sugar analysis.—Partially *O*-acetylated/methylated alditols were prepared using approximately 2 mg of saponin according to Harris *et al.* (7). Reference samples of appropriate standards were also prepared according to Doars *et al.* (8). The alditols were assigned by gc retention time and by their characteristic ms.

Biological activity.—The activity used to guide fractionation was that detected in an in vitro antiviral assay described earlier (9). Compounds **1–8** showed similar levels of activity causing whole-well inhibition

of cytopathic effects at 40 μg/disk. In vitro cytotoxicity vs. murine P388 leukemia cells for compounds **1**–**8**: ID_{50} (μg/ml) **1** 0.85, **2** 0.85, **3**>12.5, **4** 10.2, **5** 7.0, **6** 8.6, **7** 7.0, **8**>12.5. Testing was performed at the Chemistry Department, University of Canterbury.

ACKNOWLEDGMENTS

Victoria University, Wellington, provided laboratory facilities for L.Q. Assistance with collection and identification of plant material was kindly provided by A.P. Druce, Landcare N.Z. Ltd., Wellington, R.P. Buxton and B.J.P. Molloy, Landcare N.Z. Ltd., Christchurch.

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Received 14 February 1994