g, 10.8 mmol) in dry benzene (20 mL) under an argon atmosphere. The mixture was then refluxed for 1 h. Water was added, and the mixture was extracted with CH_2Cl_2 (150 mL). The organic layers were combined, washed with saturated $NaHCO_3$ solution, dried, and evaporated to dryness. Subsequent flash chromatography gave an oily liquid, which solidified on standing (528 mg, 48%). **38**: ^1H NMR (300 MHz, $CDCl_3$) δ 6.19–6.11 (m, 2 H), 5.93–5.86 (m, 2 H), 3.97–3.84 (m, 4 H), 2.71–2.61 (m, 2 H), 2.07–1.92 (m, 4 H), 1.66–1.57 (m, 2 H); GC/IR 3036 (m), 2954 (s), 1114 (s) cm^{-1}; UV (pentane) λ_{max} 276 nm (ϵ 4834); HRMS (M^+) calcd 204.1188, obsd 204.1169.

Tricyclo[5.4.0.0^{2,8}]undeca-3,5-dien-9-one (39). An aqueous solution of 10% oxalic acid[28] (0.3 g, 11 drops) was added with continuous magnetic stirring to a suspension of 3 g of silica gel (Merck, silica 60, 70–230 mesh) in dry CH_2Cl_2 (4 mL). After 2–3 min, the water phase disappeared due to absorption on the silica gel surface. **38** (500 mg, 2.45 mmol) dissolved in CH_2Cl_2 (5 mL) was added, and the mixture was stirred until no more ketal **38** could be detected (TLC control) (ca. 2 h). The reaction mixture was filtered and the solid washed several times with the solvent. The solvent was removed, and subsequent flash chromatography on silica gel (ether/petroleum ether, 1:1) gave a colorless, oily liquid (341 mg, 87%). **39**: ^1H NMR (300 MHz, $CDCl_3$) δ 6.20–6.11 (m, 2 H), 6.05–5.99 (m, 2 H), 2.79–2.75 (m, 2 H), 2.59–2.54 (m, 2 H), 2.16–2.09 (m, 3 H), 1.92–1.88 (m, 1 H); ^{13}C NMR (75.4 MHz, acetone-d_6) δ 212.6 (s), 134.6 (d, double intensity), 126.2 (d, double intensity), 42.9 (d), 42.3 (d, double intensity), 31.2 (t), 28.8 (d), 24.8 t); UV (pentane) λ_{max} 276 nm (ϵ 4879); HRMS (m^+) calcd 160.0868, obsd 160.0878.

Tricyclo[5.4.0.0^{2,8}]undeca-3,5-dien-9-one (*p*-Tolylsulfonyl)hydrazone (40). A mixture of **39** (300 mg, 1.87 mmol) and tosylhydrazine (348 mg, 1.87 mmol) in dry ethanol (20 mL) was stirred for 24 h at room temperature. A white precipitate was formed, which was filtered and identified as pure product **40** (500 mg, 81%): mp 186 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, 2 H), 7.30 (d, 2 H), 7.19 (s, br, 1 H), 6.1–6.04 (m, 2 H), 5.99–5.90 (m, 2 H), 2.49–2.37 (m, 7 H), 2.24 (d, 1 H), 2.13–2.07 (m, 2 H), 1.78–1.74 (m, 1 H); IR (KBr) 3215 (s), 2988 (s), 1333 (s), 1165 (s) cm^{-1}. Anal. Calcd for $C_{18}H_{20}N_2O_2S$: C, 65.83; H, 6.14; N, 8.53. Found: C, 65.40; H, 6.24; N, 8.54.

Tricyclo[5.4.0.0^{2,8}]undeca-3,5,9-triene (12). To a stirred solution of **40** (500 mg, 1.65 mmol) in dry THF (40 mL) at −10 °C was added dropwise a 1.6 M solution of BuLi (4.1 mL, 6.6 mmol) in hexane during 15 min.[18] The mixture was allowed to warm up to room temperature (1 h) and then stirred for another 30 min. Ice water (100 mL) was carefully added, the mixture was extracted with ether (100 mL), and the organic layers were dried. The solvent was carefully removed, and the residue was purified by flash chromatography on silica gel (pentane) to give a colorless, volatile liquid (45 mg, 19%). **12**: ^1H NMR (200 MHz, $CDCl_3$) δ 6.45–6.36 (m, 1 H), 6.14–6.03 (m, 2 H), 5.87–5.78 (m, 2 H), 5.49–5.41 (m, 1 H), 2.57–2.50 (m, 4 H), 1.94–1.89 (m, 1 H), 1.65–1.58 (m, 1 H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 138.5 (d), 136.0 (d, double intensity), 123.9 (d, double intensity), 122.1 (d), 46.2 (d, double intensity), 35.1 (t), 28.8 (d), 25.5 (d); GC/IR 3032 (s), 2966 (s), 694 (m); UV ($CDCl_3$) λ_{max} 293 nm; HRMS (M^+) calcd 144.0977, obsd 144.0958.

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Pouosides A–E, Novel Triterpene Galactosides from a Marine Sponge, *Asteropus* sp.

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Five new triterpene galactosides, pouosides A–E (**1–5**), have been isolated from a Pacific marine sponge, *Asteropus* sp. The carbon skeleton of the pouoside aglycons is new for naturally occurring triterpenes and parallels that of the C_{40} carotenoids. Structures were determined from spectroscopic data, especially extensive ^1H and ^{13}C NMR data and 2D NMR experiments. A novel saponin in which amino sugars are incorporated was isolated from the same sponge. Pouoside A, **1**, is cytotoxic.

Despite the great diversity that already exists among the carbon skeletons of known triterpenes, new variants continue to emerge.[1] Some of the most novel skeletal modifications occur among the less common metabolites and arise from partial cyclization of squalene. Interestingly, to date no naturally occurring triterpenes have been found that have a carbon skeleton patterned after that of the C_{40} carotenoids, i.e., terminal cyclohexane rings linked by a symmetrical, acyclic chain.[2] We report here the isolation, from an *Asteropus* sp. of sponge,[3] of five triterpene galactosides whose aglycons do have a carotenoid-type carbon skeleton. In addition to the pouosides, a novel, cytotoxic saponin (sarasinoside A_1) that contains amino sugars, was also been isolated from this sponge.[4,5]

(1) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **1986**, *3*, 421 and earlier reviews cited. Devon, T. K.; Scott, A. I. *Handbook of Naturally Occurring Compounds*; Academic: New York, 1972; Vol. II, p 281.

(2) Although there are no reports of such naturally occurring compounds, synthetic carotenoid-like diterpene compounds have been synthesized before; cf. the following: Sopher, D. W.; Utley, J. H. P. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1361. Foote, C. S.; Chang, Y. C.; Denny, R. W. *J. Am. Chem. Soc.* **1970**, *92*, 5216. Feichtmayr, F.; Heilbronner, E.; Nuerrenbach, A.; Pommer, H.; Schlag, J. *Tetrahedron* **1969**, *25*, 5383.

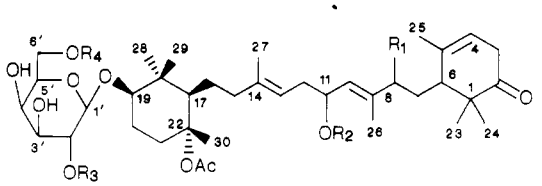
(3) Tentative sponge identification was made by Dr. Jane Fromont, Sir George Fisher Centre for Tropical Marine Studies, James Cook University, Townsville, Australia. Voucher specimens (79-GS-61; BB3) are on hand in the principal author's collection. The galactosides are named after the small bay, Pou Bay, near where the sponge was collected since the sponge genus name could not be determined from the specimen available.

(4) Schmitz, F. J.; Ksebati, M. B.; Gunasekera, S. P.; Agarwal, S. *J. Org. Chem.*, in press. Interestingly, the pouosides were not found in specimens of *Asteropus* collected at Guam Is., although sarasinoside A_1 was.

(5) Kitagawa, I.; Kobayashi, M.; Yoshihiro, O.; Yoshikawa, M.; Hamamoto, Y. *Chem. Pharm. Bull.* **1987**, *35*, 5036.

0022-3263/88/1953-3917$01.50/0

The sponge was collected near Pou Bay, Moen, Truk, Federated States of Micronesia. The ethanol extract of the sponge was concentrated and partitioned between 30% aqueous methanol and chloroform. The chloroform solubles were chromatographed over Sephadex LH-20 with chloroform–methanol (1:1). Trituration of the third fraction with $CHCl_3$ dissolved a mixture of triterpene compounds (^1H NMR), leaving behind an insoluble saponin fraction containing sarasinoside A_1.[4,5] HPLC of the triterpene mixture on a reverse-phase C_{18} column yielded glycosides **1–5**.



	R_1	R_2	R_3	R_4
1	OAc	Ac	H	H
2	OAc	H	H	H
3	H	Ac	H	H
4	OAc	Ac	Ac	H
5	OAc	Ac	H	Ac

The formulas for all the pouosides were established by HR FAB MS; for **1**, $C_{42}H_{66}O_{13}$ [801.4449, $C_{42}H_{66}O_{13}Na$, $(M + Na)^+$, calcd, 801.4401]. This was corroborated by ^{13}C NMR data (Table II). Infrared absorptions compatible with hydroxyl (3420, br), acetate (1735), and ketone groups (1710 cm^{-1}) were observed. Only end absorption was noted in the UV spectrum denoting the absence of conjugated unsaturation. ^{13}C NMR spectra provided evidence for the following groups: a saturated ketone, 218.1 (s), three acetates, 173.2 (2, s), 173.3 (s) ppm [^1H NMR: 1.93, 2.00, 2.03 ppm (s)], three trisubstituted double bonds (Table II), and one acetal group, 108.7 (d) ppm. These functionalities account for seven of the ten degrees of unsaturation in **1**. The presence of an acetal carbon and the ready loss of the elements of a sugar in the high-resolution FAB mass spectrum pointed to the presence of one sugar (see also below) and confirmed that the carbon skeleton of the aglycon portion of **1** contained two rings.

Information gleaned from a COSY plot and difference double resonance (DDR) experiments conducted in CD_3OD led to the formulation of partial structures A–D.[6] Proton–carbon correlation experiments optimized separately for detecting one- and two-/three-bond couplings confirmed these partial structures and unambiguously identified the chemical shifts for the three pairs of geminal protons attached to C-7, C-15, and C-16. These shifts could not be confidently assigned from proton NMR data alone because of inadequate dispersion in the upfield region of the spectrum.

The connectivities in the sugar moiety, partial structure A, were gleaned from an $^1H/^1H$ COSY map; proton coupling values (Table I) indicated that the sugar was galactose and this was confirmed by comparison of ^{13}C NMR chemical shifts of the sugar carbons with literature values.[7] Evidence for assigning the β-configuration to the acetal carbon consisted of the anomeric carbon chemical shift (108.7 ppm)[8], anomeric $^1H/^{13}C$ coupling of 157 Hz,[9] and H/H $J_{1,2}$ of 8 Hz. The ^{13}C NMR chemical shifts of the sugar carbons also established that the sugar was not acetylated, and hence all three acetate groups are located on the triterpene skeleton.

Table I. Proton NMR Data for Compounds 1–3[a]

H at C:	δ (mult, J in Hz)[b] 1	2	3
3	2.96 (ddq, 22, 7, 3)	2.96 (ddq, 22, 7, 3)	2.96 (ddq, 22, 7, 3)
3	2.73 (d quint, 22, 3)	2.72 (d quint, 22, 3)	2.70 (d quint, 22, 3)
4	5.47 (br s)	5.50 (br s)	5.53 (br s)
6	2.13 (br t, 5)	2.12 (br t, 5)	2.20 (br t, 4)
7	1.85 (m)[c]	1.85 (m)[c]	
7	1.41 (ddd, 4, 7, 15)[c]	1.44 (ddd, 4, 7, 15)[c]	
8	4.98 (dd, 4, 8)	4.98 (dd, 4, 8)	
8			
10	5.33 (br d, 8)	5.33 (br d, 6)	5.07 (br d, 7)
11	5.41 (m)	4.30 (br q, 6)	5.45 (br q, 7)
12	2.40 (quint, 7)	2.32 (quint, 7)	2.35 (quint, 6)
12	2.25 (quint, 7)	2.25 (quint, 6)	2.20 (quint, 6)
13	5.09 (br t, 6)	5.13 (br t, 6)	5.07 (br t, 6)
15	2.12 (m)[c]		
15	2.0 (m)[c]		
16	1.50 (m)[e]		
16	1.50 (m)[e]		
17	1.58 (br t, 6)[d,e]		
19	3.30 (dd, 4, 13)	3.30 (dd, 4, 13)	3.30 (dd, 4, 13)
20	2.05 (br dt, 13, 4)[c]	2.05 (dt, 13, 4)[c]	2.05 (dt, 13, 4)[c]
20	1.58 (q, 13)[c]	1.60 (q, 13)[c]	1.60 (q, 13)[c]
21	2.50 (br dt, 12, 4)	2.50 (br dt, 12, 4)	2.50 (br dt, 12, 4)
21	1.80 (dt, 4, 12)[c]	1.80 (dt, 4, 12)[c]	1.78 (dt, 12, 4)[c]
23	1.22 (s)	1.23 (s)	1.21 (s)
24	1.09 (s)	1.09 (s)	1.09 (s)
25	1.80 (br s)	1.82 (br s)	1.81 (br s)
26	1.65 (br s)	1.62 (br s)	1.65 (br s)
27	1.67 (br s)	1.64 (br s)	1.65 (br s)
28	1.11 (s)	1.11 (s)	1.10 (s)
29	0.89 (s)	0.90 (s)	0.89 (s)
30	1.48 (s)	1.50 (s)	1.48 (s)
1′	4.30 (d, 8)	4.29 (d, 8)	4.28 (d, 8)
2′	3.51 (dd, 8, 10)[c]	3.51 (dd, 8, 10)[f]	3.51 (dd, 10, 8)[c]
3′	3.44 (dd, 3, 10)[c]	3.44 (dd, 10, 3)[f]	3.44 (dd, 3, 10)[c]
4′	3.83 (d, 3)	3.82 (d, 3)	3.83 (d, 3)
5′	3.49 (t, 6)[c]	3.49 (t, 6)[f]	3.49 (t, 7)[c]
6′	3.71 (d, 6)	3.71 (d, 6)	3.72 (t, 6)[c]
OAc	1.93 (s)	1.95 (s)	1.94 (s)
	2.0 (s)	2.04 (s)	1.98 (s)
	2.03 (s)		

[a] In CD_3OD at 300 MHz. [b] Values given in same order as multiplicity designation. [c] Observed by difference double resonance and by $^1H/^1H$ COSY experiments. [d] Assignments by NOE experiments. [e] Assignments by ^1H–^{13}C correlation experiment. [f] Assignments by analogy to compound **1**.

The vicinally coupled sequence of protons in partial structure B (H's 19, 20, 21) was confirmed from COSY and relayed coherence transfer (RCT)[10] 2D spectra (Figure 1). In a long-range COSY spectrum[10] cross-peaks were observed between the signal at 3.30 ppm (H-19) and two quaternary methyl resonances (Me-28, Me-29), which in turn were related themselves by a cross-peak, thus confirming the presence of geminal dimethyl groups on a carbon next to the methine proton (3.30 ppm). Long-range $^1H/^{13}C$ correlations (structure B′) provided evidence for constructing the remaining parts of this ring and revealed that the sugar was attached to the secondary alcohol. Evidence for closing ring B as shown derived from the observation of *W*-type coupling between the proton signal

(6) The optimum solvent for dispersion of the entire ^1H NMR spectrum of **1** was CD_3OD. Signals for the sugar unit were more clearly resolved in C_6D_6–10% CD_3OD, but the olefinic resonances were poorly separated in this solvent. For the sake of clarity, the spectral discussion centers on spectra taken in CD_3OD.

(7) Wehrli, F. W.; Nishida, T. *Prog. Chem. Nat. Prod.* **1979**, *36*, 1.

(8) Beier, R. C.; Mundy, B. P.; Strobel, G. A. *Can. J. Chem.* **1980**, *58*, 2800.

(9) Hansen, P. E. In *Progress in NMR Spectroscopy*; Emsley, J.; Feeney, J.; Sutcliffe, L. H., Eds.; Pergamon: New York, 1981; Vol. 14, p 249.

(10) Derome, A. *Modern NMR Techniques for Chemistry Research*; Pergamon: New York, 1987.

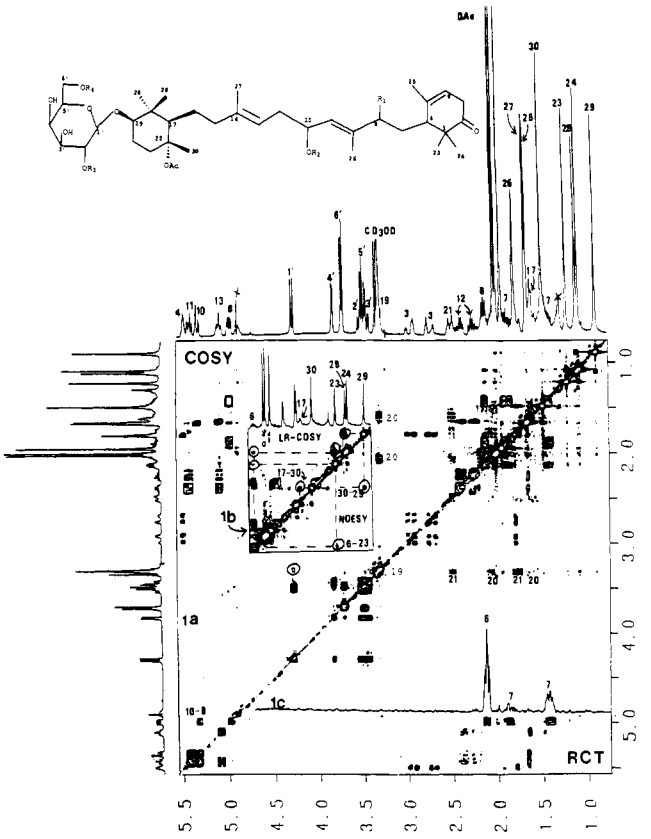


Figure 1. Combined ^1H COSY (2D correlated spectroscopy)–RCT (2D relay coherence transfer spectroscopy) plot of pouoside A in CD_3OD. The RCT mixing time was incremented from 25 to 37 ms; 512 FIDS, 32 and 16 scans each were recorded for RCT and COSY, respectively. The individual spectra were cut along the diagonal into two triangles, and the combined plot was assembled by using the upper left triangle of COSY and the lower right triangle of RCT.

at 1.58 (H-17)[11] and the quaternary methyl signal at 1.48 ppm (Me-30) in a long-range COSY spectrum (Figure 2). Nuclear Overhauser enhancements were observed between the quaternary methyl resonances 1.48 (Me-30) and 0.89 ppm (Me-29) (NOESY spectrum, Figure 2). NOE was also observed for the proton signal at 1.58 (H-17) upon irradiation of the resonance at 3.30 ppm (H-19). Thus, these two methine protons, H-19 and H-17, are cis 1,3 diaxial on one face (α-face as drawn) and the two methyl groups are cis 1,3 diaxial on the opposite face. This establishes the relative stereochemistry as shown on B and in the final structure 1.

Most of the acyclic segment C was obvious from COSY and DDR data. The vinyl methyl signal at 1.67 ppm showed long-range $^1H/^{13}C$ cross-peaks with both C-14 and C-15, thus identifying the precise location of this methyl group and also providing evidence for joining C-14 to C-15. Detection of allylic coupling between the signals at 5.33 ppm (H-10) and the 4.98 ppm (H-8) ($^1H/^1H$ COSY spectrum, Figure 1) proved that C-8 is bonded to C-9. A selective INEPT experiment[12] confirmed that there was an acetate group attached to the methine carbon absorbing at 80.0 ppm (irradiation 4.98 ppm, H-8, observed carbonyl carbon signal at 173.2 ppm). The carbon chemical shifts of the vinyl methyl groups revealed that both double bonds are of the *E* configuration.[13]

The methylene group, C-16, shown to the left of partial structure C could not be connected to C-15 from spectroscopic data, but biogenetic considerations and evidence cited below relegated this methylene group to position 16. The proton assignments for H-16 were made from the $^1H/^{13}C$ correlation spectrum.

Cross-peaks in the LR COSY spectrum identified the geminal dimethyl groups in partial structure D (Figure 3) and outlined the connectivity from the vinyl methyl group through the olefinic proton to the methylene group (H-3). No proton–proton coupling connectivity to the methine proton at 2.13 ppm (H-6) was observed, but an NOE was detected between this signal and the 1.22 ppm methyl signal (H-23, Figure 2). Partial structure D was finalized on the basis of the long-range $^1H/^{13}C$ correlations shown on D′.

Due to overlap of the signals in the 2.2–1.5 ppm region of the proton NMR spectrum, it was not possible to determine from the COSY spectrum the correct order of connecting rings B and D and the C-16 methylene group to the unsymmetrical unit C. However, connection be-

Table II. ^{13}C NMR Data for 1–3[a,b]

C no.	1[c]	2[e]	3[c]
1	50.5[d]	50.0	50.5
2	218.1[d]	218.0	218.0
3	40.6	40.5	40.7
4	121.2[d]	121.0	121.3
5	140.9[d]	140.8	143.0[e]
6	52.5[d]	52.5	55.7
7	37.2	37.5	31.6
8	80.0	79.8	37.8
9	140.9[d]	138.0	141.7[e]
10	129.1	133.0	126.1
11	73.6	70.3	74.2
12	35.8	32.3	36.0
13	121.1	122.0	121.1
14	142.2[d]	141.0	141.7[e]
15	45.5[d]	45.5	45.5
16	28.0	28.0	28.2
17	56.1[d]	56.1	56.0
18	43.9[d]	43.6	43.6[e]
19	90.5	90.4	90.4
20	29.8	29.8	29.8
21	38.0	38.0	38.4
22	89.9[d]	89.7	89.7[e]
23	28.3[d]	28.0	28.7
24	23.5[d]	23.2	24.5
25	25.8	25.7	25.1
26	14.9	14.8	18.2
27	18.5	18.3	18.8
28	30.2[d]	30.1	30.1
29	18.5[d]	18.3	18.3
30	22.3[d]	22.7	22.8
OAc	173.2, 173.3	173.4, 173.6	173.6, 173.8
	23.0, 23.1, 24.9	22.1, 24.5	22.0, 24.5
1′	108.7	108.7	108.7
2′	74.6	74.6	74.6
3′	76.6	76.6	76.8
4′	71.7	71.8	71.8
5′	77.8	78.0	78.0
6′	63.9	64.0	64.0

[a] CD_3OD at 75.4 MHz. [b] Multiplicities by DEPT experiments. [c] Protonated carbon assignments by 2D $^1H-^{13}C$ correlation experiments. [d] Assignments by long-range $^1H-^{13}C$ correlation experiments. [e] Assignments by analogy to compound 1.

(11) Signals for H-17 and H-20α both occur at ~1.58 ppm. The chemical shift and multiplicity of H-20α were readily obtained from the COSY spectrum shown in Figure 1 and a difference decoupling spectrum resulting from irradiating H-19. Long-range $^1H/^{13}C$ correlations identified C-17 (see B′) and then review of the one-bond $^1H/^{13}C$ correlations showed that both C-20 and C-17 were corrected with proton signals at 1.58 ppm. Therefore both H-17 and H-20 must absorb at 1.58 ppm. The multiplicity of H-17 was derived from the difference NOE spectrum obtained by irradiating H-19.

(12) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314.

(13) Cf. Crews, P.; Naylor, S. *Prog. Nat. Prod.* **1985**, *48*, 203.

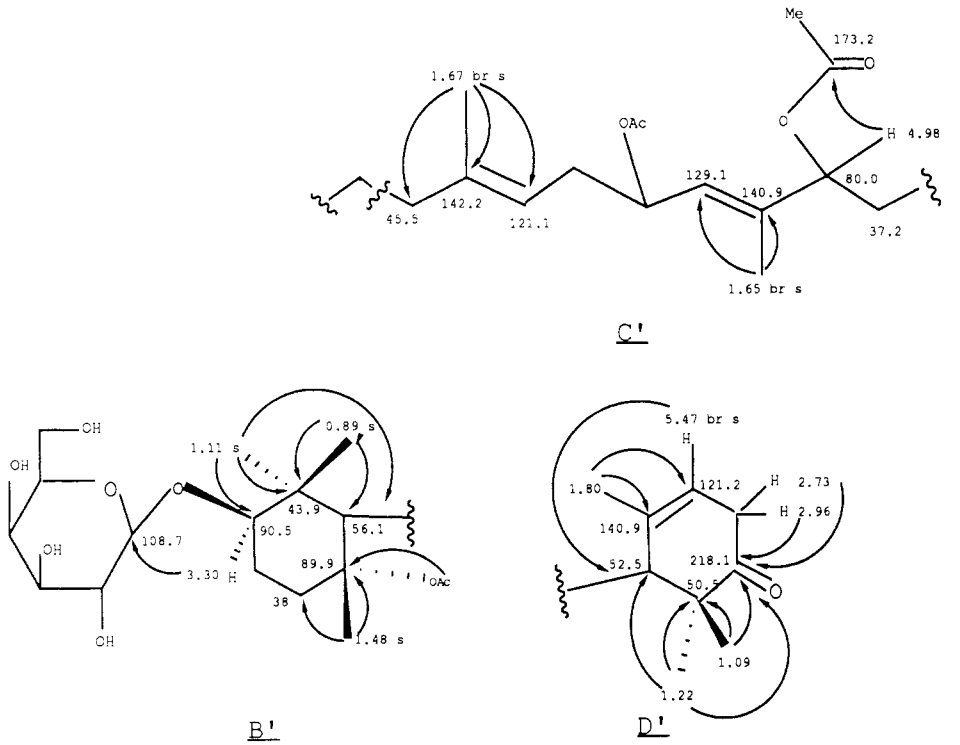


Figure 2. Combined long-range COSY–NOESY plot of pouoside A in CD_3OD. For the long-range COSY experiment, a flip angle of 90° was used for the mixing pulse; delays $\Delta = 256$ ms before data acquisition in the t1 and t2 dimensions; 512 FIDS, 16 scans each for both LR-COSY and NOESY were recorded. The mixing time for the NOESY experiment was incremented from 256 to 300 ms.

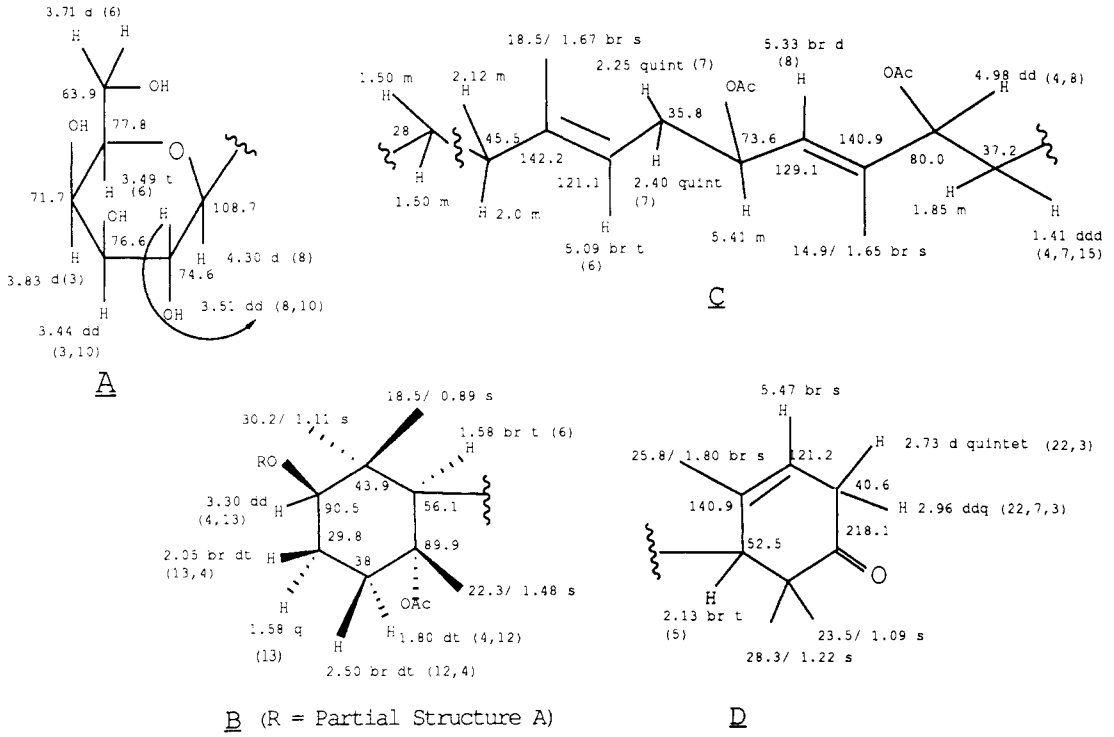


Figure 3. A slice at 4.98 ppm from RCT spectrum.

tween C-6 of ring D and C-7 of partial structure C was unequivocally established by an RCT experiment in which a cross-peak between the signals at 4.98 (H-8) and 2.13 ppm (H-6) was apparent (lower right triangle of Figure 1). A slice taken at 4.98 ppm (H-8) in this plot revealed this cross-peak was a broad triplet, $J \sim 5$ Hz (Figure 1c). With this firm evidence for connecting ring D to C-6 of partial structure C, it follows that the open valences of partial structure B and C-15 of partial structure C must both be joined to the C-16 methylene group to give the final structure 1. The stereochemistry at C-6, C-8, and C-11 remains undetermined.

Analysis of all the spectral data for **2**, pouoside B, $C_{40}H_{64}O_{12}$ (Tables I and II and the Experimental Section), revealed that this metabolite has the same structure as **1** except that it possesses one less acetyl group. Comparison of the NMR chemical shifts of H-11 and C-11 in **1** and **2** reveals that the additional hydroxyl group in **2** is at C-11. Triterpene **2** was assigned the same stereochemistry as **1** on the basis of the close correlation of all the proton and

carbon chemical shifts and proton multiplicities for these two compounds. Proton assignments and connectivities between most protonated segments of **2** were confirmed by decoupling and DDR experiments.

Pouoside C, **3**, $C_{40}H_{64}O_{11}$, was shown to be the 8-deacetoxy analogue of **1** by extensive proton NMR decoupling, $^1H/^1H$ and $^1H/^{13}C$ 2D correlation experiments, and comparison of IR and NMR data for the two compounds. The ^1H NMR spectra of **3** and **1** were virtually identical except that an acetate methyl signal and the 4.98 dd characteristic of the acetoxy deshielded H-8 in **1** and **2** were missing in the spectrum of **3**, and the olefinic proton absorption for H-10 in the spectrum of **3** was shifted upfield to 5.07 ppm. Likewise, the carbon NMR spectra for **1** and **3** differ only in the following respects: the spectrum of **3** contains only two carbonyl carbon peaks, the C-8 signal is a triplet at 37.8 ppm instead of a doublet at ~80 ppm as in the spectrum of **1**, and there are some minor shift differences between the spectra for carbons in the vicinity of C-8. Since the proton and carbon NMR data for **1** and **3** are nearly identical except for that of the H-8 and C-8, **3** is assigned the same stereochemistry as **1**.

Pouoside D, **4**, $C_{44}H_{68}O_{14}$, contained one more acetyl group than **1** (confirmed by an extra methyl singlet in the 1.94–2.08 ppm region of the ^1H NMR spectrum). Comparison of the ^1H NMR signals of **4** and **1** confirmed that all the signals corresponding to the aglycon of **1** were also present in the spectrum of **4** (all shifts within +0.01 ppm) and accordingly it is proposed that the two compounds have the same aglycon structure, including acetylation at C-8, -11, and -22. Chemical shift and coupling data (DDR experiments, see J values in the Experimental Section) confirmed the presence of a six-carbon sugar with galactose stereochemistry and a β-anomeric configuration (H/H $J_{1,2}$ = 8 Hz). Proton chemical shift comparisons between **4** and **1** revealed that the sugar H-2′ resonance of **4** occurred at 5.04 ppm compared to 3.51 ppm for the analogous signal of **1** and that the H-1′ and H-3′ signals were also shifted slightly downfield (see the Experimental Section). Other sugar proton absorptions were the same for the two compounds. Hence **4** is the 2′-acetyl analogue of **1**.

The aglycon portion of **5**, pouoside E (same molecular formula as for **4**), was shown to be identical with that of **1** by comparison of their ^1H NMR spectra (multiplicities identical and all signals within +0.05 ppm). Proton NMR coupling data (see J values in the Experimental Section) for the sugar portion of **5** confirmed that the stereochemistry of a galactose unit was present and that the anomeric configuration was β (H/H $J_{1,2}$ = 8 Hz). Absorptions for H-1′ to H-4′ were at the same positions as for **1**, but the H-6′ signals were shifted downfield [4.14 (dd, 4.8, 11.4), 4.35 (dd, 7.8, 11.4 ppm)]. Glycoside **5** is thus the 6′-acetyl derivative of **1**.

Glycoside **1** exhibits cytotoxicity, ED_{50} (PS) = 1.5 μg/mL.[14] The β,γ-unsaturation in pouoside A showed little or no propensity to isomerize to the α,β-conjugated position upon standing in CD_3OD solution in a refrigerator for approximately 2 months, even though it underwent almost complete proton exchange at C-3.

(14) Gueran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbot, B. J. *Cancer Chemother. Rep., Part 2* **1972**, *3*, No. 2, 1. Effective doses (ED_{50}) in tissue culture tests are expressed as concentrations in micrograms/milliliter of test material in the growth medium that causes 50% inhibition of cell growth. "Active" materials display an $ED_{50} \leq 10$ μg/mL. PS (P388) refers to in vitro lymphocytic leukemia.

Experimental Section

^1H NMR spectra were recorded at 300 MHz and ^{13}C spectra at 75.4 MHz on a Varian XL-300 spectrometer; chemical shifts are reported in parts per million (δ) downfield from internal tetramethylsilane. IR spectra were measured on a Perkin-Elmer Model 298 spectrometer. High-resolution FAB mass spectra were recorded on a VG ZAB-E mass spectrometer. An Altex 5 μm × 9.6 mm × 29.9 cm semipreparative Adsorbosphere reverse-phase C_{18} column was used for separations.

Extraction and Isolation Procedures. Frozen sponge specimens (642 g) collected in Truk Lagoon in 1979 were soaked in ethanol for 2 days, and the liquid recovered by filtration was concentrated in vacuo. The residue was freeze-dried to give 141 g of red solids. A portion (50 g) of the solids was partitioned between $CHCl_3$ and 30% aqueous methanol (1 L) to give 9 g of organic solubles, which was chromatographed on a Sephadex LH-20 column using $CHCl_3$/MeOH (1:1) into five fractions. Trituration of the third fraction (1.2 g) with $CHCl_3$ afforded an insoluble residue (0.8 g) consisting of a saponin mixture[4] and a soluble fraction (0.4 g). The latter was subjected to HPLC with a C-18 column and MeOH/H_2O (75:25) as eluent to give pure pouosides A–E. ^1H and ^{13}C NMR data for pouosides A–C are in Tables I and II.

Pouoside A (1): 40 mg; colorless oil; for ^1H and ^{13}C NMR data, see Tables I and II; IR ($CHCl_3$) 3420, 1735, 1710 cm^{-1}; HRFABMS, m/z (composition, interpretation, calculated millimass) 801.4449 [$C_{42}H_{66}O_{13}Na$, (M + Na)$^+$, 801.4401], 599.3955 ($C_{36}H_{55}O_7$, M$^+$ – galactose, 599.3947), 436.3316 ($C_{30}H_{44}O_2$, M$^+$ – galactose – 2AcOH – Ac, 436.3341), 418.3224 ($C_{30}H_{42}O$, M$^+$ – galactose – 2AcOH – Ac – H_2O, 418.3236), 297.2184 ($C_{21}H_{29}O$, M$^+$ – galactose – 2AcOH – Ac – H_2O – C_9H_{13}, 297.2218).

Pouoside B (2): 2 mg; colorless oil; for ^1H and ^{13}C NMR data, see Tables I and II; IR (neat) 3400, 1730, 1705 cm^{-1}; HRFABMS, m/z (composition, interpretation, calculated millimass) 759.4306 [$C_{40}H_{64}O_{12}Na$, (M + Na)$^+$, 759.4295].

Pouoside C (3): 5 mg; colorless oil; for ^1H and ^{13}C NMR data, see Tables I and II; IR (neat) 3410, 1733, 1710 cm^{-1}; HRFABMS, m/z (composition, interpretation, calculated millimass) 743.4350 [$C_{40}H_{64}O_{11}Na$, (M + Na)$^+$, 743.4346].

Pouoside D (4): 1.2 mg; colorless oil; ^1H NMR (CD_3OD) protons on galactose 5.04 (1 H, dd, 8.0, 10.0, H-2), 4.47 (1 H, d, 8.0, H-1), 3.87 (1 H, br d, 4.0, H-4), 3.73 (2 H, d, 6, H-6), 3.64 (1 H, dd, 10.0, 4.0, H-3), 3.53 (1 H, br t, 6, H-5), 2.01 (3 H, s, OAc), ^1H assignments were established by ^1H decoupling and DDR experiments, the protons on the aglycon were virtually identical with those of **1** except for two signals: 1.01 (3 H, s, Me-28) and 0.78 (3 H, s, Me-29); IR (neat) 3405, 1735, 1710 cm^{-1}; HRFABMS, m/z (composition, interpretation, calculated millimass) 843.4531 [$C_{44}H_{68}O_{12}Na$, (M + Na)$^+$, 843.4507].

Pouoside E (5): 1.0 mg; colorless oil; ^1H NMR (CD_3OD) on galactose, 4.35 (1 H, dd, 7.8, 11.4, H-6), 4.28 (1 H, d, 8.0, H-1), 4.14 (1 H, dd, 4.8, 11.4, H-6′), 3.78 (1 H, br d, 3.5, H-4), 3.71 (1 H, m, H-5), 3.51 (1 H, dd, 8.0, 10.0, H-2), 3.45 (1 H, dd, 10.0, 3.5, H-3), 2.01 (3 H, s, OAc), ^1H assignments were established by ^1H decoupling and DDR experiments; IR (neat) 3400, 1735, 1710 cm^{-1}; HRFABMS, m/z (composition, interpretation, calculated millimass) 843.4527 [$C_{44}H_{68}O_{12}Na$, (M + Na)$^+$, 843.4507].

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