Sarasinoside A_1: A Saponin Containing Amino Sugars Isolated from a Sponge

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A new saponin designated sarasinoside A_1, **1**, has been isolated from a sponge, *Asteropus* sp., collected from Guam Island and also from Truk Lagoon. Acid hydrolysis of sarasinoside A_1 afforded the aglycon **2**, identified as 3β-hydroxy-4,4-dimethylcholesta-8,24-dien-23-one by spectral analysis. Methanolysis of **1** yielded the methyl glycosides of xylose, glucose, *N*-acetyl-2-amino-2-deoxygalactose, and *N*-acetyl-2-amino-2-deoxyglucose. Glycosidic connections were established by a combination of chemical degradations and spectroscopic methods. ^1H and ^{13}C NMR assignments were made with the aid of $^1H/^{13}C$ NMR correlations, relayed coherence transfer, and ROESY experiments. This appears to be the first saponin isolated from a sponge.

Among marine organisms, saponins are most commonly found in various classes of echinoderms, especially starfish and holothurians.[1] Sponges, on the other hand, have been a source of numerous novel sterols, but not saponins.[2,3] We report here the isolation and structure determination of a cytotoxic saponin from the sponge, *Asteropus sarasinosum*[4] collected at Guam Island and another *Asteropus* sp.[5] from Truk Lagoon. Whereas the saccharide moiety of saponins rarely contains amino sugars, this new saponin contains two amino sugars, *N*-acetyl-2-amino-2-deoxygalactose and *N*-acetyl-2-amino-2-deoxyglucose. Independent work in Japan has resulted in isolation of the same saponin plus eight related ones from a Palauan sponge.[6] In view of the larger number of related saponins being reported by the Osaka group and to avoid name duplication in the literature, we accept the name sarasinoside A_1 for the saponin described below whose structure was elucidated independently and by slightly different methods by our respective groups.

Sponges preserved by freezing were thawed, cut, and extracted at room temperature with chloroform/methanol (1:1) or ethanol. The concentrated extracts were partitioned between $CHCl_3$ and 30% aqueous methanol, and the organic solubles were chromatographed over Sephadex LH-20 ($MeOH/CHCl_3$) followed by high-pressure liquid chromatography using a C_{18} reversed-phase column with $MeOH/H_2O$ (9:1) to give pure sarasinoside A_1, **1** (Chart I), mp 208–212 °C. High-resolution FAB mass spectral measurements revealed a formula of $C_{62}H_{100}O_{26}N_2$ [1311.6422, $(M + Na)^+$, 4.0 mmu error], which was corroborated by elemental analysis. The infrared spectrum of **1** exhibited absorptions for hydroxyl (3300, br, s) and carbonyl groups (1680–1640 cm^{-1}, possibly an α,β-unsaturated ketone and/or amides), and there was UV absorption at 237 nm (ε 10192). The ^{13}C NMR spectrum of **1** in pyridine-d_5 showed a peak at ~200 ppm, consistent with the carbonyl of an α,β-unsaturated ketone, and signals at 172.0 and 171.7 ppm, compatible with amide carbonyls. The 200 ppm peak was observed in spectra taken before and after any type of chromatography, indicating that the α,β-unsaturated ketone was not generated by any facile elimination during the purification steps. Signals for four olefinic carbons and five anomeric carbons were also ob-

Table I. ^{13}C NMR Data for 1–4[a]

C	1[b]	1[c]	2[d]	3[e]	4[b]
1	37.4	36.2	36.9	36.8	37.2
2	27.8	26.4	27.9	27.6	27.6
3	90.6	88.6	79.0	89.9	90.1
4	40.1	41.6	39.0	39.1	40.0
5	51.0	49.9	50.2	50.4	50.9
6	18.9	17.8	18.4	18.2	18.8
7	28.6	28.4	28.4	28.2	28.3
8	127.8	126.9	127.8	127.6	127.7
9	136.6	135.6	135.9	135.8	136.5
10	37.2	38.0	37.0	36.6	37.0
11	22.5	21.5	22.0	21.9	22.3
12	36.3	35.3	35.8	35.7	36.2
13	42.6	*f*	42.2	42.1	45.5
14	52.3	51.3	52.0	51.8	52.3
15	24.1	23.2	23.8	23.7	24.2
16	29.4	27.9	29.0	29.0	28.6
17	55.4	52.3	55.0	54.9	55.3
18	11.5*[g]	11.0	11.3	11.2	11.6
19	16.7*	15.9	15.4	16.1	16.8
20	33.9	32.8	33.6	33.7	33.9
21	20.2*	19.4	19.8	19.5	20.3
22	52.0	50.7	51.5	51.4	51.9
23	200.9	200.4	201.6	202.5	200.8
24	124.9	124.0	124.4	124.2	125.0
25	154.2	153.8	154.7	155.4	154.0
26	20.8*	19.5	19.8	19.7	20.3
27	27.4*	27.0	27.7	26.4	27.3
28	28.2*	27.3	28.0	27.8	28.0
29	20.2*	20.2	20.7	20.6	20.6
$CH_3C(O)$	22.7 (2 C)	23.0, 27.0			23.6

[a] 75 MHz. [b] Pyr-d_5, room temperature; chemical shifts measured at 60 °C were within ±0.3 ppm of room temperature values. [c] DMSO-d_6. [d] $CDCl_3$. [e] $CDCl_3/CD_3OD$. [f] Signal obscured by DMSO. [g] * Assignments confirmed by $^1H/^{13}C$ correlation.

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(4) We thank Dr. P. Bergquist, University of Auckland, N.Z., for sponge identification.

(5) Tentative identification was kindly provided by Dr. Jane Fromont, Sir George Fischer Center for Tropical Marine Studies, Townsville, Australia.

(6) At the time of submission of this paper, we became aware through Dr. I. Kitagawa, Osaka University, Japan, that his group had deduced the same structure we report herein for sarasinoside A_1; see: Kitagawa, I.; Kobayashi, M.; Okamoto, Y.; Yoshikawa, M.; Hamanoto, Y. *Abstracts*, 29th Symposium on the Chemistry of Natural Products, Sapporo, Japan, Aug 26–27, 1987, and *Chem. Pharm. Bull.* **1987**, *35*, 5036. On the basis of comparison of spectral data exchanged in late 1986, both research groups then believed that we were dealing with different compounds. Specifically, there were significant differences in the glycosidic region of the ^{13}C NMR spectrum reported in pyr-d_5 at 75 and 125 MHz, from ambient temperature to 60 °C. Illustrative examples are as follows (this work/Osaka University): 106.5/104.6; 85.0/81.6; 71.2, 70.75/coincident peaks at 69.8 ppm. The discrepancy was resolved when we established that we could duplicate the values reported to us by the Osaka group by adding ~30% D_2O to a pyr-d_5 sample (~120 mg/0.6 mL) of the saponin. Dr. Kitagawa has since confirmed that their ^{13}C NMR spectra were obtain in pyr-d_5/D_2O mixtures although the spectra provided us initially were inadvertently labeled as having been measured in pyr-d_5 at 40 °C.

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Chart I

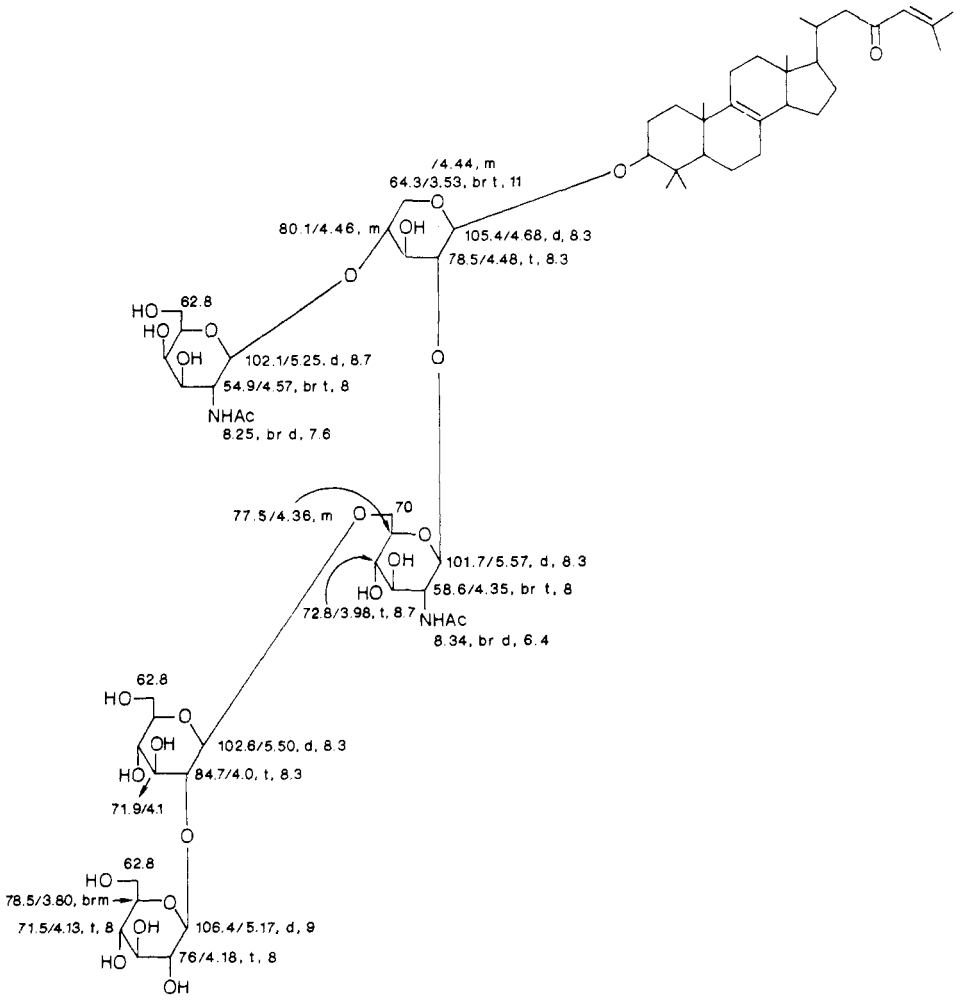

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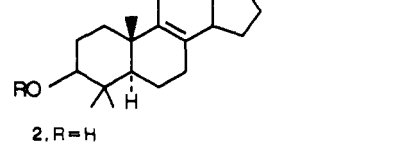
Table II. ^{13}C NMR Data for Saponin Sugars[a]

	Xyl			2-NAc-Glc			2-NAc-Gal			Glc[1]			Glc[2]		
C	1[b]	1[c]	4[b]	1[b]	1[c]	4[b]	1[b]	1[c]	4[b]	1[b]	1[c]	4[b]	1[b]	1[c]	4[b]
1	106.2	104.2	105.9	102.2	100.1	101.8	102.5	101.1		103.1	101.2	103.0	106.4	104.3	107.2
2	78.3	77.4	78.7	58.6	56.4	59.1	54.9	54.3		85.4	82.4	84.8	76.5	74.7	76.8
3	77.8	75.9	77.3	76.7	75.0	77.3	72.5	71.2		77.8	75.1	77.6	77.9	75.9	78.0
4	80.0	78.2	70.9	72.8	67.5	72.7	71.6	70.8		69.9	69.7	71.5	71.4	64.5	71.3
5	64.2	62.7	66.5	78.5	75.9	78.9	76.9	75.9		77.8	75.9	79.0	78.1	75.2	77.8
6				70.0	68.2	69.8	62.6	60.9		62.6	60.6	62.3	62.8	60.5	62.8

[a] 75 MHz. [b] Pyr-d_5, room temperature; chemical shifts observed at 60 °C were within ±0.3 ppm of room temperature values except that all anomeric signals shifted upfield 0.3–0.8 ppm. [c] DMSO, room temperature.

served; see Tables I and II. The ^1H NMR spectrum (pyridine-d_5) contained singlet signals for four quaternary methyl groups on a 4,4-dimethyl steroid (0.64, 1.07, 1.24, and 1.33 ppm), one secondary methyl (1.04 ppm), and four additional methyl singlets which could be assigned to two acetamide groups (2.00, 2.06 ppm) and an α,β-unsaturated ketone moiety of the type $C(O)CH{=}C(CH_3)_2$ (1.75, 2.20 ppm). Consistent with the last hypothesis was the occurrence of a one-hydrogen singlet at 6.15 ppm. Such an α,β-unsaturated ketone moiety constitutes the terminus of the side chain in the starfish saponin marthasterone.[7]

The aglycon was obtained by hydrolysis of the saponin by refluxing in 0.5 N HCl/toluene. The resulting emulsion was extracted with 1-butanol, and the 1-butanol solubles were chromatographed over silica gel to give two major fractions. The less polar of these was purified further by reversed-phase HPLC to give pure aglycon 2, mp 145–147



2, R = H
3, R = Xyl
4, R = Xyl(2→1)NAc-glc(6→1)glc(2→1)glc
5, R = 3-OMeXyl
6, R = 3-OMeXyl(OAc)$_2$

°C after recrystallization from methanol: $C_{29}H_{46}O_2$ (high-resolution mass spectrum); UV (95% EtOH) 237 nm (ϵ 11 200); IR 3600, 3480 (OH), 1680 cm^{-1} (α,β-unsaturated

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Table III. ^1H NMR Data for 1–4[a]

H at C	1[b]	1[c]	2[d]	3[d]	4[b]
3	3.18 (5, 12)	3.23 (5, 12)	3.23 (5, 11.4)	3.17 (4, 10)	3.24 (5, 12)
18	0.64	0.70	0.64	0.64	0.66
19	1.24	1.26	1.00	0.98	1.22
21	1.04 (7)	1.09	0.84 (7)	0.94 (7)	1.04 (7)
22	2.52 (2, 14)	2.60 (2, 14)	2.49 (2, 13.6)	2.50 (2, 14)	
24	6.15	6.20	6.05	6.06	6.15
26	2.20	2.22	2.14	2.13	2.20
27	1.75	1.82	1.88	1.88	1.77
28	1.33	1.36	0.98	0.98	1.34
29	1.07	1.11	0.81	0.85	1.06
Xyl-1	4.72 (8)	4.68 (8.3)		4.52 (6.5)	4.77 (8)
2-NAc-Gal-1	5.24 (8)	5.25 (8.3)			5.28[d] (7)
2-NAc-Glc-1	5.66 (9)	5.57 (8.3)			
Glc-1	5.66 (9)	5.50 (8.3)			5.79[d]
Glc[1]-1	5.21 (8)	5.17 (9)			5.45[d]
NH-Gal	8.68 (8)	8.52 (7.6)			
NH-Lc	8.48 (8)	8.34 (6.4)			8.84 (8)
NAc	2.00	2.07			2.00
	2.06	2.10			

[a] 300 MHz. [b] Pyr-d_5, room temperature. [c] Pyr-d_5, 60 °C. [d] $CDCl_3$. [d] Assignments with like letter may be exchanged.

CO). These data indicated the presence of a C(O)CH=C$(CH_3)_2$ moiety in **2**, and this was supported by ^1H NMR data ($CDCl_3$; methyl singlets at 1.88, 2.14; olefinic proton singlet at 6.05 ppm) and ^{13}C NMR data ($CDCl_3$; 201.6 s, 154.7 s, and 124.4 d). The high-resolution mass spectrum also showed intense peaks at m/z 83.04969 for the C(O)-CH=C$(CH_3)_2$ fragment and at m/z 313.253 14 corresponding to a loss of a methyl group and a $CH_2C(OH)C$-H=$(CH_3)_2$ moiety. In the ^1H NMR spectrum of **2**, resonances were observed for one secondary methyl at 0.94 and four quaternary methyls at 0.64, 0.81, 0.98, and 1.00 ppm, typical of those in a 3-hydroxylated triterpene;[8] see Table III. Further evidence for this type of structure was the presence of a one-proton double doublet, J = 5, 11 Hz, at 3.21 ppm, typical of the axial proton of a 3β-hydroxy-4,4-dimethyl arrangement. A doublet signal was also observed in the ^{13}C NMR spectrum at 79.0 ppm, which is in good agreement for the C-3 signal observed for lanosterol.[9]

In addition to the side-chain unsaturation, **2** possessed one fully substituted double bond (^{13}C NMR singlets at 127.8 and 135.9 ppm). The two double bonds and one carbonyl group discussed thus far account for three of the seven degrees of unsaturation implied by the formula of **2** and confirmed the presence of four rings as expected for a triterpene or sterol. The tetrasubstituted double bond was assigned to the Δ^8 position since the ^{13}C NMR shifts were very compatible with those of (5α)-cholest-8-en-3β-ol [128.0 (C-8) and 134.8 (C-9)] and quite different from those of (5α)-cholest-8(14)-en-3β-ol [126.1 (C-8) and 142.4 (C-14)].[10] Since the signals for C-8 and C-9 in lanosterol both occur at 134.0 ppm,[9] the occurrence of separate signals for the nuclear tetrasubstituted olefinic carbons in **2** reinforced the view that it lacks a methyl group at C-14. The remaining carbon signals for the methyl groups and the nuclear carbons match closely the corresponding ones of Δ^8-cholestenol and lanosterol[9-11] and provide support for the structure and stereochemistry shown in **2**.

The more polar component from acid hydrolysis of **1** was purified by reversed-phase HPLC to give an amorphous white solid, mp 227–228 °C, whose mass spectrum exhibited a molecular ion at m/z 558 corresponding to the weight of **2** plus a 5-carbon sugar. Since xylose was identified as one of the sugars in the hydrolysis of the intact saponin (see below), this product could be formulated as **3**. NMR data (see Tables I–III) and IR data (see Experimental Section) supported this conclusion. Acid hydrolysis of **3** yielded **2**.

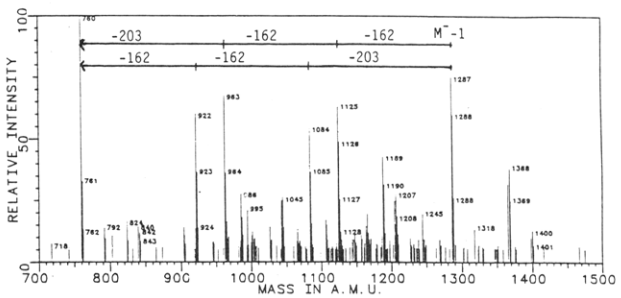

Figure 1. Negative ion laser desorption Fourier transform mass spectrum of **1**.

The sugars in **1** were liberated by methanolysis (anhydrous HCl/MeOH), and the resulting crude methyl glycoside mixture was then reacted with excess *p*-bromobenzoyl chloride. From the complex mixture of products obtained, four fully benzoylated β-OMe glycosides were isolated, and these were identified as derivatives of xylose, glucose, *N*-acetyl-2-amino-2-deoxyglucose, and *N*-acetyl-2-amino-2-deoxygalactose by comparison of their ^1H NMR spectra with literature data.[12] That all of the sugars in **1** are joined through β-linkages was revealed by the ^{13}C NMR chemical shifts (pyr-d_5; δ > 100 ppm) and $^1J_{CH}$ coupling constants (J = 161–162 Hz) of their anomeric carbons.[13,14]

The negative ion laser desorption Fourier transform mass spectrum of **1** showed prominent peaks (see Figure 1) corresponding to consecutive loss of two glucose units followed by a *N*-acetyl-2-amino-2-deoxy sugar (–162, –162, –203 amu) and the reverse order of fragmentation (–203, –162, –162 amu). This was compatible with the two pos-

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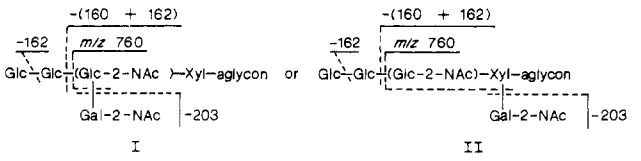
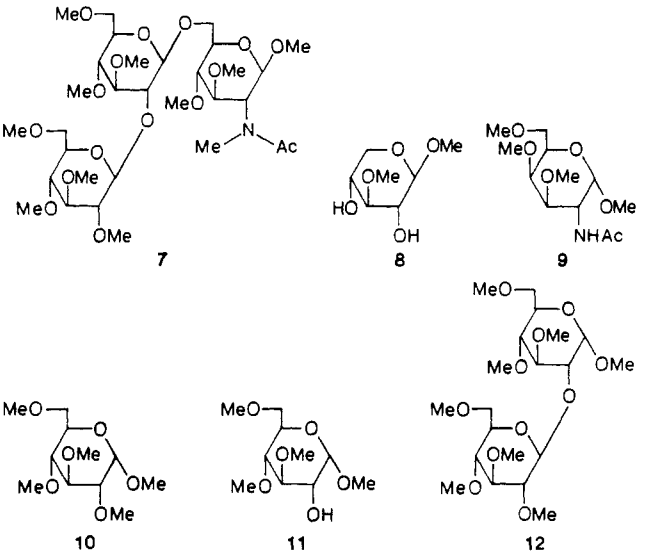


sible structures I or II. Digestion of 1 with a commercial grade of β-glucosidase gave a new saponin 4 which contained only four sugars as judged from the presence of only four anomeric carbons in its ^{13}C NMR spectrum. The sugar which had been selectively removed was confirmed to be *N*-acetyl-2-amino-2-deoxygalactose by the following facts: NMR spectra of 4 revealed that only one acetyl group remained (^1H NMR 2.0 ppm; ^{13}C NMR 171.2 ppm), and the ^{13}C NMR doublet at 54.9 ppm which is diagnostic of C-2 in a β-linked *N*-acetyl-2-amino-2-deoxygalactose[15] was missing while the 58.6 ppm signal characteristic of *N*-acetyl-2-amino-2-deoxyglucose[15] was still present; cf. spectra in pyr-d_5 at room temperature, Table II. The point of attachment of the *N*-acetyl-2-amino-2-deoxygalactose in 1 could be inferred from comparison of the ^{13}C NMR data of 1 and 4 in pyr-d_5 at room temperature. First, it was established by comparison of the ^{13}C NMR spectra of 1 taken in *DMSO* before and after partial deuteriation of the hydroxyl groups that the two sugar methylene groups at 62.9 and 68.4 ppm were both alkylated while the three methylene carbons at 60.7, 60.9, and 61.1 ppm have OH groups (signals doubled after partial deuteriation[16]). Since the C-6 carbons of any of the glucose or galactose units are invariably shifted to ~68–69 ppm upon glycosidation, the 62.9 ppm signal must be assigned to C-5 of xylose. Because the latter shift is upfield from the normal position of C-5 in xylose,[17] glycosidation at the xylose C-4 was indicated. The sugar methylene carbons absorb in the same relative order in DMSO as in pyr-d_5 at room temperature (see Table II), and so the same relative assignments are presumed to apply, i.e., 64.2 ppm in pyr-d_5 corresponds to the C-5 of xylose in 1. Comparison of the spectra of 1 and 4 in pyr-d_5 reveals that, in 4; (a) the sugar methine signal at 54.9 ppm is missing (corresponding to loss of *N*-acetyl-2-amino-2-deoxygalactose) and (b) the 64.2 ppm peak (xylose C-5) has been shifted to 66.5 ppm. The remaining signals for 4 are virtually unchanged from their positions in 1. The downfield shift of the xylose C-5 peak in 4 is indicative of the loss of a sugar at the xylose C-4 in 1 since this would constitute removal of a γ-shielding effect[18] from xylose C-5. Hence the *N*-acetyl-2-amino-2-deoxygalactose in 1 is attached at C-4 of xylose.

Exhaustive methylation of sarasinoside A_1 with MeI/NaOH/DMSO[19] gave a mixture of products which differed in the degree of N-methylation (^1H NMR analysis).[20] Methanolysis (anhydrous MeOH/HCl) of this total mixture at 60 °C overnight yielded a mixture of products, one of which was the monomethoxyxylose product **5**. In the ^1H NMR spectrum of **5** (see Experimental Section), the signals for all of the xylose protons were sufficiently resolved so that complete decoupling could be carried out. Comparison of the ^1H NMR chemical shifts for the xylose protons in **5** and the derived diacetate **6** (pyr, Ac_2O) confirmed that the methoxy group is located at the xylose C-3 position, and hence this sugar must be glycosidated at C-2 and C-4 in the intact saponin.

A second product from the above methanolysis of 1 was tentatively identified as the trisaccharide **7** on the basis of mass (*m/z* 684, $M^+ - 15$) and ^1H NMR analysis (three anomeric protons, one OAc).



From methanolysis of 1 (anhydrous MeOH/HCl) at a higher temperature, but for a shorter period of time (70 °C, 3 h), products identified by ^1H and some ^{13}C NMR data as **8–12** were isolated by HPLC. Products **10–12** confirmed that the terminal sugar in 1 was glucose, that neither glucose is glycosidated at C-6, and that the terminal glucose is linked to the next glucose at C-2.

Since the negative ion mass spectral data and chemical analysis both place the two glucose units as a chain-terminating pair, *N*-acetyl-2-amino-2-deoxyglucose must be situated between xylose and the glucose units as shown to give structure 1.

The ^1H and ^{13}C NMR assignments (Pyr-d_5, 60 °C) shown on structure 1 were confirmed by H/H and H/C COSY,[21,22] RCT[23] (Figure 2), RCT2,[23] and NOE (Figure 3) data. The xylose anomeric proton was identified by a NOE interaction with the aglycon 3α-H (see Figure 3). In the 1D difference NOE experiment, this interaction and all other NOEs were negative.[24] With the xylose anomeric proton as a reference point, one- and two-bond $^1H/^{13}C$ correlations[22,25] then identified the xylose C-1 and C-2 signals. The xylose C-4 signal was identified by its correlation (H/C relay) with proton absorptions at 3.53 and 4.44 ppm, which in turn were shown to be correlated (H/C COSY) with xylose C-5 (64.3 ppm).

NMR assignments for *N*-acetyl-2-amino-2-deoxygalactose and *N*-acetyl-2-amino-2-deoxyglucose were determined through H/H and H/C correlations which ul-

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(20) In the ^{13}C NMR spectrum of the product, all the anomeric carbon signals were doubled. The two signals characteristic of C-2 in *N*-acetyl-2-amino-2-deoxygalactose and *N*-acetyl-2-amino-2-deoxyglucose were seen, 54.9 and 59.13 ppm, but two additional methine signals at 63.90 and 66.18 ppm were also observed consistent with the C-2's of the amino sugars after methylation. This suggests that the two possible *N*-methyl-*N*-acetyl products were present as well as possibly some product with both nitrogens methylated.
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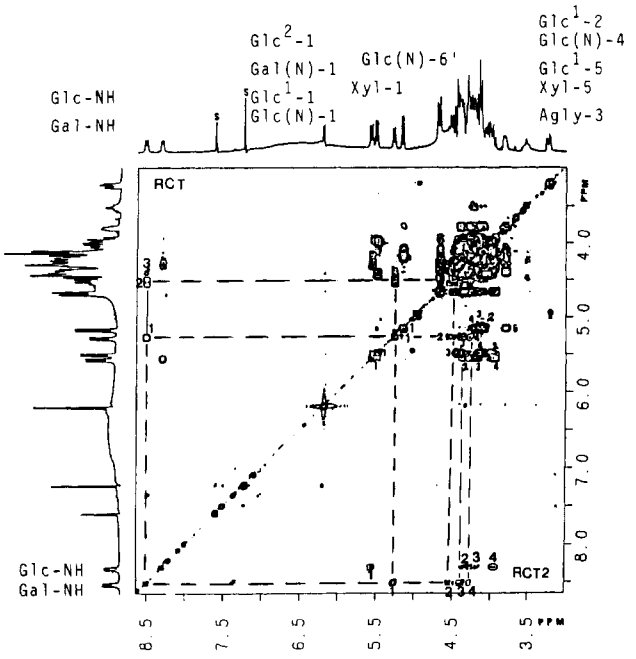

Figure 2. Combined ^1H RCT (2D relay coherence transfer spectroscopy)–RCT2 (2D double relay coherence transfer spectroscopy) plot (downfield region only) of 1 in pyr-d_5 at 60 °C. The RCT and RCT2 mixing time was incremented from 25 to 37 ms; 512 FIDS; 32 and 64 scans each were recorded for RCT and RCT2, 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 RCT and the lower-right triangle of RCT2.

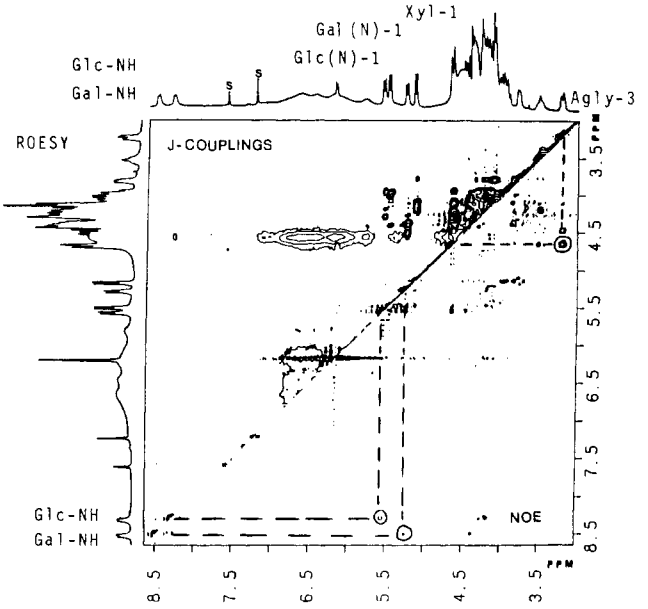

Figure 3. ROESY (2D rotating frame Overhauser enhancement spectroscopy) plot (downfield region only) of 1 in pyr-d_5 at 60 °C. The mixing time was 200 ms; 2 × 256 FIDS, 16 scans each, were recorded for the ROESY experiment. The individual positive and negative contours of ROESY were cut along the diagonal into two triangles, and the combined plot was assembled by using the upper-left triangle of positive contours (J couplings) of ROESY and the lower-right triangle of negative contours (nuclear Overhauser enhancements) of ROESY.

timately established connections to their characteristic C-2 signals (see above). For *N*-acetyl-2-amino-2-deoxygalactose, the connections were made as follows. In the DQF-COSY[26] spectrum, both the amide proton signal at 8.52 and the anomeric proton signal at 5.25 ppm correlated with the 4.57 broad triplet (H-2 of *N*-acetyl-2-amino-2-deoxygalactose), which in turn was found to correlate (H/C COSY) with the carbon signal at 54.9 ppm, the expected shift for C-2 of *N*-acetyl-2-amino-2-deoxygalactose.[17] In the RCT and ROESY[27] spectra, this same amide proton signal at 8.52 ppm correlated with the anomeric proton signal at 5.25 ppm, which in turn correlated with the carbon absorption at 102.1 ppm. The same strategy was used to identify H-1, H-2, C-1, and C-2 in *N*-acetyl-2-amino-2-deoxyglucose.

By comparison with literature values the 106.4 ppm[17] signal was assigned to the anomeric carbon of the terminal glucose unit. The remaining anomeric signal could then be assigned to the penultimate glucose unit. H/C correlations confirmed the assigned H-2, C-1, and C-2 resonances of these sugars.

So far as we are aware, sarasinoside A_1 is the first saponin to be reported from a sponge. It is also unique in that it contains two amino sugars. Sarasinoside A_1 is mildly cytotoxic, ED_{50} = 2.8 (PS).[28]

Experimental Section

Melting points are uncorrected and were taken on a Kofler hot-stage or Thomas-Hoover melting point apparatus. IR spectra were recorded on a Perkin-Elmer 298 spectrophotometer; UV spectra were taken on a Perkin-Elmer Lambda 3 UV/vis spectrophotometer; optical rotation was measured on a Perkin-Elmer Model 141 digital instrument. Mass spectra were obtained on Hewlett-Packard Model 5985A, CEC 110 (Du Pont, Monrovia, high resolution), and VG ZAB E (high resolution) spectrometers. The negative ion laser desorption Fourier transform spectrum was obtained on a Nicolet 2000 FT mass spectrometer and was kindly provided by Nicolet Instruments. Silica gel, 230–400 mesh (E. Merck), was used for column chromatography. NMR spectra were recorded at 300 MHz for ^1H and 75 MHz for ^{13}C on a Varian XL-300 spectrometer. ^1H NMR data are reported as δ (number of H's, multiplicity, J in hertz, assignment).

Isolation of Sarasinoside A_1 (1) from *Asteropus* sp. (a) Frozen sponge specimens (642 g), from Truk Lagoon in 1979, were soaked in ethanol for 1–2 days, and the liquid recovered by filtration was concentrated in vacuo. The residue was freeze-dried to give 141 g of 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. The second fraction (2 g) was triturated with $CHCl_3$, and the undissolved material (1.7 g) was subjected to HPLC on a C-18 column with MeOH/H_2O (75:25) as eluant to give ~500 mg of pure sarasinoside A_1.

(b) Sponges (~3.8 kg) collected at Guam Island in May 1979 and preserved at −20 °C were thawed, cut into small pieces, and soaked three times in $CHCl_3$/MeOH (1:1) at room temperature. The combined extracts, after filtration and concentration on a rotary evaporator, were diluted with water and partitioned successively against $CHCl_3$ and then 1-butanol. The butanol extracts were evaporated to dryness on a rotary evaporator (<50 °C, acetone/dry ice condenser) using a vacuum pump. This residue was dissolved in methanol, and a small amount of hexane was added to cause precipitation of a light tan solid, which was precipitated twice more by using MeOH/EtOAc. A portion (2.5 g) of this light tan solid was chromatographed over Sephadex LH-20 (450 g × 2 in. × $3^1/_4$ ft column; 50-mL fractions) by using

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methanol. Some (40 mg) of the solid obtained in fractions 3–5 was resolved by HPLC using a reversed-phase C-18 column (5 μm; 9.4 mm i.d. × 25 cm long) with a mobile phase of H_2O/MeOH (10:90) to give 15 mg of pure sarasinoside A_1. 1 precipitated as a white powder from $CHCl_3$/MeOH (4:1) at −10 °C: mp 208–212 °C; $[\alpha]_D$ −7.4 (c 0.3, MeOH); IR (KBr) 3380, 1640, 1550, 1375, and 1065 cm^{-1}; UV (MeOH) λ_{max} 237 nm (ε 10192); MS (FD, low resolution), m/z 719, 558, 426.

Anal. (after HPLC purification). Calcd for $C_{62}H_{100}O_{26}N_2 \cdot 2H_2O$: C, 56.19; H, 7.85; N, 2.12. Found: C, 56.31; H, 7.52; N, 2.44.

Hydrolysis of Sarasinoside A_1 (1) to 3β-Hydroxy-4,4-dimethylcholesta-8,24-dien-23-one (2) and 3β-Hydroxy-4,4-dimethylcholesta-8,24-dien-23-one 3-β-D-Xyloside (3). Sarasinoside A_1 (1) (90 mg) was heated under reflux in a mixture of 0.5 N HCl (20 mL) and toluene (20 mL) for 2 h. 1-Butanol was added to the cooled reaction mixture to facilitate separation of the emulsion. The 1-butanol/toluene layer was concentrated and the residue chromatographed over silica gel by using $CHCl_3$ and then $CHCl_3$ with increasing proportions of MeOH (up to 10% MeOH). The $CHCl_3$ eluates afforded 2, and the $CHCl_3$/MeOH washings yielded 3. Both 2 and 3 were further purified by reversed-phase (C-18) HPLC using 10% aqueous MeOH as eluant.

For 2: mp 153–154 °C (from 10% $CHCl_3$ in hexane); IR (KBr) 3610, 3480, 1680, 1618, 1445, 1384, 1235, 1025 cm^{-1}; UV (MeOH) λ_{max} 237 nm (ε 11200); ^1H NMR (see Table II); MS (70 eV; GC/MS, 260 °C isothermal, SE-30), m/z (relative intensity) 426 (M^+, 16), 408 (5), 393 (11), 343 (3), 365 (2), 329 (5), 328 (19), 327 (8), 326 (16), 313 (24), 295 (5), 259 (2), 241 (6), 175 (18), 173 (14), 161 (16), 159 (13), 147 (15), 145 (14), 135 (18), 133 (19), 125 (21), 121 (23), 98 (8), 83 (100); high-resolution MS [obsd mass (calcd formula, millimass error)] 426.346 36 ($C_{29}H_{46}O_2$, −3.41), 408.342 70 ($C_{29}H_{44}O$, 3.48), 393.315 74 ($C_{28}H_{41}O$, 4.61), 329.282 66 ($C_{23}H_{27}O$, −1.77), 327.267 32 ($C_{23}H_{35}O$, −1.46), 326.264 35 ($C_{23}H_{34}O$, 3.38), 313.252 93 ($C_{22}H_{33}O$, −0.20), 299.239 89 ($C_{21}H_{31}O$, 2.40), 273.221 84 ($C_{19}H_{29}O$, 0.54), 259.208 43 ($C_{18}H_{27}O$, 2.24), 1235.117 37 ($C_{10}H_{15}$, 1.26), 98.073 53 ($C_6H_{10}O$, 0.37), 83.049 69 (C_5H_7O, 1.51).

For 3: (powder) mp 227–228 °C; IR (KBr) 3440 (OH), 2950, 1690, 1625, 1450, 1385, 1370, 1045 cm^{-1}; UV (EtOH) λ_{max} 237 nm (ε 9750); ^1H NMR (300 MHz, $CDCl_3$) (for aglycon absorptions, see Table III) xylose absorptions 3.40 (1 H, dd, 7, 13, H-5), 3.54 (1 H, dd, 6, 7, H-2), 3.64 (1 H, t, 7, H-3), 3.76 (1 H, m, H-4), 4.12 (1 H, dd, 4, 13, H-5′), 4.52 (1 H, d, 6.5, H-1); MS (70 eV, GC/MS), m/z (relative intensity) 558 (M^+, 3), 540 (1), 426 (1), 409 (4), 393 (5), 327 (3), 311 (6), 175 (13), 147 (10), 83 (100).

Identification of the Sugar Units in Sarasinoside A_1. Sarasinoside A_1 (20 mg) was treated with 4% HCl in MeOH (4 mL) at 70 °C for 36 h. The reaction mixture was neutralized with Ag_2CO_3 and the precipitated AgCl removed by filtration through a Celite bed on a sintered-glass funnel. Concentration of the filtrate at reduced pressure provided a pale brown gum. This was dissolved in 10% aqueous MeOH (1 mL), and the solution was passed through a C_{18} Sep-Pak cartridge with 10% aqueous MeOH whereupon the polar sugar methyl glycosides (10 mg) were separated from the less polar aglycon material (8 mg).

According to the procedure of Golic et al.,[12] the methyl glycoside mixture was treated with a catalytic amount of 4-(dimethylamino)pyridine (100 μL) and p-bromobenzoyl chloride (150 mg) in dry, freshly distilled pyridine (1.5 mL), and the resulting solution was maintained at 60–65 °C for 48 h. Evaporation of the solvents in vacuo on a water bath at 40 °C afforded a white solid. The white solid was dissolved in $CHCl_3$ (1 mL) and chromatographed in that solvent on a column of SiO_2 gel (5 g, 240–300 mesh) to give four fractions. ^1H NMR analysis revealed that fractions 1 and 2 were identical. HPLC of fraction 2 [5-μm SiO_2, ether/hexane (1:8)] yielded (a) methyl 2,3,4-tri-O-(p-bromobenzoyl)-β-D-xylopyranoside, (b) methyl 2,3,4,6-tetra-O-(p-bromobenzoyl)-α-D-glucopyranoside, and their anomeric counterparts. Fractions 3 and 4, identical by ^1H NMR analysis, were combined and chromatographed by HPLC [5-μm SiO_2, $CHCl_3$/EtOAc (25:4)] to give a benzoylated sugar fraction and p-bromobenzoic acid. HPLC [5-μm SiO_2, hexane/$CHCl_3$ (1:1)] of the benzoylated sugar fraction yielded, in order of elution, (c) methyl 2,3,4,6-tetra-O-(p-bromobenzoyl)-β-D-glucopyranoside, (d) methyl 2-(p-bromobenzamido)-3,4,6-tri-O-(p-bromobenzoyl)-2-deoxy-α-D-galactopyranoside, (e) methyl 2-(p-bromobenzamido)-3,4,6-tri-O-(p-bromobenzoyl)-2-deoxy-α-D-glucopyranoside, and a mixture of (f) methyl 2-(p-bromobenzamido)-3,4,6-tri-O-(p-bromobenzoyl)-2-deoxy-β-D-galactopyranoside and (g) methyl 2,3,4,6-tetra-O-(p-bromobenzoyl)-β-D-glucopyranoside. The ^1H NMR spectra of the sugar derivatives a, b, d, and e were identical with those reported.[10]

Hydrolysis of Sarasinoside A_1 with β-Glucosidase. A solution of sarasinoside A_1 (16 mg) and β-glucosidase (50 mg, 6 units/mg solid; Sigma Chemical Co., type II from almonds) in AcOH/AcONa buffer solution (pH 5; 2 mL) was incubated at 37 °C for 12 h. MeOH (3 mL) was added to the reaction mixture, the protein which precipitated was separated by centrifugation, and the supernatant was concentrated in vacuo. The residue was chromatographed by HPLC [C-18, H_2O/MeOH (18:82)] to give 4 (5 mg) and unreacted 1 (6 mg). This procedure was repeated several times to obtain more of 4.

Permethylation of Sarasinoside A_1. Powdered NaOH (250 mg) and methyl iodide (6 mL)[19] were added to a solution of sarasinoside A_1 (80 mg) in DMSO (15 mL). The reaction mixture was stirred for 2 h in a closed flask at room temperature, then diluted with H_2O (275 mL), and extracted with $CHCl_3$ (3 × 175 mL). The $CHCl_3$ solution was evaporated to obtain an oily residue (90 mg), which was purified by preparative TLC (elution with 3.5% MeOH/$CHCl_3$) to give 75 mg of permethylated sarasinoside A_1 (2 spots on analytical TLC).[20] This was used without further purification for the following reaction.

Methanolysis of Permethylated Sarasinoside A_1. A solution of permethylated sarasinoside A_1 (60 mg) in anhydrous HCl/MeOH (12 mL) was stirred at 70 °C for 3 h. After cooling, the MeOH was evaporated in vacuo at 30 °C, the oily residue was diluted with 20 mL of water, and the resulting mixture was neutralized with 5% NaOH. This aqueous solution was extracted with $CHCl_3$ (3 × 50 mL) to yield 45 mg of oily residue, which was subjected to silica gel HPLC using 1% MeOH/$CHCl_3$ to give 8–12 and a mixture in which 2 was identified by characteristic proton NMR data. When the methanolysis was performed at 60 °C for 12 h, only 5 and 7 were isolated.

5: ^1H NMR ($CDCl_3$) protons on xylose δ 4.29 (1 H, d, 7.4, H-1), 3.98 (1 H, dd, 5.3, 11.4, H-5), 3.68 (1 H, m, H-4), 3.66 (3 H, s, OMe), 3.50 (1 H, dd, 7.4, 7.4, H-2), 3.26 (1 H, dd, 11.4, 11.4, H-5), 3.16 (1 H, t, 7.4, H-3), protons on aglycon δ 6.1 (1 H, br s, H-24), 3.16 (1 H, dd, 4.12, H-3), 2.14 (3 H, s, H-26), 1.87 (3 H, s, H-27), 1.0 (3 H, s, H-19), 0.98 (3 H, s, H-28), 0.92 (3 H, d, 7, H-21), 0.86 (3 H, s, H-29), 0.63 (3 H, s, H-18). Assignments of xylose protons were established by ^1H decoupling and difference decoupling experiments.

6: ^1H NMR ($CDCl_3$) protons on xylose δ 4.92 (1 H, dd, 8.0, 7.5, H-2), 4.90 (1 H, dt, 3.5, 8, H-4), 4.46 (1 H, d, 8, H-1), 4.10 (1 H, dd, 3.5, 11.5, H-5), 3.44 (3 H, s, OMe), 3.44 (1 H, t, 7.5, H-3), 3.23 (1 H, dd, 8, 11.5, H-5′), 2.09 (3 H, s, OAc), 2.08 (3 H, s, OAc); protons on aglycon δ 6.1 (1 H, br s, H-24), 3.04 (1 H, dd, 4, 12, H-3), 2.14 (3 H, s, H-26), 1.87 (3 H, s, H-27), 0.97 (3 H, s, H-19), 0.92 (3 H, s, H-28), 0.92 (3 H, d, 7, H-21), 0.88 (3 H, s, H-29), 0.63 (3 H, s, H-18). Proton assignments were made by analogy to 5.

7: ^1H NMR ($CDCl_3$) δ 4.66 (1 H, d, 9), 4.42 (1 H, d, 8.5), 4.29 (1 H, d, 8.5), 4.10 (1 H, m), 3.76 (1 H, dd, 3, 10), 3.39→3.63 (10 s due to 10 OMe), 2.86 (3 H, s, N-Me), 2.14 (3 H, s, Ac).

8: ^1H NMR ($CDCl_3$) δ 4.18 (1 H, d, 7.5, H-1), 4.01 (1 H, dd, 5, 12, H-5), 3.68 (1 H, m, H-4), 3.65 (3 H, s, OMe on C-3), 3.53 (3 H, s, OMe on C-1), 3.48 (1 H, t, 7.5, H-2), 3.29 (1 H, dd, 12, 12, H-5′), 3.16 (1 H, t, 7.5, H-3). Assignments were made by analogy to 5.

9: ^1H NMR ($CDCl_3$) δ 5.50 (1 H, br d, 8.5, N-H), 4.79 (1 H, d, 3.5, H-1), 4.50 (1 H, ddd, 3.5, 8.4, 8.4, H-2), 3.81 (1 H, br t, 6, H-5), 3.73 (1 H, br d, 3.5, H-4), 3.57 (3 H, s, OMe), 3.55 (2 H, AB quartet, H-6), 3.43 (3 H, s, OMe), 3.41 (3 H, s, OMe), 3.40 (1 H, dd, 8.4, 3.5, H-3), 3.36 (3 H, s, OMe), 2.0 (3 H, s, N-Ac). ^1H assignments were established by ^1H decoupling and difference decoupling experiments.

10: ^1H NMR ($CDCl_3$) δ 4.82 (1 H, d, 3.5, H-1), 3.63, 3.52, 3.50, 3.42, and 3.41 all singlets, OMe's.

11: ^1H NMR ($CDCl_3$) δ 4.76 (1 H, d, 3.8, H-1), 3.66 (3 H, s, OMe), 3.59 (2 H, m, H-6), 3.58 (1 H, ddd, 9, 10, 3.8, H-2), 3.53 (3 H, s, OMe), 3.42 (6 H, s, 2 OMe), 3.36 (1 H, t, 9, H-3), 3.20 (1 H, tt, 9, 2, H-4), 2.16 (1 H, d, 10, OH at C-2); ^1H assignments were established by ^1H decoupling and difference decoupling experiments; ^{13}C NMR ($CDCl_3$) δ 99.3 (d), 84.5 (d), 79.3 (d), 72.4 (d),

70.2 (d), 70.9 (t), 60.9 (q), 60.3 (q), 59.2 (q), 55.3 (q).

12: ^{1}H NMR ($CDCl_3$) δ 4.89 (1 H, d, H-1), 4.30 (1 H, d, 8.5, H-1′), 3.62 (6 H, s, 2 OMe), 3.53 (1 H, dd, 3, 9, H-2), 3.54 (3 H, s, OMe), 3.51 (3 H, s, OMe), 3.41 (3 H, s, OMe), 3.38 (3 H, s, OMe), 3.34 (3 H, s, OMe), 3.12 (1 H, dd, 8.5, 8.5, H-2′); ^{13}C NMR ($CDCl_3$) δ 104.8 (d), 99.6 (d), 86.7 (d), 83.3 (d), 82.5 (d), 80.5 (d), 79.9 (d), 79.4 (d), 74.4 (d), 71.5 (t), 71.0 (t), 69.6 (d), 60.8 (q), 69.6 (q), 60.5 (q), 60.4 (q), 60.3 (q), 59.2 (q), 59.1 (q), 55.1 (q).

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Synthesis of 10β-Oxiranyl and 10β-Thiiranyl Steroids†

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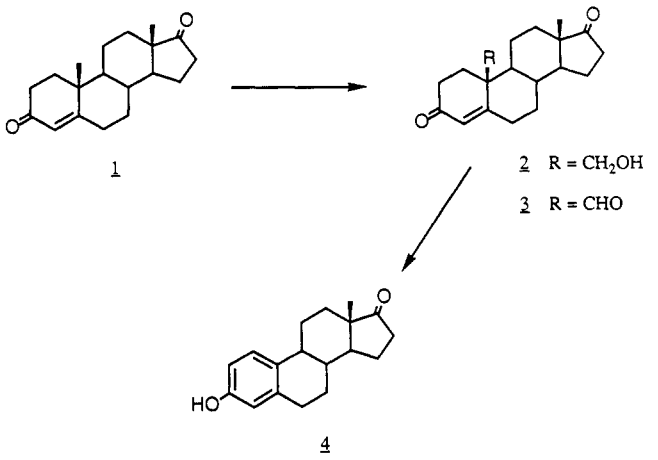
The 10β-oxiranylestr-4-ene-3,17-diones (**7a** and **7b**) have been prepared from 19-oxo intermediate **5** by ylide reactions. Compounds **7a** and **7b** were converted to the thiiranes **8b** and **8a**, respectively, with stereochemical inversion at C-19 by a modified triphenylphosphine sulfide (TPS) reaction using TPS–picric acid. Reaction of **7a** and **7b** with TPS–trifluoroacetic acid gave, respectively, (19*R*)- and (19*S*)-5,19-(thiomethylene)-19-(trifluoroacetoxy)-5β-androstane-3,17-dione (**9a** and **9b**) through internal trapping of a reaction intermediate. Hydrolysis of **9a** and **9b** gave, respectively, the corresponding 19-hydroxy heterocycles, **10a** and **10b**, which could also be obtained by reaction of oxiranes **7a** and **7b** with TPS–methanesulfonic acid. The reactions involving formation or hydrolysis of **9a**, **9b**, **10a**, and **10b** proceeded stereospecifically.

Human placental aromatase[1] is a cytochrome P-450 enzyme complex that is responsible for the conversion of androgens (**1**) to estrogens (**4**) (Scheme I). This biologically important process is of great mechanistic interest. Furthermore, inhibitors of this enzyme may be useful in the treatment of breast cancer.[2] As part of continuing studies on the mechanism and inhibition of this enzyme, we describe below the synthesis of the 10β-oxiranyl and 10β-thiiranylestr-4-ene-3,17-diones **7a**, **7b** and **8a**, **8b**, respectively. The oxiranes **7a**, **7b** were synthesized as analogues of intermediate **2** in the enzymatic reaction sequence, and their powerful inhibitory effects[3] on aromatase induced us to prepare the corresponding thiiranes **8a**, **8b**, which proved[4] to be even more potent inhibitors.

The two most obvious approaches to the desired epoxides **7a** and **7b** would involve either peracid epoxidation of a 10β-vinyl steroid or reaction of a 10β-formyl steroid with the sulfur-based ylides described[5] by Corey and Chaykovsky. Attempts to epoxidize selectively the 10β-vinyl group in the presence of a 5,6-olefin or a 4-ene-3-one grouping proved unsuccessful. Therefore we studied the reaction of the 10β-formyl steroid[6] (**5**) with dimethylsulfonium and dimethylsulfoxonium methylide (Scheme II). The 3- and 17-hydroxyl groupings were protected as tetrahydropyranyl ethers (THP) in order to avoid destruction of the basic ylides or methylation of the hydroxyl groups. Treatment of **5** with dimethylsulfonium methylide and dimethylsulfoxonium methylide in dimethyl sulfoxide/tetrahydrofuran gave comparable overall yields of total epoxidized products. The ratios of C-19 diastereomeric epoxides **6a** and **6b** resulting from each reagent were determined by high-pressure liquid chromatography (HPLC) analysis after removal of the THP protecting groups with pyridinium *p*-toluenesulfonate–methanol. Reaction with dimethylsulfonium methylide gave a **6a**:**6b** ratio of 9:1 while the corresponding ratio for dimethyl-

Scheme I



† Dedicated to Sir Derek Barton on the occasion of his 70th birthday.

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