Starfish Saponins. Part 14.[1] Structures of the Steroidal Glycoside Sulphates from the Starfish *Marthasterias glacialis* †

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The major sulphated asterosaponins, marthasterosides A_1, A_2, B, and C, isolated from the starfish *Marthasterias glacialis*, have been completely characterized. Marthasterosides A_1 and A_2 are hexaglycosides and contain the same aglycone, thornasterol A. They have been established as 6α-*O*-{β-D-fucopyranosyl-(1 ⟶ 3)-β-D-fucopyranosyl-(1 ⟶ 2)-β-D-galactopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-quinovopyranosyl}-20-hydroxy-23-oxo-5α-cholest-9(11)-en-3β-yl sodium sulphate (**6**) and 6α-*O*-{β-D-fucopyranosyl-(1 ⟶ 3)-β-D-fucopyranosyl-(1 ⟶ 2)-β-D-quinovopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-quinovopyranosyl}-20-hydroxy-23-oxo-5α-cholest-9(11)-en-3β-yl sodium sulphate (**7**) respectively. Marthasterosides B and C are the pentaglycosides (**8**) and (**9**); they contain the same oligosaccharide moiety, *i.e.* β-D-fucopyranosyl-(1 ⟶ 2)-β-D-fucopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-quinovopyranosyl-(1 ⟶ 3)-β-D-glucopyranosyl, which is attached to C-6 of 3β-sulphomarthasterone, *i.e.* 3β,6α-dihydroxy-5α-cholesta-9(11)24-dien-23-one, and of 3β-sulpho-24,25-dihydromarthasterone, respectively.

The saponins are responsible for the general toxicity of sea cucumbers and starfishes.[2] The saponins from starfishes were shown to belong to three structural types:[3] (a) the steroidal cyclic glycosides recently discovered in starfish of the genus *Echinaster*;[4–6] they are devoid of the sulphate group and their structures include a Δ^7-3β,6β-dioxygenated steroidal nucleus and a cyclic trisaccharide moiety which bridges the C-3 and C-6 atoms of the steroid; (b) the non-sulphated 24-*O*-glycosidal steroids, first discovered in two starfishes, *Protoreaster nodosus*[7] and *Hacelia attenuata*;[8,9] and (*c*) the asterosaponins which are sulphated steroidal glycosides.[10,11] These latter are widely distributed and have been described fron various species. Their general structure includes a $\Delta^{9,11}$-3β,6α-dioxygenated-23-oxosteroidal moiety; the oligosaccharide portion is attached at C-6 and the sulphate group is at C-3. Only a few complete structures of asterosaponins have been described (Chart 1). Kitagawa and Kobayashi[12] in 1978 determined the complete structure of the major *Acanthaster planci* asterosaponin, thornasteroside A (**1**), mainly based on the accurate analysis of the partial oligoglycosides obtained by mild acid hydrolysis. Ikegami *et al.*[13] described the structure of glycoside B_2 (**2**), an asterosaponin from the ovaries of *Asterias amurensis*; it is almost identical with thornasteroside A, except that the terminal fucose (6-deoxy-D-galactose) is replaced by quinovose (6-deoxy-D-glucose). Very recently a Japanese group from Kyushu published a series of papers in which they described the acanthaglycoside A (**5**), a minor component of *Acanthaster planci*,[14] and two more asterosaponins (**3**) and (**4**) from the starfish *Astropecten latespinosus*.[15] The latter structures were mainly based on extensive ^{13}C n.m.r. studies. We would note that the saccharide portions of all five saponins in Chart 1 contain an invariant structural feature, *i.e.* the (1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-quinovopyranosyl moiety.

In another paper[16] we have reported the isolation of the four principal asterosaponins, marthasterosides A_1, A_2, B, and C, from the starfish *Marthasterias glacialis* and have clarified the sugar composition and the structure of the aglycones. The structures of the genuine aglycones have been established by ^1H and ^{13}C n.m.r. spectra of the intact saponins, which also allowed determination of the location of the sulphate group at C-3 of the steroid. Marthasterosides A_1 and A_2 are hexaglycosides of the aglycone thornasterol A, *i.e.* 3β,6α,20ξ-trihydroxy-5α-cholest-9(11)-en-23-one;[17] marthasterosides B and C are pentaglycosides of the aglycones marthasterone, *i.e.* 3β,6α-dihydroxy-5α-cholesta-9(11),24-dien-23-one,[18] and 24,25-dihydromarthasterone,[18] respectively.

The carbohydrate constituents of the four saponins were established[16] as follows: marthasteroside A_1, $[\alpha]_D = +3.6°$: D-fucose‡ (× 2), D-quinovose (× 2), D-galactose, and D-xylose; marthasteroside A_2, $[\alpha]_D = +6.5°$: D-fucose (× 2), D-quinovose (× 3), and D-xylose; marthasteroside B, $[\alpha]_D = +9.0°$, and C, $[\alpha]_D = +13.3°$: D-fucose (× 2), D-quinovose (× 2), and D-glucose. In all compounds the glycosidic linkages are β-orientated (^{13}C n.m.r.) and the oligosaccharide chain is linked to C-6 of the steroid. This paper deals with a study of the carbohydrate moieties of marthasterosides A_1, A_2, B, and C, which led to the complete structures (**6**), (**7**), (**8**), and (**9**), respectively.

Determination of the Interglycosidic Linkages.—Each saponin was permethylated by treating a solution in DMF (dimethyl-

† This contribution is part of the Progetto Finalizzato 'Chimica Fine Secondaria' del C.N.R., Roma. Presented in part at the Fifth Conference of the European Society for Comparative Physiology and Biochemistry, Taormina, Italy, September 5—8, 1983.

‡ Since L-fucose is known to be a constituent of brown seaweed, D-fucose obtained here was determined by the exciton-split c.d. curve of methyl 2,3,4-tri-*O*-(*p*-bromobenzoyl)-α-D-fucopyranoside [c.d.: 236/254, Δε − 26.8/+98.7, A +125.5; calc. A: +140 (ref. 19)].

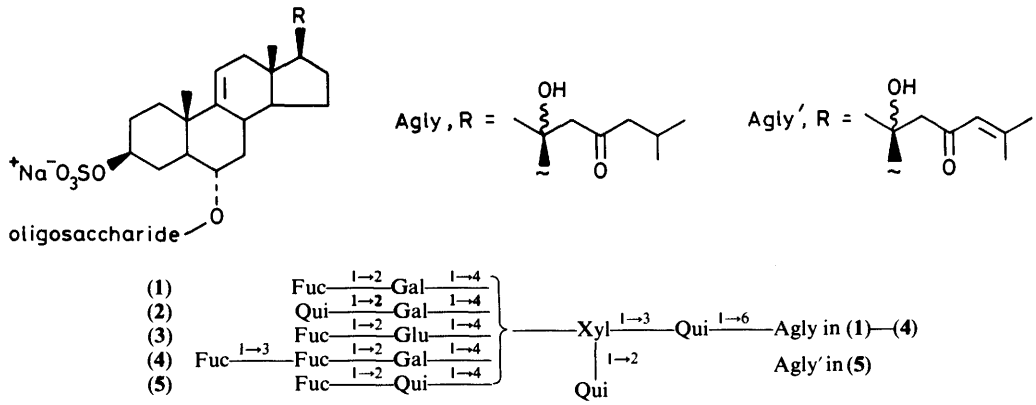


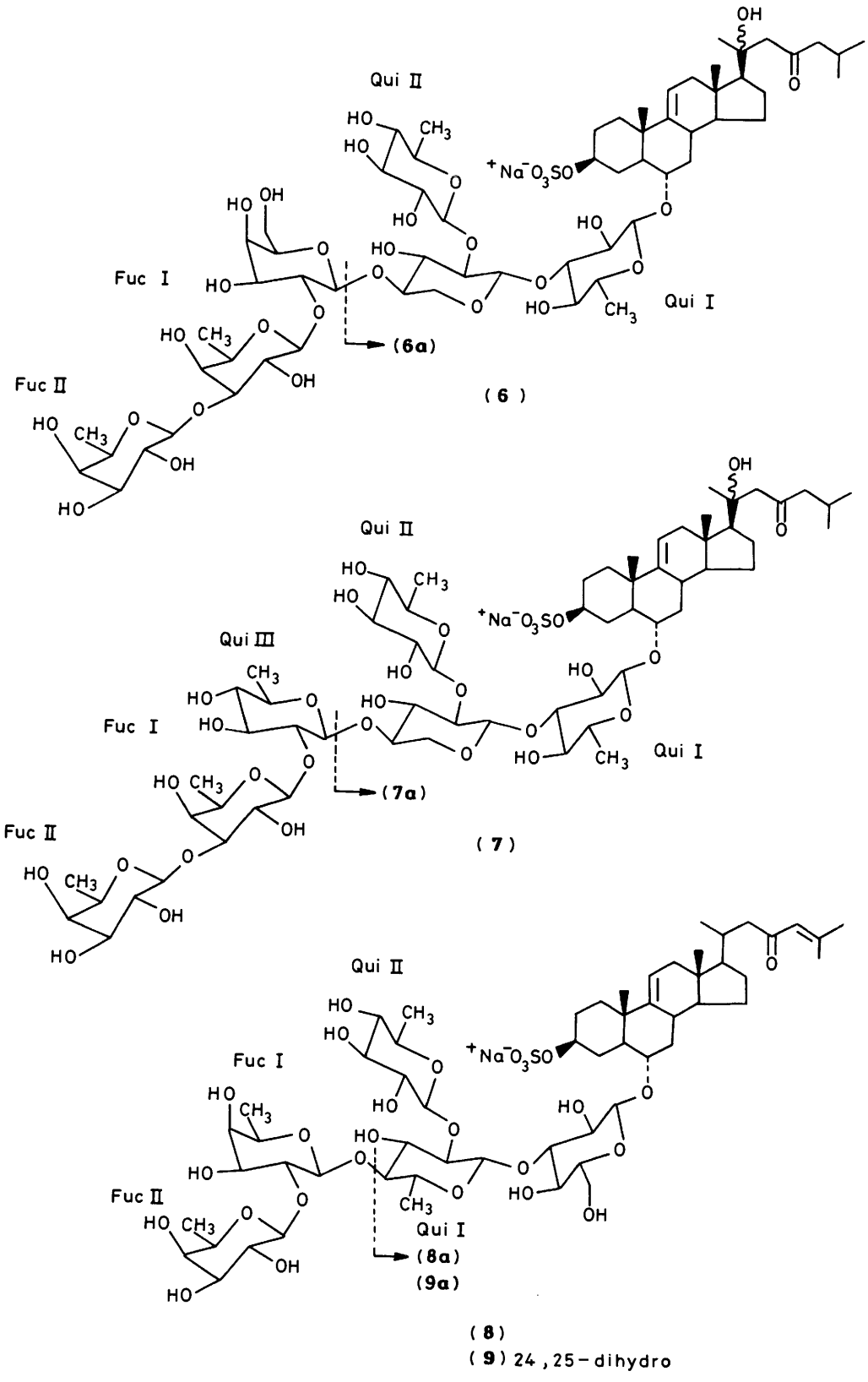
Chart 1. Reported structure of asterosaponins; (**1**) and (**5**) from *Acanthaster planci*;[12,14] (**2**) from *Asterias amurensis*;[13] and (**3**) and (**4**) from *Astropecten latespinosus*.[15] Qui = quinovose (6-deoxy-D-glucose); Xyl = xylose; Fuc = fucose (6-deoxy-D-galactose); Gal = galactose; Glu = glucose

Table 1. ^{13}C N.m.r. shifts (δ_C/p.p.m.) of sugar carbon atoms in (**6**), (**6a**), (**6b**), (**7**), and (**7b**) for solutions in [$^2H_5$]pyridine. The peaks due to the aglycones have been reported in a previous paper (ref. 16).[a] Pertinent shifts discussed in the text are shown in italics

Carbon		(6)	(6a)	(6b)[b]	(7)	(7b)[b]	Methyl-β-D-glycopyranoside	
Qui I	1	105.0	105.2	104.6	105.1	104.6	105.3	
	2	74.4	74.5	74.3	74.4	74.4	76.6	
	3	90.5	91.0	90.4	90.5	90.4	78.0	Qui
	4	74.5	74.5	74.4	74.6	74.5	77.2	
	5	72.6	72.2	72.7	72.6	72.7	73.8	
	6	18.0	18.0	18.1	18.0	18.2	18.5	
Xyl	1	104.4	104.8	104.3	104.5	104.8	105.2	
	2	*82.4*	83.4	*84.8*	*82.3*	*85.0*	74.2	
	3	75.5	77.7	75.3	75.4	75.5	77.3	Xyl
	4	79.2	*70.7*	79.0	78.7	78.6	70.8	
	5	64.3	*67.2*	64.7	64.4	64.7	66.4	
Qui II	1	*105.3*	105.2	*107.4*	*105.1*	*107.4*		
	2	75.7	75.8	76.3	75.7	76.4		
	3	77.0	77.3	77.6	77.1	77.6		
	4	76.3	76.5	77.0	76.3	76.9		
	5	73.8	74.0	73.0	73.8	73.1		
	6	18.5	18.6	18.6	18.2	18.6		
Gal in (**6**) and (**6b**); Qui III in (**7**) and (**7b**)	1	102.4		102.3	101.4	101.6	106.1	
	2	82.8		83.2	84.3	84.9	72.4	
	3	74.7		74.4	76.1	76.2	75.3	Gal
	4	69.8		69.6	77.6	77.8	70.2	
	5	76.7		76.9	73.2	73.3	76.9	
	6	62.3		62.3	18.5	18.6	62.4	
Fuc I	1	105.9		106.4	106.0	106.5	105.6	
	2	71.8		71.8	71.7	71.8	72.1	
	3	84.4		84.6	84.3	84.6	75.2	Fuc
	4	71.9		71.9	71.8	72.0	72.6	
	5	71.9		72.0	71.9	72.0	71.4	
	6	17.1		17.2	17.1	17.2	16.8	
Fuc II	1	106.1		106.6	106.2	106.5		
	2	72.2		71.9	72.2	72.1		
	3	75.0		75.0	75.1	75.3		
	4	72.8		72.7	72.9	72.7		
	5	72.3		72.3	72.4	72.3		
	6	17.1		17.1	17.0	17.1		

[a] Signals for C-3, C-6, and C-20 of the aglycones have been now assigned; (**6**), C-3; 78.3, C-6: 80.5, C-20: 74.1; (**7**), C-3: 78.1, C-6: 80.5, C-20: 74.1; (**6a**), C-3: 77.8, C-6: 80.7, C-20: 74.2 p.p.m. [b] (**6b**) Is the desulphated marthasteroside A_1 and (**7b**) is the desulphated marthasteroside A_2; the shifts of the aglycone carbons are within 0.1 p.p.m. of those of the full saponins[16] except for C-2, C-3, and C-4 which in (**6b**) and (**7b**) resonate at δ_C 32.5 (*vs.* 29.3), 70.9 (*vs.* 78.3, 78.1), and 33.7—33.8 (*vs.* 30.5, 30.6) p.p.m. respectively; shifts were also observed for C-6 (79.4—79.5 *vs.* 80.5 p.p.m.).

formamide) and NaH with methyl iodide. The methylated saponin was purified by chromatography of silica gel and then was methanolysed by being refluxed with 2M HCl–methanol. After neutralization with Ag_2CO_3, centrifugation, and evaporation of solvent, the residue was in part used for g.l.c. analysis (identification of permethylated sugars), and in part



was per-*p*-bromobenzoylated, and the reaction mixture was separated by h.p.l.c. The major u.v.-absorbing h.p.l.c. peaks were then subjected to ^{1}H n.m.r. spectroscopy and the mono- and di-benzoates identified. All four saponins gave permethylated methyl fucoside and permethylated methyl quinovoside and this indicated that the saccharide chains of all marthasterosides contain one branching point (two terminal sugars). Chart 2 shows the methyl *O*-methyl-*O*-(*p*-bromobenzoyl)glycosides obtained from marthasteroside A_1 (**6**). The α (axial)-anomers are the principal products; in some cases β

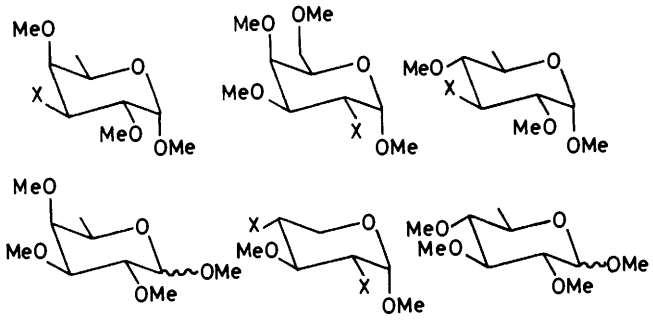


Chart 2. Products obtained by methylation, methanolysis, and *p*-bromobenzoylation of marthasteroside A_1 (**6**). X = *p*-bromobenzoyloxy

(equatorial)-anomers have been isolated in smaller amounts and characterized. Marthasteroside A_2 (**7**) gave the same products given by marthasteroside A_1 (**6**), except that methyl 3,4,6-tri-*O*-methyl-2-*O*-(*p*-bromobenzoyl)-α-D-galactopyranoside was replaced by methyl 3,4-di-O-methyl-2-*O*-(*p*-bromobenzoyl)-α-quinovopyranoside. Marthasterosides B (**8**) and C (**9**) gave the same products and these are shown in Chart 3.

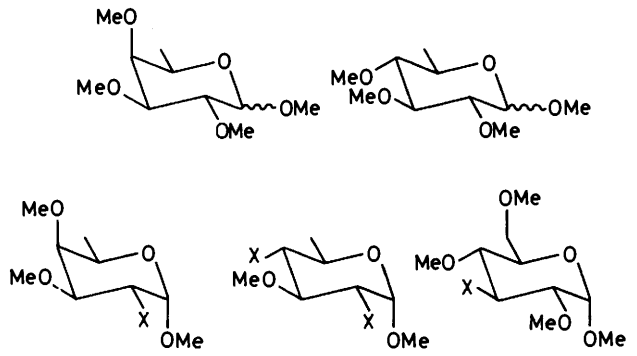


Chart 3. Products obtained by methylation, methanolysis, and *p*-bromobenzoylation of marthasteroside B and C. X = *p*-bromobenzoyloxy

Enzymatic Hydrolysis with Glycosidase Mixture of Charonia lampas.—Each saponin (*ca.* 20 mg) in citrate buffer at pH 4.5 was incubated with *ca.* 10 mg of the glycosidase mixture of *Charonia lampas* at 40 °C for 1—3 d. The reaction mixture was then extracted with n-butanol and the partial glycosides separated by t.l.c. and/or h.p.l.c. Marthasteroside A_1 (**6**) and A_2 (**7**) gave the same major prosapogenol sulphate (**6a**) = (**7a**). In some experiments it was the only detected product. The fast atom bombardment (FAB)-mass spectrum of (**6a**) showed a sodium-containing molecular ion species at *m/z* 981, which indicated a molecular weight of 958 corresponding to a triglycoside (containing xylose and two 6-deoxyhexose units) of thornasteryl A sodium sulphate. Acid methanolysis provided methyl xyloside and methyl quinovoside in the ratio 1:2. In addition to the usual aglycone signals, the ^{1}H n.m.r. spectrum showed three anomeric proton doublets at δ 4.56 (*J* 7.5 Hz), 4.55 (*J* 7.5 Hz), and 4.42 (*J* 7.5 Hz), two 3-H doublets at δ 1.30 (*J* 6 Hz) and 1.39 (*J* 6 Hz) for the methyl protons of the quinovosyl units and, more significantly, a double doublet (*J* 12.0 and 4.0 Hz) at δ 3.92 ascribable to 5-H_{eq} of the xylosyl unit. Its high-field position relative to the same signal observed in the spectrum of the full saponin (δ 4.16, *J* 11.0 and 4.0 Hz) suggested the removal of the substituent at C-4 on passing from the hexaglycosides (**6**) and (**7**) to the triglycoside. We have noted that in terminal and in 2-*O*-substituted xylopyranosyl units 5-H_{eq} resonates at δ *ca.* 3.90.[7,20] This was confirmed by the ^{13}C n.m.r. spectrum (Table 1) of the triglycoside (**6a**), in which the signals for C-4 and C-5 of the xylosyl unit are observed at δ_C 70.7 and 67.2 p.p.m. respectively, as expected for a 4-*O*-unsubstituted xylose.[21] Thus the structure of the major prosapogenol obtained from both marthasteroside A_1 and A_2 is (**6a**) [= (**7a**)]. On enzymic hydrolysis marthasteroside A_1 (**6**) also gave smaller amounts of a tetraglycoside, which on acid methanolysis gave methyl quinovoside, methyl xyloside, and methyl galactoside in the proportions 2:1:1. Consequently the saccharide chain of marthasteroside A_1 is defined as β-D-fucopyranosyl-(1 ⟶ 3)-β-D-fucopyranosyl-(1 ⟶ 2)-β-D-galactopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-quinopyranosyl and the complete structure of the saponin is (**6**), which is identical with that assigned to the oligoglycoside (**4**) from *Astropecten latespinosus* by Itakura *et al.*,[15] mainly based on ^{13}C n.m.r. data and comparison with a previous pregnane pentaglycoside having the same saccharide chain as in (**1**).[22]

On enzymic hydrolysis marthasteroside A_2 (**7**) also gave in trace amounts a shortened oligoglycoside, which on acid methanolysis yielded methyl quinovoside and methyl xyloside in the ratio 3:1. Intense peaks at *m/z* 477 and 461 observed in the FAB-mass spectrum (Figure 2) of marthasteroside A_2 suggest the presence of a linear sequence of three 6-deoxyhexoses. In the spectrum (Figure 1) of marthasteroside A_1 the corresponding fragments, which include a galactosyl residue instead of a quinovosyl residue, occur at *m/z* 493 and 477. These observations and all the above data established the saccharide chain of marthasteroside A_2 as β-D-fucopyranosyl-(1 ⟶ 3)-β-D-fucopyranosyl-(1 ⟶ 2)-β-D-quinovopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-xylopyranosyl-(1 ⟶ 3)-β-D-quinovopyranosyl and the structure of the saponin as (**7**). The alternative structure with the terminal quinovose linked at C-4 of the branched sugar, and the linear sequence Fuc–Fuc–Qui linked at C-2, could be eliminated on comparison of the ^{13}C n.m.r. spectrum of (**7**) with that of its desulphated derivative in which the resonances of C-1 of the terminal quinovosyl unit (107.4 p.p.m.) and of C-2 (85.0 p.p.m.) of the xylosyl unit are shifted downfield relative to (**7**) (105.1 p.p.m. and 82.3 p.p.m.) (see below and Table 1).

On enzymic hydrolysis marthasteroside B (**8**) gave one major prosapogenol sulphate (**8a**); FAB-MS: *m/z* 993 $(M + Na)^+$. Acid methanolysis provided methyl quinovoside and methyl glucoside in the ratio 2:1. The ^{1}H n.m.r. spectrum showed, in addition to the usual signals of the 3β-sulphomarthasterone, three anomeric proton doublets at δ 4.56 (*J* 7.5 Hz), 4.54 (*J* 7.5 Hz), and 4.46 (*J* 7.5 Hz) and two 3-H doublets at δ 1.40 (*J* 6 Hz) and 1.32 (*J* 6 Hz) for the methyl protons of the quinovosyl units. Exhaustive methylation, methanolysis, and per-*p*-bromobenzoylation gave two major u.v.-absorbing products, which were identified as methyl 3,4-di-*O*-methyl-2-*O*-(*p*-bromobenzoyl)-α-D-quinovopyranoside and the previous methyl 2,4,6-tri-*O*-methyl-3-*O*-(*p*-bromobenzoyl)-α-D-glucopyranoside. Thus the saccharide chain of the prosapogenol sulphate (**8a**) is β-D-quinovopyranosyl-(1 ⟶ 2)-β-D-quinopyranosyl-(1 ⟶ 3)-β-D-glucopyranosyl and consequently that of marthasteroside B (**8**) is β-D-fucopyranosyl-(1 ⟶ 2)-β-D-fucopyranosyl-(1 ⟶ 4)-[β-D-quinovopyranosyl-(1 ⟶ 2)]-β-D-quinovopyranosyl-(1 ⟶ 3)-β-D-glucopyranosyl. Marthasteroside C (**9**) contains the same oligosaccharide chain. On enzymic hydrolysis marthasteroside C gave one major prosapogenol sulphate (**9a**), FAB-MS: *m/z* 995 $(M + Na)^+$; ^{1}H n.m.r.: in the sugar region the spectrum was virtually superimposable on that of (**8a**).

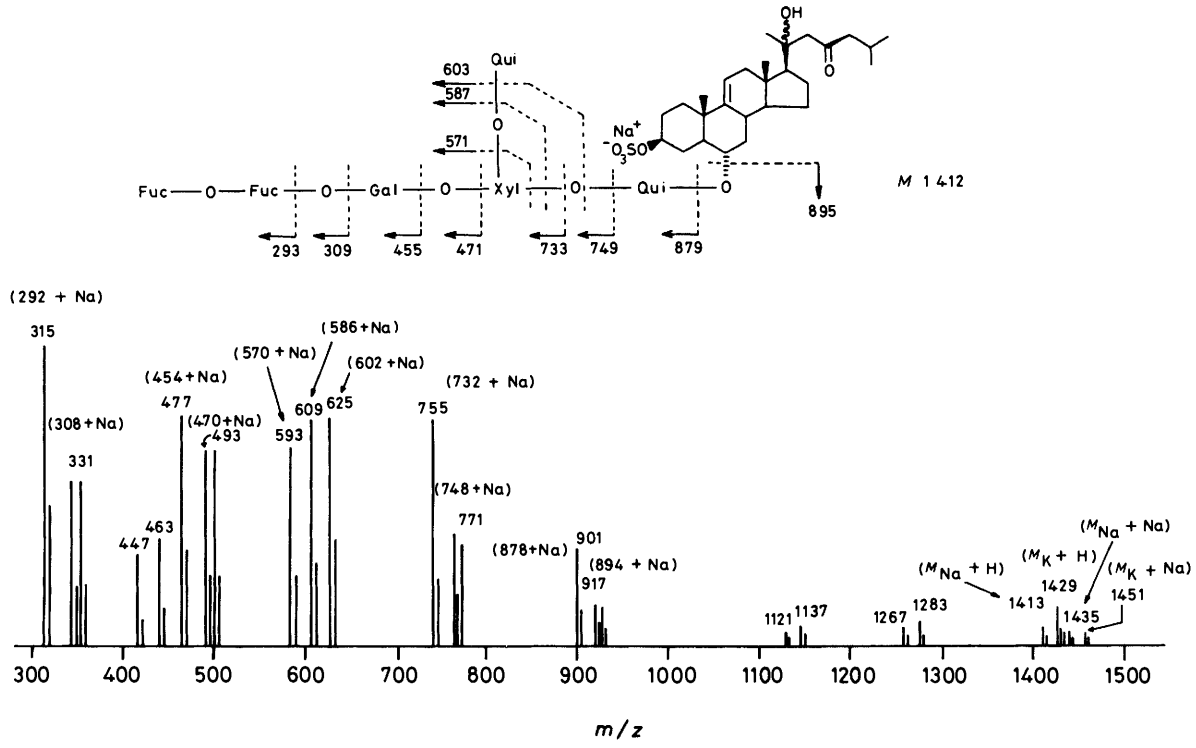


Figure 1. FAB-mass spectrum of marthasteroside A_1 (**6**)

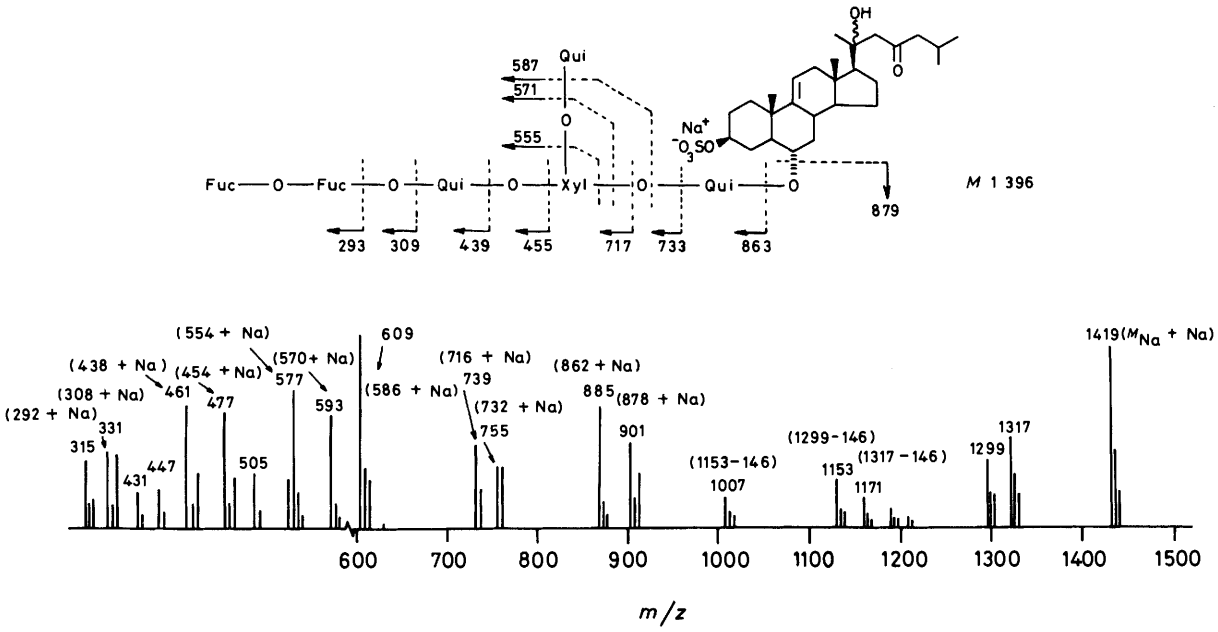


Figure 2. FAB-mass spectrum of marthasteroside A_2 (**7**)

We note that the glycosidase mixture of *Charonia lampas* we have used easily removes the sugar residues linked at C-4 of the branched unit (xylose or quinovose) leaving a triglycoside which is more resistant to further enzymic cleavage. The triglycoside (**6a**) [= (**7a**)] appears to be an invariant structural feature of the thornasterol A-containing asterosaponins; in marthasteroside B and C, which contain the aglycones marthasterone and dihydromarthasterone, respectively, the branched xylose is replaced by quinovose also branched at C-2 and C-4, while quinovose directly attached to the aglycone is replaced by glucose also with the 3-hydroxy function involved in interglycosidic linkage.

We think that the triglycoside portion of the asterosaponins can assume a spatial arrangement giving rise to a sort of cavity which interacts with the sodium cation of the sulphate residue. The comparison of the ^{13}C n.m.r. spectra (Tables 1 and 2) of the

Table 2. ^{13}C N.m.r. shifts (δ_C/p.p.m.) of sugar carbon atoms in (**8**), (**8b**), and (**9**) for solutions in [$^2H_5$]pyridine. The peaks due to the aglycones have been reported in a previous paper (ref. 16).[a] Pertinent shifts discussed in the text are shown in italics

Carbon		(8)	(8b)[a]	(9)	Methyl-β-D-glycopyranoside	
Glu	1	105.1	104.5	104.8	105.4	
	2	75.2	75.3	74.9	75.0	
	3	*91.8*	*91.3*	*91.6*	78.4	Glu
	4	70.1	69.7	69.9	71.4	
	5	77.4	77.4	77.3	78.0	
	6	62.9	62.8	62.7	62.7	
Qui I	1	104.0	103.7	103.7	105.3	
	2	*83.0*	*85.0*	83.1	76.6	
	3	75.2	74.9	74.9	78.0	Qui
	4	85.7	85.7	85.4	77.2	
	5	73.8	73.8	73.7	73.8	
	6	18.0	18.1	17.9	18.5	
Qui II	1	*105.2*	*107.5*	105.3		
	2	75.7	76.2	75.6		
	3	77.1	77.6	76.9		
	4	76.5	77.0	76.3		
	5	73.7	74.3	73.5		
	6	18.4	18.3	18.2		
Fuc I	1	102.6	102.7	102.3	105.6	
	2	82.0	82.3	81.7	72.1	
	3	74.1	74.1	74.1	75.2	Fuc
	4	72.0	72.0	71.9	72.6	
	5	71.9	71.9	71.8	71.4	
	6	16.7	16.7	16.6	16.8	
Fuc II	1	106.7	107.0	106.4		
	2	71.8	71.8	71.7		
	3	75.2	75.2	74.9		
	4	72.7	72.6	72.5		
	5	72.0	71.9	71.9		
	6	17.0	17.0	16.9		

[a] Signals for C-3 and C-6 of the aglycones have been now assigned; (**8**), C-3: 78.2, C-6: 80.8; (**9**), C-3; 78.3, C-6: 80.4 p.p.m. [b] (**8b**) Is the desulphated marthasteroside B; the shifts of the aglycone carbons were within 0.1 p.p.m. of those of marthasteroside B[16] except for C-2, C-3, C-4, which in (**8b**) resonated at δ_C 32.5 (*vs.* 29.0), 70.8 (*vs.* 78.2), and 33.7 (*vs.* 30.5) p.p.m. respectively; a shift was also observed for C-6 (79.5 *vs.* 80.8 p.p.m.).

full saponins with those of their desulphated analogues has shown that the resonances of C-2 of the branched sugar unit and C-1 of the terminal quinovosyl residue are significantly shifted on passing from the full to the desulphated saponins, thus giving support to our view.

^{13}C N.M.R. Spectra.—The ^{13}C n.m.r. spectra were recorded at 62.90 MHz in perdeuteriated pyridine. Assignments of aglycone carbons are reported in a previous paper.[16] Here we report the assignments of the sugar carbon atoms (Tables 1 and 2) based upon (a) comparison with the spectra of the appropriate methyl β-D-monoglycosides;[23,24] (b) the known glycosidation shifts;[21,25] and (c) the assignments reported for similar glycosides, which were supported by measuring the spin-lattice relaxation times (T_1).[14,15] The data are consistent with the proposed structures and we should like to make a few remarks. First we would note the unusual downfield position of the substituted C-3 of the unit directly attached to the aglycone, lower than expected for the substituted C-3 of β-D-glucopyranosyl and β-D-quinovopyranosyl units which are reported to resonate at δ_C *ca.* 87—88.5 p.p.m.[24] This seems a distinctive feature of the ^{13}C n.m.r. spectra of asterosaponins; other than those reported in this paper and in other papers,[14,15] the ^{13}C n.m.r. spectra of the asterosaponins isolated from various sources that we have run all contain a signal at δ_C *ca.* 90—91 p.p.m.

Secondly, we would draw attention to the downfield shifts observed for C-2 of the branching sugar units and C-1 of the terminal quinovosyl units on passing from the full saponins to the desulphated ones. Similar shifts were also reported by Komori *et al.*[14] on describing the ^{13}C n.m.r. spectra of acanthaglycoside A (**5**) (82.1 and 105.1 p.p.m.) and its desulphated analogue (85.0 and 107.1 p.p.m.). We would also note that the comparison of the spectra of the full saponins with those of the desulphated ones further confirm the location of the sulphate group at C-3; for example, in the spectrum of the desulphated marthasteroside B, C-3 is shifted upfield by 7.4 p.p.m. and C-2 and C-4 are shifted downfield by 2.7 and 3.0 p.p.m., respectively, relative to the sulphated compound (**8**). Similar shifts were observed in the spectra of desulphated marthasteroside A_1 and A_2.

FAB-Mass Spectra.—In a previous paper[16] we have reported only the molecular ion species observed in the FAB-mass spectra of the four principal saponins of *Marthasterias glacialis*, from which their molecular weights could be readily derived. In this paper we report the full spectra (Figures 1—3). In addition to the protonated and cationized ions in the form $(M_s + H)^+$ and $(M_s + C)^+$ where M_s is the molecular weight of the intact sodium or potassium salt and C is the mass of the cation (Na or K), the spectra also contain a series of fragment ions. The principal fragmentations are considered to be due to the cleavages of the glycosidic bonds and two fragmentation pathways are apparent. In addition to the ions with the positive charge located on the aglycone-containing fragments, ions are also produced with the positive charge located on the sugar fragments. The comparison of the spectrum of (**8**) with that of (**9**) (Figure 3) gives strong supporting evidence: the aglycone-containing fragments are shifted by two mass units on passing from (**8**) to (**9**), while the sugar fragments have identical masses. This process is the main process and produces mainly sodium-cationized fragments. Cleavages can occur on both sides of glycosidic linkages with proton transfer. Komori *et al.*[14] also described the FAB-mass spectrum of acanthaglycoside A (**5**) and have suggested that cleavages occur on one side of the glycosidic oxygen and that the series of doublets separated by 16 mass units is due to potassium- and sodium-cationized ions, respectively. We think that cleavages on both sides of the glycosidic oxygen are more probable. For example, one spectrum of marthasteroside A_2 (**7**) (Figure 2), showed the intense $(M + Na)^+$ ion as the sole molecular ion species but all the sugar fragment ions are still doublets separated by 16 mass units. We would also observe that the most intense peak above *m/z* 300, at *m/z* 609, should be due to potassium-cationized ions according to the view of Komori *et al.*[14]

The isomeric nature of fucosyl and quinovosyl units results in ambiguity in the interpretation of the very complex spectra and the saccharide sequence cannot be determined solely by mass spectrometry. Even so, some information on the saccharide sequences can be extracted from the spectra. The peaks at *m/z* 785—769 and *m/z* 623—607 (loss of 162 mass units: glucose) in the spectrum of marthasteroside C (Figure 3) indicate that glucose is directly linked to the aglycone, as is the case in marthasteroside B, previously demonstrated by the isolation of marthasterone 6α-glucoside among the acid hydrolysis products of glycoside M_2.[26] The series of ions observed in the spectra of all four saponins, *viz.* *m/z* 625, 609, and 593 in the spectrum of marthasteroside A_1 (Figure 1), *m/z* 609, 593, and 577 in that of marthasteroside A_2 (Figure 2), and *m/z* 477, 461, and 445 in the spectra of the pentaglycosides (Figure 3) might suggest the location of the branching point in each molecule as being on the second monosaccharide unit starting from the aglycone, although no firm conclusions can be drawn without studying a wide range of branched saponins.

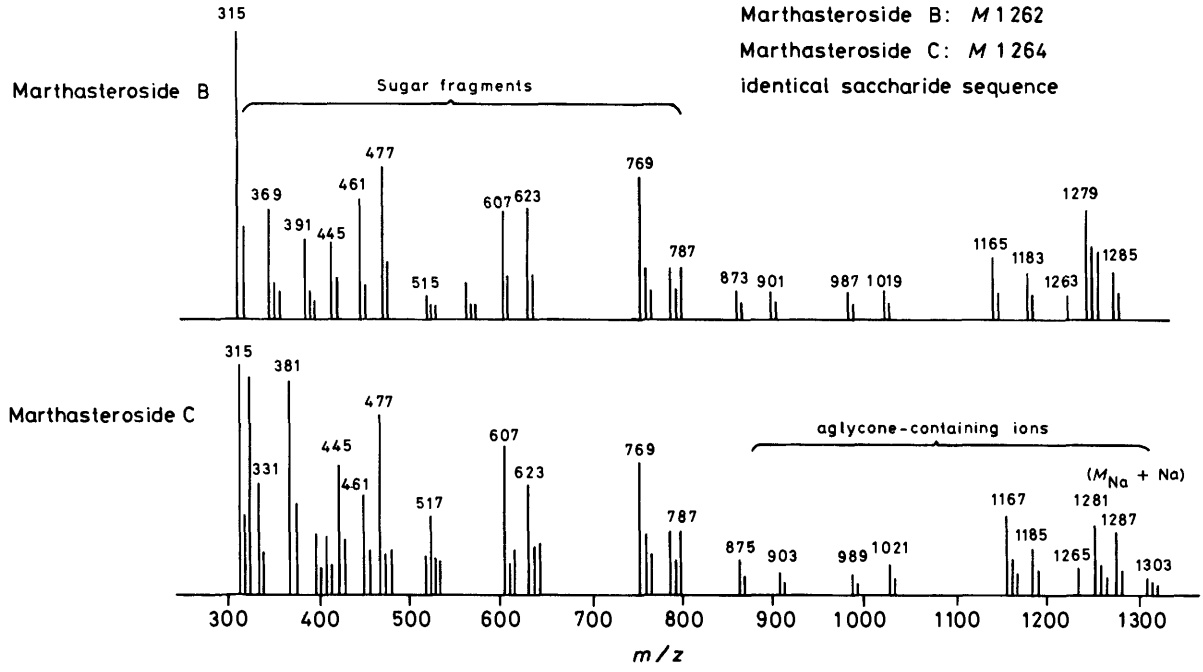


Figure 3. FAB-mass spectra of marthasteroside B (**8**) and C (**9**)

Experimental

The following instruments were used: n.m.r., Bruker WM-250; h.p.l.c., Waters Model 6000A pump equipped with a U6K injector and a differential refractometer, model 401, together with a UVILOG-5 III variable-wavelength detector; g.l.c., Carlo Erba Fractovap 2900 capillary column; c.d., Cary 61 spectropolarimeter; mass spectrometry, Kratos MS 902 mass spectrometer equipped with Kratos FAB source.

The FAB-mass spectra were obtained by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardment with Xe atoms of energy 2—6 kV.

The ^{1}H n.m.r. spectrum of the oligoglycosides were run in CD_3OD, the ^{1}H n.m.r. spectra of the methyl methylated/benzoylated monoglycosides in $CDCl_3$, and the ^{13}C n.m.r. spectra in [$^2H_5$]pyridine.

Methylation of Saponins.—A solution of a saponin (*ca.* 25 mg) in DMF (1 ml) was slowly added under nitrogen to a stirred mixture of NaH (100 mg) in dry DMF (1 ml) cooled in an ice-bath. The mixture was stirred for 10 min, and then CH_3I (0.5 ml) was added in two portions. The reaction mixture was kept for a further 10 min in the ice-bath and then for 4 h at room temperature. The excess of NaH was destroyed by a few drops of methanol, and after addition of water, the mixture was extracted with chloroform (3 × 1.5 ml). The combined extracts were washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue obtained from each saponin was loaded on a silica gel (2 g) column and eluted with chloroform–methanol (95:5). Each permethylated saponin with R_F *ca.* 0.7 on SiO_2 t.l.c. with chloroform–methanol (82:15) was eluted to give a residue of *ca.* 20 mg.

Methanolysis with Hydrochloric Acid.—Each 'permethylated' saponin (*ca.* 20 mg) was dissolved in anhydrous 2M HCl–methanol (1—2 ml) and the solution was heated at 80 °C in a stoppered reaction vial for 8 h. After being cooled the reaction mixture was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under reduced pressure. The residue was in part used for a g.l.c. analysis and in part (major) was *p*-bromobenzoylated. G.l.c. analyses were run at 84 °C using a 25 m column of OV-101 (hydrogen carrier, flow 10 ml min^{-1}). G.l.c. peaks in each 'permethylated' saponin methanolysate co-eluted with those of methyl 2,3,4-tri-*O*-methylfucoside and methyl 2,3,4-tri-*O*-methylquinovoside standards.

p-*Bromobenzoylation and Separation of Benzoates by H.P.L.C.*—Solutions of each of the aforementioned residues in dry pyridine (1.5 ml) were treated with *p*-bromobenzoyl chloride (100 mg) and a few mg of 4-dimethylaminopyridine, the mixtures were stirred overnight at 60 °C under nitrogen, and chilled water was then added to the solutions, which, after 30 min, were extracted with chloroform. In each case the extract was washed successively with saturated aqueous $NaHCO_3$ and water, and the solvent was evaporated off under reduced pressure. The dark brown polar materials were removed by dissolving the residue in chloroform and passage of the solution through a Pasteur pipette filled with a slurry of silica gel in hexane–diethyl ether (7:3). The fastest moving portion was eluted with hexane–diethyl ether (7:3), and the eluate was evaporated to give a residue (*ca.* 10 mg) containing the benzoates.

The benzoate mixture obtained from each saponin was separated by h.p.l.c. under the following conditions: Whatman Partisil PXS M9 10/25; diethyl ether in hexane, flow rate 5 ml min^{-1}; 260-nm detection. The results from each saponin are given below.

(a) *From marthasteroside* A_1. (Elution with 25% diethyl ether in hexane); methyl 2,4-di-*O*-(*p*-bromobenzoyl)-3-*O*-methyl-α-D-xylopyranoside: elution time 5 min: δ_H 7.5—7.95 (8 H, m, ArH), 5.18 (1 H, ddd, *J* 10.5, 9.0, and 6.0 Hz, 4-H), 5.02—5.05 (2 H, complex m, 1- and 2-H), 4.02 (1 H, t, *J* 9.0 Hz, 3-H), 3.97 (1 H, dd, *J* 10.5 and 6.0 Hz, 5-H_{eq}), 3.69 (1 H, t, *J* 10.5 Hz, 5-H_{ax}), 3.53 (OMe), and 3.43 (OMe); methyl 3-*O*-(*p*-bromobenzoyl)-2,4-di-*O*-methyl-β-D-quinovopyranoside: elution time 9.6 min; δ_H 8.0 (2 H, d, *J* 9.0 Hz; ArH), 7.58 (2 H, d, J 9.0 Hz, ArH), 5.29 (1 H, t, *J* 9.5 Hz, 3-H), 4.31 (1 H, d, *J* 7.5 Hz, 1-H), 3.57 (OMe), 3.44 (OMe), 3.39 (OMe), 3.17 (1 H, dd, *J* 9.5 and 7.5 Hz, 2-H), 3.5 (m

partially overlapped, 5-H), 3.05 (1 H, t, *J* 9.5 Hz, 4-H), 1.45 (d, 5-Me); methyl 2,4-di-*O*-(*p*-bromobenzoyl)-3-*O*-methyl-β-D-xylopyranoside: elution time 13.4 min; δ_H 7.5—7.85 (8 H, m, ArH), 5.13 (2 H, m, 2- and 4-H), 4.69 (1 H d, *J* 3.5 Hz, 1-H), 4.31 (1 H, dd, *J* 12.0 and 3.5 Hz, 5-H_{eq}), 3.75 (1 H, t, *J* 4.5 Hz, 3-H), 3.67 (1 H, dd, *J* 12.0 and 4.5 Hz, 5-H_{ax}), 3.55 (OMe), and 3.49 (OMe); methyl 3-*O*-(*p*-bromobenzoyl)-2,4-di-*O*-methyl-α-D-quinovopyranoside: elution time 17.6 min; δ_H 7.96 (2 H, d, *J* 9.0 Hz, ArH), 7.58 (2 H, d, *J* 9.0 Hz, ArH), 5.60 (1 H, t, *J* 9.7 Hz, 3-H), 4.85 (1 H, d, *J* 3.1 Hz, 1-H), 3.81 (1 H, dq, *J* 9.7 and 6.0 Hz, 5-H), 3.47 (OMe), 3.45 (OMe), 3.39 (OMe), 3.40 (dd partially overlapped, 2-H), 3.05 (1 H, t, *J* 9.7 Hz, 4-H), and 1.33 (d, *J* 6 Hz, 5-Me); methyl 3-*O*-(*p*-bromobenzoyl)-2,4-di-*O*-methyl-α-D-fucopyranoside: elution time 24 min; δ_H 7.96 (2 H, d, *J* 9.0 Hz, ArH), 7.60 (2 H, d, *J* 9.0 Hz, ArH), 5.42 (1 H, dd, *J* 10.5 and 3.0 Hz, 3-H), 4.92 (1 H, d, *J* 3.7 Hz, 1-H), 4.05 (1 H, br q, *J* 6.0 Hz, 5-H), 3.88 (1 H, dd, *J* 10.5 and 3.7 Hz, 2-H), 3.61 (1 H, br d, *J* 3.0 Hz, 4-H), 3.45—3.5 (overlapping singlets, OMe), and 1.33 (d, *J* 6.0 Hz, 5-Me); methyl 2-*O*-(*p*-bromobenzoyl)-3,4,6-tri-*O*-methyl-α-D-galactopyranoside: elution time 31.2 min; δ_H 7.96 (2 H, d, *J* 9.0 Hz, ArH), 7.58 (2 H, d, *J* 9.0 Hz, ArH), 5.37 (1 H, dd, *J* 10.2 and 3.7 Hz, 2-H), 5.06 (1 H, d, *J* 3.7 Hz, 1-H), 3.94 (1 H, br t, *J* 6.0 Hz, 5-H), 3.78—3.81 (2 H, 3- and 4-H), 3.60 (m partially overlapped, 6-H_2), 3.60 (OMe), 3.51 (OMe), 3.44 (OMe), and 3.38 (OMe).

(b) *From marthasteroside* A_2. (Elution with 5% diethyl ether in hexane); methyl 2,4-di-*O*-(*p*-bromobenzoyl)-3-*O*-methyl-α-D-xylopyranoside: elution time 42 min; δ_H (see above); methyl 2-*O*-(*p*-bromobenzoyl)-3,4-di-*O*-methyl-α-D-quinovopyranoside: elution time 49.6 min; δ_H 7.96 (2 H, d, *J* 9.0 Hz, ArH), 7.60 (2 H, d, *J* 9.0 Hz, ArH), 4.90—4.96 (2 H, m, 1- and 2-H), 3.71 (1 H, t, *J* 9.5 Hz, 3-H), 3.67 (m, 5-H), 3.60 (OMe), 3.57 (OMe), 3.35 (OMe), 2.90 (1 H, t, *J* 9.5 Hz, 4-H), and 1.32 (3 H, d, *J* 5.5 Hz, 5-Me).

Elution with diethyl ether yielded a mixture of benzoates which were separated by h.p.l.c. with 25% diethyl ether in hexane and identified as methyl 2,4-di-*O*-(*p*-bromobenzoyl)-3-*O*-methyl-β-D-xylopyranoside; methyl 3-*O*-(*p*-bromobenzoyl)-2,4-di-*O*-methyl-α-D-quinovopyranoside;methyl3-*O*-(*p*-bromobenzoyl)-2,4-di-*O*-methyl-α-D-fucopyranoside.

(c) *From marthasteroside B and C.* Both saponins yielded the same methyl benzoylated methylated glycosides; elution with 20% diethyl ether in hexane gave methyl 2,4-di-*O*-(*p*-bromobenzoyl)-3-*O*-methyl-α-D-quinovopyranoside, elution time 4.2 min; δ_H 7.58—7.96 (8 H, m, ArH), 5.01—5.09 (3 H, m, 1-, 2-, and 4-H), 3.98 (1 H, t, *J* 9.0 Hz, 3-H), 3.98 (m overlapping with 3-H, 5-H), 3.45 (OMe), 3.40 (OMe), and 1.32 (d, *J* 6.0 Hz, 5-Me); methyl 2-*O*-(*p*-bromobenzoyl)-3,4-di-*O*-methyl-α-D-fucopyranoside, elution time 14.4 min; δ_H 7.96 (2 H, d, *J* 9.0 Hz, ArH), 7.58 (2 H, d, *J* 9.0 Hz, ArH), 5.37 (1 H, dd, *J* 10.5 and 3.7 Hz, 2-H), 5.02 (1 H, d, *J* 3.7 Hz, 1-H), 3.95 (1 H br q, *J* 6.0 Hz, 5-H), 3.80 (1 H, dd, *J* 10.5 and 3.0 Hz, 3-H), 3.63 (OMe), 3.54 (1 H, br d, *J* 3.0 Hz, 4-H), 3.50 (OMe), 3.36 (OMe), and 1.32 (3 H, d, *J* 6.0 Hz, 5-Me). Methyl 3-*O*-(*p*-bromobenzoyl)-2,4,6-tri-*O*-methyl-α-D-glucopyranoside, which was eluted with 30% diethyl ether in hexane, had δ_H 7.96 (2 H, d, *J* 9.0 Hz, ArH), 7.58 (2 H, d, *J* 9.0 Hz, ArH), 5.63 (1 H, t, *J* 9.0 Hz, 3-H), 4.94 (1 H, d, *J* 3.7 Hz, 1-H), 3.77 (1 H, dt, *J* 9.5 and 3 Hz, 5-H), 3.65 (2 H, d, *J* 3 Hz, 6-H_2), 3.51 (OMe), 3.46 (OMe), 3.45 (OMe), 3.39 (OMe), and 3.45—3.55 (m, 2- and 4-H).

Enzymatic Hydrolysis of Saponins.—Each saponin (*ca.* 20 mg) in citrate buffer (pH 4.5) (1.5 ml) was incubated with glycosidase mixture (20 mg) from *Charonia lampas* (Scikagaku Kogyo) at 40 °C for 1—3 d. Then the mixture was extracted with n-butanol. The extract was evaporated to dryness under reduced pressure to give a residue which was separated by SiO_2 t.l.c. with n-butanol–acetic acid–water (60:15:25) and then purified by h.p.l.c. under the following conditions: Waters C-18 μ-bondapack; 52% methanol in water, flow rate 6 ml min^{-1}; refractometer detector.

(a) *From marthasteroside* A_1. SiO_2 t.l.c. showed two bands with R_F 0.4 (major, 5 mg) and 0.38 (minor, 0.2 mg). The minor compound was analysed for the sugar constituents (see below) and shown to contain quinovose (× 2), xylose, and galactose. The major component was purified by h.p.l.c. to give the prosapogenol (**6a**) (4 mg), FAB-MS: *m/z* 981 $(M + Na)^+$, 463—465 and 447 (trisaccharide ion), 317—319 and 301 (disaccharide ion, qui–xyl); δ_H (aglycone) 0.81 (3 H, s, 18-H_3), 0.93 (3 H, d, *J* 6.5 Hz, 26- or 27-H_3), 0.95 (3 H, d, *J* 6.0 Hz, 27- or 26-H_3), 1.02 (3 H, s, 19-H_3), 1.37 (3 H, s, 21-H_3), 2.41 (2 H, d, *J* 7.0 Hz, 24-H_2), 2.63 (2 H, ABq, *J* 16.0 Hz, 22-H_2), 4.23 (1 H, m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), and 5.37 (1 H, br d, *J* 5.5 Hz, 11-H); δ_H (sugars) 1.30 (3 H, d, *J* 6.0 Hz, 5-CH_3 of quinovosyl), 1.39 (3 H, d, *J* 6.0 Hz, 5-CH_3 of quinovosyl), 3.92 (1 H, dd, *J* 12.0 and 4.0 Hz, 5-H_{eq} of xylopyranosyl), 4.42 (1 H, d, *J* 7.5 Hz, anomeric H), 4.55 (1 H, d, *J* 7.5 Hz, anomeric H), and 4.56 (1 H, d, *J* 7.5 Hz, anomeric H); sugar analysis: quinovose (× 2) and xylose; δ_C in Table 1.

(b) *From marthasteroside* A_2. SiO_2 t.l.c. showed two bands with R_F 0.4 (major, 4.5 mg) and 0.37 (minor, 0.4 mg). The mixture was separated by h.p.l.c.; the minor component was shown to contain quinovose (× 3) and xylose, and the major component was shown to be identical with (**6a**) (^{1}H n.m.r., FAB-MS, and sugar analysis).

(c) *From marthasteroside* B. SiO_2 t.l.c. showed only one spot with R_F 0.42; the residue was purified by h.p.l.c. to give (**8a**) (3.5 mg), elution time 13.2 min; FAB-MS: *m/z* 993 $(M + Na)^+$, 873 $(M + H - 98)^+$, 493—495 and 477 (trisaccharide ion), 331—333 and 315 (disaccharide ion; qui–qui); δ_H (aglycone) 0.70 (3 H, s, 18-H_3), 0.95 (3 H, d, *J* 6.0 Hz, 21-H_3), 1.03 (3 H, s, 19-H_3), 1.94 (3 H, s, 26-H_3), 2.15 (3 H, s, 27-H_3), 4.22 (1 H, m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), 5.34 (1 H, br d, *J* 5.5 Hz, 11-H), and 6.20 (1 H, br s, 24-H); δ_H (sugars) 1.32 (3 H, d, *J* 6.0 Hz, 5-CH_3 of quinovosyl), 1.40 (3 H, d, *J* 6.0 Hz, 5-CH_3 of quinovosyl), 4.46 (1 H, d, *J* 7.5 Hz, anomeric H), 4.54 (1 H, d, *J* 7.5 Hz, anomeric H), and 4.56 (1 H, d, *J* 7.5 Hz, anomeric H); sugar analysis: glucose, quinovose (× 2).

(d) *From marthasteroside* C. SiO_2 t.l.c. showed only one spot with R_F 0.41; the residue was purified by h.p.l.c. to give the prosapogenol (**9a**) (3 mg), elution time 13.5 min; FAB-MS: *m/z* 995 $(M + Na)^+$, 493—495 and 477 (trisaccharide ion), 331—333 and 315 (disaccharide ion; qui–qui); δ_H (aglycone) 0.70 (3 H, s, 18-H_3), 0.92 and 0.95 (9 H, overlapping doublets, 21-, 26-, and 27-H_3), 1.03 (3 H, s, 19-H_3), 2.34 (2 H, d, *J* 7.5 Hz, 24-H_2), 4.22 (1 H, m, $w_{\frac{1}{2}}$ 22 Hz, 3α-H), and 5.37 (1 H, br, d, *J* 5.5 Hz, 11-H); δ_H (sugars) 1.32 (3 H, d, *J* 6.0 Hz, 5-CH_3 of quinovosyl), 1.40 (3 H, d, *J* 6.0 Hz, 5-CH_3 of quinovosyl), 4.46 (1 H, d, *J* 7.5 Hz, anomeric H), 4.56 (2 H, d, *J* 7.5 Hz, 2 × anomeric H); sugar analysis: glucose, quinovose (× 2).

Methanolysis of Prosapogenols: Sugar Analysis.—A solution of each prosapogenol (0.2—1 mg) in anhydrous 2M HCl–methanol was heated at 80 °C in a stoppered reaction vial for 8 h. After being cooled, the reaction mixture was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under N_2. The residue was dissolved in TRISIL Z (0.1 ml; *N*-trimethylsilylimidazole in pyridine, Pierce Chemical Co.), left at room temperature for 15 min, and analysed by g.l.c. (139 °C; SE-30; 25 m). G.l.c. peaks in the silylated prosapogenol hydrolysate co-eluted with those in silyated standards.

Configuration of D-*Fucose from the Hydrolysate of Marthasteroside* A_2 (**7**).—A solution of marthasteroside A_2 (*ca.* 10 mg) in anhydrous 2M HCl–methanol (2 ml) was heated at 80 °C in a stoppered reaction vial for 8 h. After being cooled, the

reaction mixture was neutralized with Ag_2CO_3, centrifuged, and the supernatant evaporated to dryness under N_2. The residue was per-*p*-bromobenzoylated and the reaction mixture purified as above. The benzoate mixture was then separated by h.p.l.c. using a Whatman Partisil PXS M9 10/25 column; 10% diethyl ether in hexane, flow rate 5 ml min^{-1}. Methyl 2,3,4-tri-*O*-(*p*-bromobenzoyl)-α-D-fucopyranoside was identified by ^1H n.m.r. spectroscopy, δ_H 7.96, 7.84, 7.66, 7.61, 7.54, and 7.41 (each 2 H, d, *J* 9.0 Hz, ArH), 5.90 (1 H, dd, *J* 3.0 and 10.5 Hz, 3-H), 5.71 (1 H, br, d, *J* 3.0 Hz, 4-H), 5.58 (1 H, dd, *J* 3.7 and 10.5 Hz, 2-H), 5.20 (1 H, d, *J* 3.7 Hz, 1-H), 4.38 (1 H, br q, *J* 6 Hz, 5-H), and 1.29 (3 H, d, *J* 6 Hz, 5-Me); c.d. (CH_3OH) Δ_{254} +98.7, Δ_{236} −26.8; *A* +125.5 (calc. *A* +140).[19]

Solvolysis of Saponins to give the Desulphated Analogues.—A solution of a saponin (50 mg) in dioxane (0.5 ml) and pyridine (0.5 ml) was heated at 120 °C for 4 h in a stoppered vial. After the solution had cooled, water (5 ml) was added and the solution was extracted with n-butanol (3 × 2 ml). The combined extracts were washed with water and evaporated under reduced pressure to give the desulphated material.

Desulphated marthasteroside B. δ_H 0.70 (3 H, s, 18-H_3), 0.95 (3 H, d, *J* 6 Hz, 21-H_3), 1.02 (3 H, s, 19-H_3), 1.28, 1.31, and 1.48 (each 3 H, d, *J* 6.5 Hz, CH_3 of quinovosyl and fucosyl units), 1.94 (3 H, s, 26-H_3), 2.15 (3 H, s, 27-H_3), 4.44, 4.48, 4.56, and 4.58 (5 H, each d, *J* 7.5 Hz, anomeric Hs), 5.36 (1 H, br d, *J* 6.5 Hz, 11-H), and 6.20 (1 H, br s, 24-H). Significantly the signal for 3α-H, at δ 4.20 in the sulphated saponin, was shifted upfield and was seen between δ 3.5—3.8; the spectrum was devoid of signals in the regions 3.8—4.4; δ_C data in Table 2.

Desulphated marthasteroside A_1. δ_H 0.81 (s, 18-H_3), 0.91—0.92 (2 d, *J* 6.0 Hz, 26- and 27-H_3), 1.01 (s, 19-H_3), 1.25—1.35 (overlapping doublets, CH_3 of quinovosyl and fucosyl units), 1.37 (s, 21-H_3), 2.39 (d, *J* 7.5 Hz, 24-H_2), 2.60 (ABq, *J* 15.0 Hz, 22-H_2), 4.16 (1 H, dd, *J* 12.0 and 4.0 Hz, 5-H_{eq} of the xylosyl unit), 4.45—5.60 (overlapping doublets, anomeric Hs), and 5.36 (br d, *J* 5.5 Hz, 11-H); δ_C data in Table 1.

Desulphated marthasteroside A_2. ^1H N.m.r. data were very similar to those of desulphated marthasteroside A_1; small differences were observed in the bulk of overlapping resonances spread out over δ 3.3—3.9; δ_C data are in Table 1.

We note that during the solvolysis of the tornasterol A-containing saponins (**6**) and (**7**), a retro-aldol reaction also occurs with the formation of asterone [3β,6α-dihydroxy-5α-preg-9(11)-en-20-one]-containing saponins, which are easily detectable by the presence of singlets at δ 0.57 (18-H_3) and 2.16 (21-H_3) in the ^1H n.m.r. spectra of the reaction mixture.[16] The extension of the retro-aldol cleavage is time dependent and during a 4 h reaction the asterone-containing glycosides account for *ca.* 15—20% of the total mixture.

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Received 7th November 1983; *Paper* 3/1974