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STEROIDAL GLYCOSIDES FROM STARFISHES

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Abstract - This paper covers recent work - much of it from the author's laboratory - dealing with novel steroidal oligoglycosides from starfishes. The steroidal glycosides until now encountered in this class of marine animals can be grouped into three structure types. Compounds of the first type, recognized for long time, include the sulphated saponins (asterosaponins), characterized by steroidal aglycones possessing a 3β,6α-diol pattern and a 9,11-double bond; the oligosaccharide moiety (four up to six sugar units) is attached at C-6 and the sulphate residue is at C-3. Compounds of the second type, recently discovered in two species of the genus Echinaster, have a number of unusual features: a Δ^7,3β,6β-dihydroxy steroidal moiety, there is no sulphate group and, most remarkably, the carbohydrate chain (three sugar units) is cyclized between C-3 and C-6 of the aglycone. Compounds of the third type include glycosides having highly hydroxylated steroidal aglycones; the carbohydrate moiety (one or two sugar units) is attached at C-24 of the side chain and there is no sulphate residue. During the course of our investigation on the steroidal glycosides from starfishes we have also encountered several polyhydroxylated sterols and their structures are presented.

INTRODUCTION

Saponins, water soluble compounds composed of sugars and steroid or triterpenoid moieties, are common constituents of terrestrial plants, but are uncommon as animal constituents. In the animal kingdom saponins have been found in the exclusively marine phylum Echinodermata and particularly in species of the classes Holothuroidea (sea cucumbers) and Asteroidea (starfishes). These compounds are apparently absent from the other three classes, Crinoidea (sea lilies), Echinoidea (sea urchins) and Ophiuroidea (brittle stars), of echinoderms.
Saponins derived from sea-cucumbers (holothurins) are triterpenoid glycosides, which, upon acid hydrolysis, give triterpenoid aglycones based on the lanostane skeleton, sugars and sulphate, whereas those from starfishes (asterosaponins) are sulphated steroidal glycosides (Refs. 1, 2, 3).

The toxicity of starfishes has been described in various ways for many years, but most of the reports can be explained by the presence of saponins. Starfish extracts and also purified saponins have been reported to exhibit a broad spectrum of physiological and pharmacological activities. Asterosaponins are highly surface active, and most of them show potent haemolitic properties (Refs. 2, 4). They also have antitumor (Ref. 5) and antibacterial activities (Ref. 6); Shimizu reported that asterosaponins from Asterias forbesi, Acanthaster planci and Asterina pectinifera inhibited influenza virus multiplication (Ref. 7). Antiinflammatory activity was reported for Asterias forbesi saponins (Ref. 8), and activity toward contraction of the rat phrenic nerve-diaphram preparation was reported for Asterias amurensis saponins (Ref. 9). Several observations concerning with the biological functions of the asterosaponins have also been described. Because of their general toxicity it is probable that saponins act primarily as chemical defence agents, discouraging many predators; they also induce escape reactions in bivalve molluscs (Ref. 10), thus reducing the predation ability of the starfishes theirself.
Asterosaponins have been identified as the spawning inhibitor in the Japanese starfish Asteria amurensis (Ref. 11) and more recently they have been reported

to inhibit the production of 1-methyladenine in the follicle cell (Ref. 12).

Thus most of the work on asterosaponins has been prompted by the toxic and, more generally, physiological properties of asterosaponins and recently we also became interested in this class of compounds. In the course of our program of screening starfishes for their saponins we have isolated several non-sulphated toxic steroidal glycosides of novel structure types: the steroidal cyclic glycosides from two species of the genus Echinaster, and a group of highly hydroxylated steroids 24-O-glycosidated from the Mediterranean species Hacelia attenuata and the Pacific species Protoreaster nodosus.
In view of the appearance of several reviews (Refs. 1, 2, 13, 14) which have covered various aspects of the "asterosaponins" field and the comprehensive review entirely devoted to echinoderm saponins produced by D. J. Burnell and J. W. ApSimon, which will appear in the fifth volume of the P.J.Scheuer's series Marine Natural Products - Chemical and Biological Perspectives, the present article will deal primarily with structure elucidation of the non-sulphated steroidal glycosides. A few remarks on the sulphated saponins will be also reported. We have also encountered a group of novel highly hydroxylated sterols (five up to eight hydroxyl functions) in starfishes and their structures are presented.

SULPHATED STEROIDAL GLYCOSIDES

This group of saponins is characterized by steroidal aglycones possessing a 3β,6α-diol pattern, the biosynthetically unusual 9,11-double bond and, very often, a 23-oxo function; the oligosaccharide (four up to six sugar units) moiety is attached by a glycosidic linkage to C-6, while the sulphate is at C-3 (see for example the structures 15, 16 and 17). The oligosaccharide portion always includes fucose (6-deoxygalactose) and quinovose (6-deoxyglucose); other common monosaccharides are xylose, galactose and glucose. The "asterosaponins" are quite delicate molecules and usually occurr as mixtures, which are difficult to separate cleanly. For this reason most of the reported works have been concerned with the aglycones produced by acid hydrolysis, which very often are artefacts and may not represent the true structure of the intact glycoside. For example the C-21 compound, asterone 1, the most widely reported steroid from asterosapogenins (Refs. 15, 16, 17, 18) is mostly an artefact generated from the 20-hydroxysteroid, thornasterol A 13, via a retro-aldol cleavage during acid hydrolysis. Thornasterol A 13 was itself isolated later, along with minor amounts of its 24-methyl analogue 14, from the saponins of Acanthaster planci by using an hydrolytic enzyme mixture from the mollusc Charonia lampas to remove the sugars (Ref. 19). The $\Delta^{20(22)}$-steroid 4, the $\Delta^{17(20)}$-isomers 5 and 6, and the rearranged 17-Me, Δ^{13}-olefin 7 (Refs. 20, 21) are all artefacts too, arising from thornasterol A 13 during acid hydrolysis (Ref. 22). Dihydromarthasterone 2 and marthasterone 3 are the principal genins from Marthasterias glacialis, which also gave minor amounts of the truncated C-24 steroid 11 (Ref. 23). This latter looks as an artefact even a 25-hydroxylated steroid, from which it may well derive, has not yet been detected. On the other hand the $\Delta^{24(25)}$-compound, marthasterone, has proved to be a genuine sapogenin (see below). The steroids 8, 9 (which is probably an artefact arising from the corresponding 20-ol) (Note a), and 10 (unusual in that it lacks the 9,11-double bond) are reported once (Refs. 24, 25, 26). Recently the triol asterogenol 12 has been reported as minor constituent of the saponins hydrolysate of the starfishes Asterias forbesi and Asterias vulgaris (Ref.27), raising again the question concerning the origin of progesterone-type compounds in saponin hydrolysates from starfish.

In contrast with the efforts toward elucidation of the aglycones, the structure determination of the intact glycosides has received less attention. Only three complete structures have been described, and all are based on the thornasterol A aglycone. Kitagawa and Kobayashi have determined the complete structure of the major Acanthaster planci saponin, thornasteroside A, 15 (Ref. 28). Glycoside B_2 16, an asterosaponin isolated from the ovaries of Asterias amurensis by Ikegami et al. (Ref. 29), is almost identical with thornasteroside A, except that the terminal fucose unit is replaced by quinovose.

Note a: A recent examination of the "asterosaponins" of the Pacific starfish Protoreaster nodosus has led to the isolation of the major three saponins; a preliminary analysis by ^{1}H NMR spectroscopy has indicated that one of them possess the 5α-cholesta-9(11),24(25)-diene-3β,6α,20ξ-triol as aglycone [δ 0.85 (s, 18-H), 1.03 (s, 19-H), 1.26 (s, 21-H), 1.67 and 1.74 (each s, 26 and 27-H), 3.62 (m, H-6), 4.22 (7-lines m, 3α-H), 5.28 (t, J=6.5 Hz, 24-H), 5.37 (br d J=4 Hz, H-11)], from which the steroid 9 may well derive during acid hydrolysis.

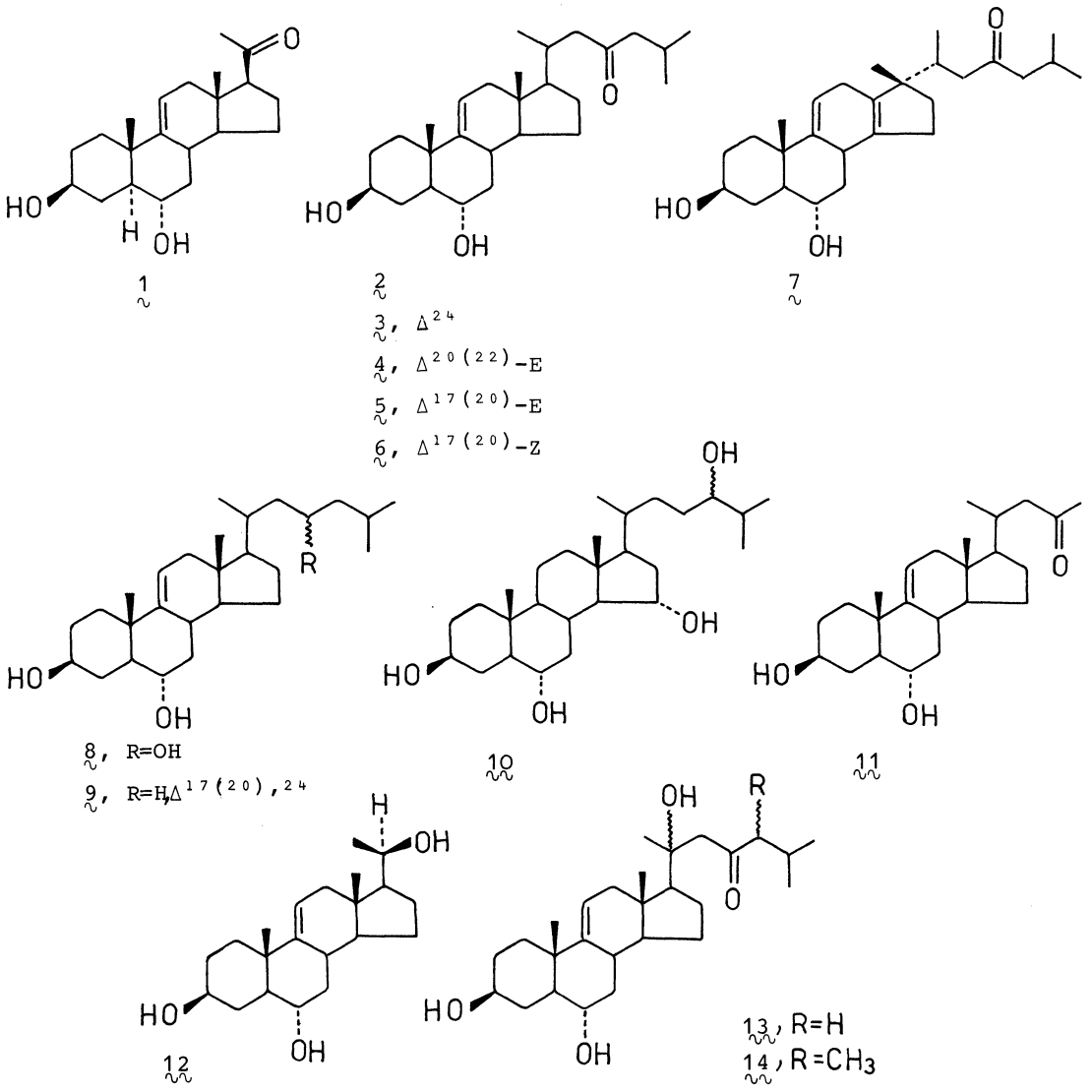
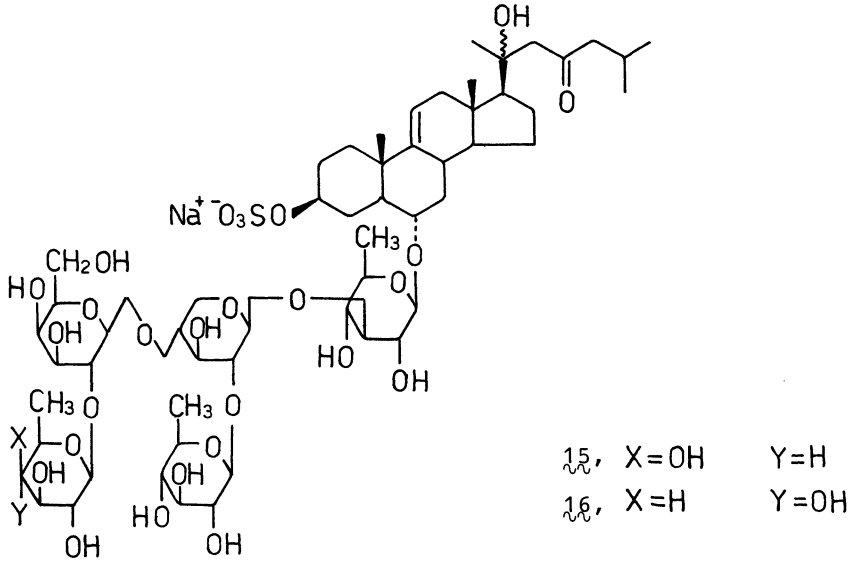
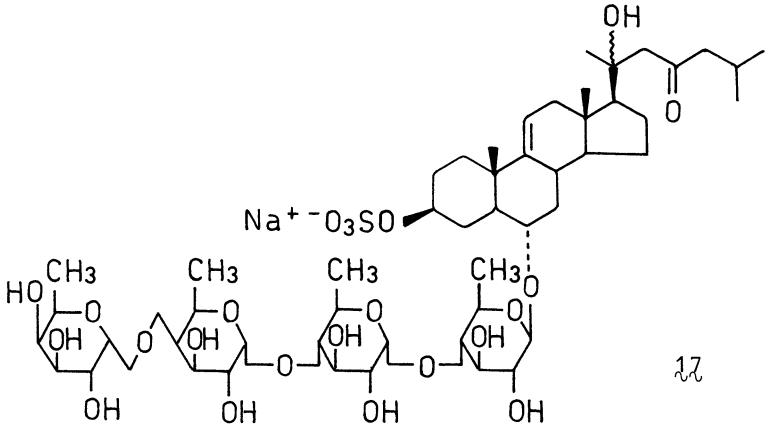


Fig. 1 - Structure of the reported steroidal aglycones from "asterosaponins"



The Ikegami's group have also determined the structure of asterosaponin A, 17, one of the two major saponins from the same starfish Asterias amurensis (Ref. 30).



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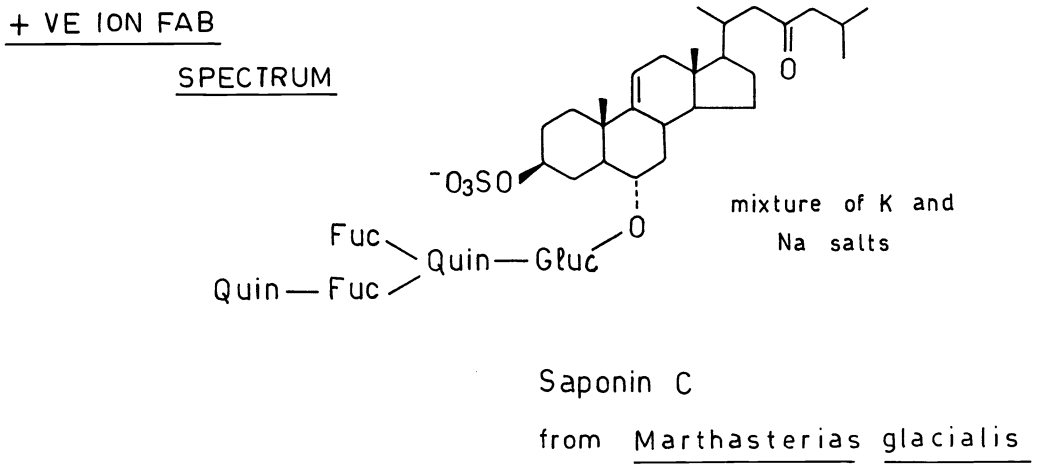
Through a series of chromatographic steps (1. Amberlite XAD-2, 2. Sephadex LH-20, 3. HPLC on C_{18} μ-bondapack) we have been able to separate the saponin mixture of Marthasterias glacialis into six individual components. The ^{1}H and ^{13}C NMR spectra have established the structures of the genuine aglycones. The ^{1}H NMR spectra also confirmed that the sulphate is at C-3, 7-line multiplet at δ 4.16-4.20 in all spectra. These compounds were examined by Dr. R. Self (Food Research Institute, Norwich, U. K.) by fast atom bombardment (FAB) mass spectrometry, a new technique for ionization of highly polar involatile compounds (Ref. 31). The FAB spectrum of every sample indicated the anion molecular weight and the associated inorganic cation species. The structural data of the "asterosaponins" from Marthasterias glacialis are summarized in Table 1. A first group of compounds contains five sugar units, and a second group, all characterized by having the same 20-hydroxysteroid 13, thornasterol A, as aglycone, possess carbohydrate chains made up by six sugars units. The + VE ion FAB spectrum of one of the sulphated saponin from Marthasterias glacialis, saponin C, is presented in Fig. 2. The spectrum is characterized by protonated and cationized (both Na and K) molecular ions; interestingly our saponin C sample is mostly a potassium salt.

TABLE 1. Structural data of the 3-O-sulphated saponins from Marthasterias glacialis

	Aglycone*	Sugars**	M. Wt. (FAB)	
			Na salt	K salt
B	M	Gluc Quin (2) Fuc (2)	1262	1278
C	DHM	Gluc Quin (2) Fuc (2)	1264	1280
C_1	DHM	Gluc Quin (2) Fuc (2)	1264	1280
A	T	Quin (3) Fuc (2) Xyl	1396	1412
A_1	T	Quin (3) Fuc (2) Xyl	1396	1412
A_2	T	Quin (2) Fuc (2) Xyl Gal	1412	1428

* M = marthasterone 3; DHM = dihydromarthasterone 2.
T = thornasterol A 13.
** Determined by GLC; Gluc = glucose, Quin = quinovose.
Fuc = fucose; Xyl = xylose; Gal = galactose.



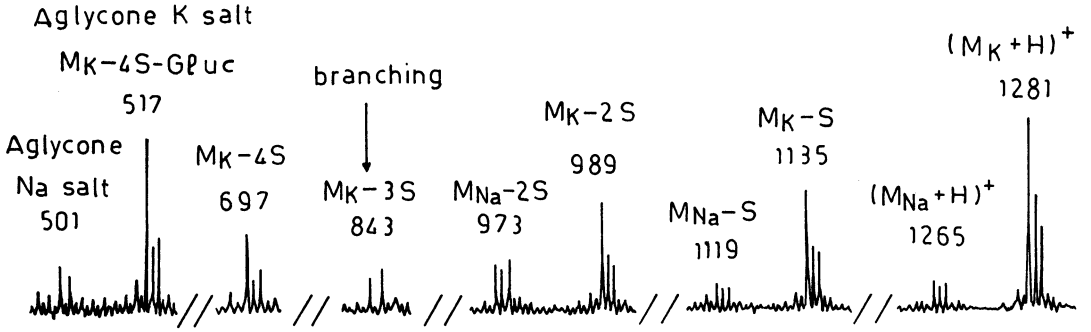


Fig. 2 - FAB spectrum of the mixture of K and Na salts of saponin C from Marthasterias glacialis; S = 6-deoxyhexase unit (fucose and/or quinovose).

In addition to the $(MK + H)^+$ and $(MNa + H)^+$ ions, the spectrum contains ions produced by sequential cleavage of glycosidic linkages; the low intensity of the ion at m/z 843, originating from the molecular ion by the consecutive loss of three 6-deoxyhexose units, might suggest the point of branching in the carbohydrate chain; the ion at m/z 697 clearly indicates that glucose is directly linked to the aglycone; the spectrum also contains an intense ion at m/z 517 indicating the mass of the aglycone. Thus FAB mass spectrometry, which is an unique technique in that it produces molecular ions of involatile organic salts, in combination with the recent advances in ^{13}C NMR spectroscopy, will enable the determination of the sequence and interglycosidic linkages of the sugars of sulphated "asterosaponins", without having to degrade the molecules. Indeed the potentiality of FAB mass spectrometry in the determination of the sequence of sugars in starfish saponins is reduced because the oligosaccharide portions of these molecules are usually composed of several sugar units (6-deoxyhexose units) having the same molecular weight.
The proposed sequence of the five monosaccharides of saponin C, shown in fig.2, is mainly derived from the results of partial hydrolysis with mixed glycosidase from Charonia lampas By a similar procedure the sequences of the sugars of saponins B and C_1, which inter alia contain identical carbohydrate chains (^{13}C NMR spectra identical in the sugar carbon region), have been determined :

(Fuc ⟶ Fuc ⟶ Quin ⟶ Gluc)-aglycone.
↑
Quin

STEROIDAL CYCLIC GLYCOSIDES

Toxic saponins of a completely new type have been recently discovered in starfishes of the genus Echinaster. 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. The saccharide portion includes an unprecedent glucuronate (Na salt) unit attached at C-3. The Echinaster species we have investigated do not contain the previous sulphated "asterosaponins".

Figure 3 presents the structure of the major toxic saponin, 18, from the Mediterranean starfish Echinaster sepositus, which we named Sepositoside A. A full account of the structure work is reported in Ref. 32.

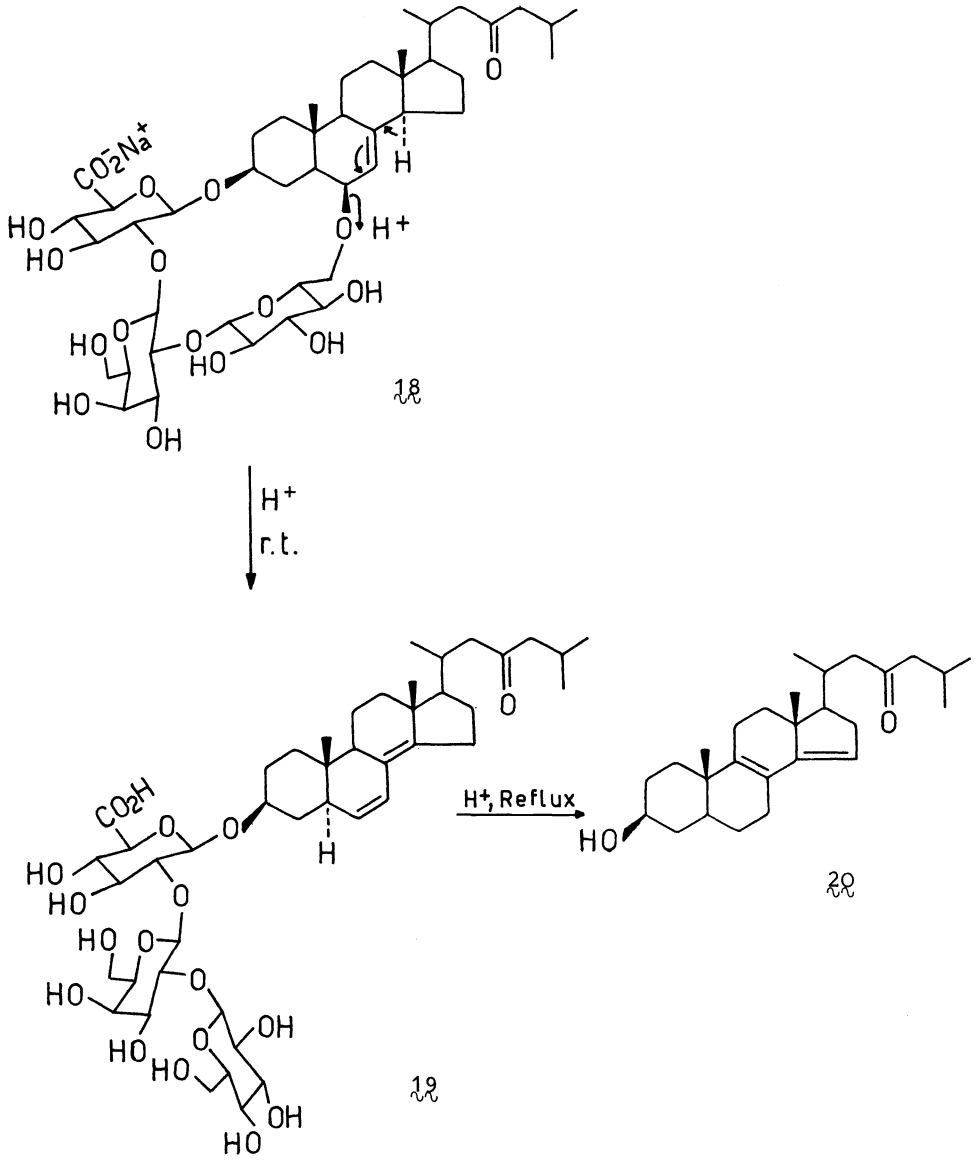


Fig. 3 - Structure of sepositoside A, the toxic major saponin of Echinaster sepositus (18).

The key step, during the structural work, was the very mild hydrolysis of 18 which resulted in the opening of macrocyclic ring, made up by the trisaccharide moiety, giving rise to the formation of UV active compound 19; more vigorous hydrolysis gave the steroid 20 (Ref. 33) in which the 6,8(14)-diene has migrated to an 8,14-diene. The structure of the steroidal portion of the opened glycoside 19 was based on spectral data and comparison with the model 5α--cholesta-6,8(14)-dien-3β-ol. The sequence of the three monosaccharides of 19 was determined by EI mass spectrometry of the permethylated derivative and acids hydrolysis of this latter, which established glucose as the terminal sugar. The interglycosidic linkages were determined by using ^{13}C NMR spectroscopy. Table 2 reports the assignments of the sugar carbon signals, which have been made by comparing the spectrum with those of methyl-β-D- glucopyranoside (Ref. 34), -β-D- galactopyranoside (Ref. 33) and -β-D-glucuronopyranoside (Ref. 32). At first the values of the anomeric carbon atoms are suggestive of β-glycopyranosyl linkages for all the monosaccharides; appearance of one anomeric carbon signal at relatively high field (δ 101.7 ppm) is explained in terms of shielding effects expected for the C-1 signal in secondary alcoholic β-D-glycopyranosides (Ref. 36); so the signal at δ 101.7 is due to C-1 of the glucuronate unit. The presence of two signals at δ 62.1 and 62.6 ppm, which may be assigned to hydroxymethylene carbons, excludes a glycosidation at C-6 of galactose. Glycosidation at C-4 and C-3 of the galactose unit can be also excluded by (i) the appearance of a signal at δ 69.9 ppm which may only be assigned to C-4 of galactose and (ii) the recent observation of Voelter et al. (Ref. 35) of a strong upfield shift of ca. 4.5 ppm for C-4 in 3-O-substituted galactopyranosyl residue; so it should be expected that a resonance line at ca. δ 65 ppm and no line between δ 62.6 and 69.9 ppm would appear in the spectrum of 19. The C-2 glycosidic linkage is also evident because the C-2 carbon in the galactose residue is shifted downfield by ca. 10 ppm (β effect) to 83.0 ppm. The signal at δ 85.5 ppm can be assigned to the glycosidated carbon of the glucuronate unit and its appearance relatively downfield suggests a substitution at C-2. Indeed the glycosidic linkages of the opened sepositoside A 19 have been confirmed by the classical chemical method of permethylation followed by acid hydrolysis and identification of the partially methylated sugars.

TABLE 2. ^{13}C NMR shifts of sugar carbons in 19 and 28+

Sugar carbon atoms	19			28		
	Gluc	Gal	Glucur	Gal	Arab	Glucur
1	106.1	104.8	101.7	106.4	103.9	101.7
2	75.2	83.0	85.4	72.5	81.7	84.0
3	77.9	76.5	77.1	73.5	74.9	77.4
4	71.3	69.9	72.4	70.5	68.6	73.2
5	79.0	76.2	76.5	76.4	65.8	77.2
6	62.6	62.1	172.7	62.6	-	172.7

+ Measured in CD_3OD; the chemical shifts are expressed as δ.

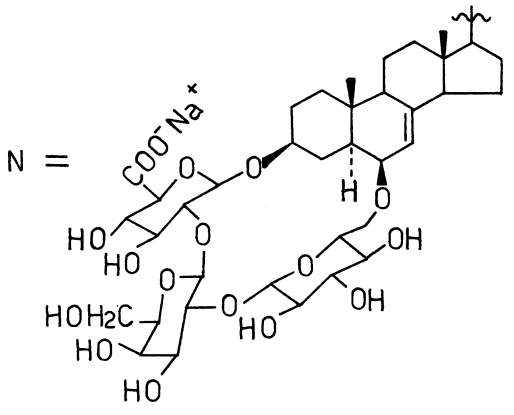
The easy formation, by very mild acid treatment, of a steroidal $\Delta^{6,8(14)}$diene immediately suggested a Δ^{7},6-O-steroidal structure for the intact saponin, whose UV spectrum showed no absorption beyond λ 210 nm and the ^{13}C NMR showed the presence of only one trisubstituted double bond, δC 143.0 (s) and 119.0 (d) ppm. The direct comparison of the chemical and spectral properties of sepositoside A with those of the models 5α-cholest-7-ene-3β,6α-diol and 5α--cholest-7-ene-3β,6β-diol supported the presence in the aglycone portion of a Δ^{7},6-O-structure and also indicated a 6β-stereochemistry. Particularly relevant in this respect were the chemical shift values for the 19- and 18-protons as well as the chemical shift and shape for the olefinic 7-H proton signal. In the spectrum of the 3β,6α-diol epimer these signal appeared at 0.85, 0.55 and 5.18 (br s, $W_{\frac{1}{2}}$ = 4 Hz) and in that of the 3β,6β-diol epimer they appeared at δ 0.94, 0.61 and 5.45 (br d, $W_{\frac{1}{2}}$ = 11 Hz), respectively. In the spectrum of the permethylated sepositoside A, recorded in $CDCl_3$, the same signals were observed at δ 0.94, 0.62 and 5.45 (br d, $W_{\frac{1}{2}}$ = 11 Hz). Both epime-

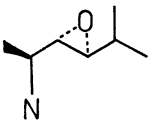
ric 5α-cholest-7-ene-3β,6-diols, on very mild acid treatment, afforded rapidly 5α-cholesta-6,8(14)-diene-3β-ol paralleling the sepositoside A's chemical behaviour.

A more significant difference between the intact saponin 18 and the opened glycoside 19, was the formation of a tri-O-methylglucose, identified as 2,3,4--tri-O-methylglucose, by acid hydrolysis of the permethylated intact saponin instead of the 2,3,4,6-tetra-O-methylglucose obtained from the permethylated opened glycoside. This indicated a substitution at the HO-C-6 carbon of glucose in 18, removable by very mild acid treatment. Moreover, in the mass spectrum of the permethylated sepositoside A the peaks at m/z 219 and 187 observed in the spectrum of the permethylated opened sepositoside A and due to the terminal permethylated glucose are replaced by peaks at m/z 205, 187 and 173 indicative of a terminal trimethylated hexose. Further, the ^{13}C NMR spectra of 18 and 19 contain marked differences in the sugar carbon region, but much more significantly the spectrum of the intact saponin 18 contains only one signal due to a primary hydroxy-bearing carbon at δ 61.7, whereas the spectrum of the opened glycoside 19 shown two signals for HO-C-6 carbons at δ 62.6 and 62.1.

The above evidence gave the cyclic structure 18 for the E.sepositus major saponin. The cyclic structure of sepositoside A appears unique; the macrocyclic ring made up by the sugar moiety bridging C-3 and C-6 of the steroid is unusual, but also the Δ^7,6β-oxygenated steroidal structure has not previously been encountered among naturally occurring steroids. We would note that the macrocyclic ring in 18 is reminiscent of a crown ether and the cavity can easily accomodate the sodium cation. Interestingly, unlike the sulphated asterosaponins which have been found associated with potassium and sodium cations, sepositoside A was obtained as pure sodium salt.

Sepositoside A is accompained by smaller amounts of three related saponins, 21-23, whose structures also include the cyclic trisaccharide moiety bridging C-3 and C-6 of the steroidal aglycones (Ref. 37). The more polar component 21





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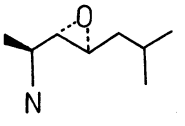

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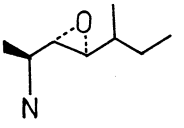

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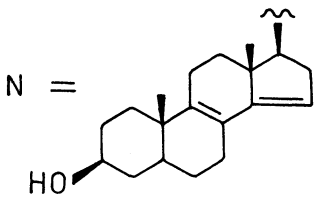
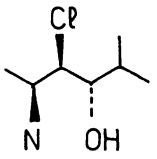
gave, on prolonged acid hydrolysis with HCl, the C-26 chlorohydrin 24, while the less polar components 22 and 23, which were obtained in admixture, yielded the C-27 chlorohydrins 25 and 26 (Ref. 38). The origin of these chlorohydrins from the corresponding epoxides was at first suspected from the formation of the bromohydrins, when the hydrolysis was carried out with HBr. The comparison of the ^1H NMR and ^{13}C NMR spectra of the epoxysteroidal cyclic glycosides and their opened derivatives with those of sepositoside A 18 and the opened 19 established the structure of the carbohydrate portion and the steroidal nucleus. A model study confirmed that the minor saponins 21-23 are {22S, 23S}-epoxides, as one would expect on the basis of the formation of the {22R, 23S} -chlorohydrins 24-26. We have prepared both {22S, 23S}- and {22R, 23R}-trans-epoxides and one 22,23-cis-epoxide and have compared their ^{13}C NMR spectra with those of the minor opened glycosides; the data are summarized in Fig. 4. The high field resonance of carbon-21 (16.8 - 16.7 ppm) and the low field resonance of carbon-20 (39.2 - 39.3 ppm) in the natural epoxides excluded a cis-stereochemistry (C-21 : 22.5; C-20 : 33.1 ppm) for them, while the resonance of carbon-17, which is significantly high field shifted in the model





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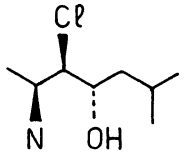

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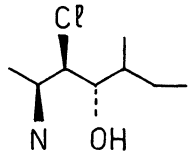

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{22R, 23R}-trans-epoxide, was the clear indicator of the {22S, 23S}-trans-stereochemistry for the natural saponins.

The structures of the minor saponins of E.sepositus combine the uniqueness of a macrocyclic ring made up by the carbohydrate moieties which bridge C-3 and C-6 of the steroid with an unusual epoxide functionality in the side chain. The occurrence of 22,23-epoxysteroids in one starfish is of biological interest because of their probable role in the biosynthesis of the 23-oxo function of the many aglycones of asterosaponins.

A further example of this novel class of steroidal cyclic glycosides has recently been discovered from a starfish of the same geneus, E.luzonicus, collected near Nouméa, Nouvelle Calédonie. The major saponin, luzonicoside, on acid hydrolysis, yielded 20, and, unlike the E.sepositus saponins, galactose, arabinose and glucuronic acid. The cyclic structure 27 for luzonicuside was deduced from the comparison of the spectral data of the intact molecule itself and its opened derivative 28 with those of sepositoside A (18) and the opened 19 (Ref. 39). The sequence of the sugar residues and the interglycosidic linkages in 28 were determined by using the same procedure described for opened sepositoside A (19), i. e. mass spectrometry and ^{13}C NMR spectroscopy (see Table 2). We would note that in both sepositoside A (18) from E.sepositus and luzonicuside (27) from E.luzonicus, the macrocyclic ring made up by the sugar portions has the same size and conformation.

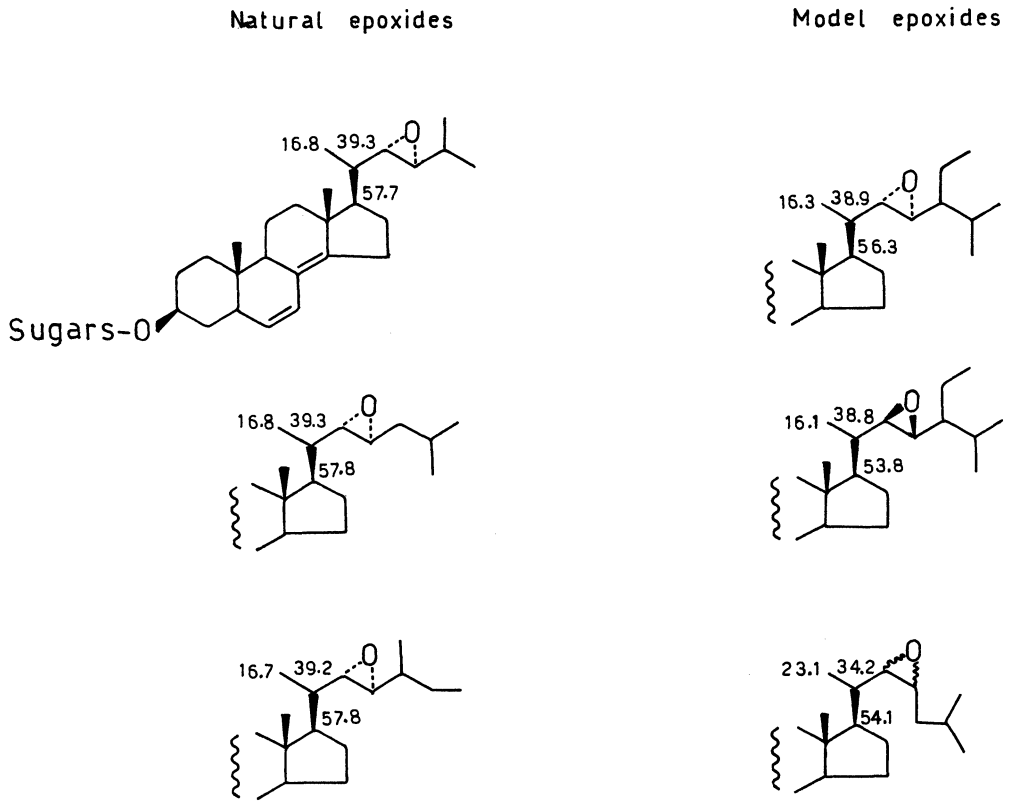
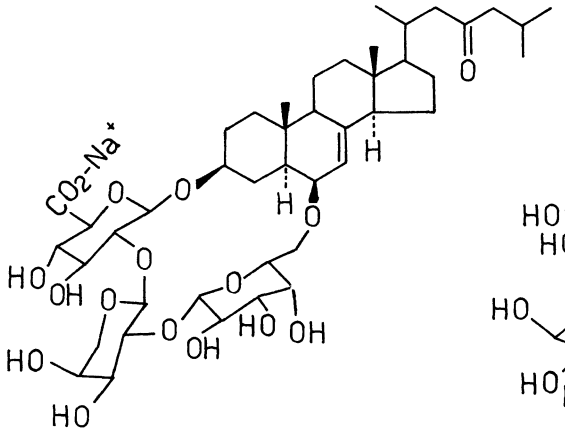


Fig. 4 - Relevant ^{13}C NMR data of the minor opened epoxysteroidal saponins and the model epoxides.



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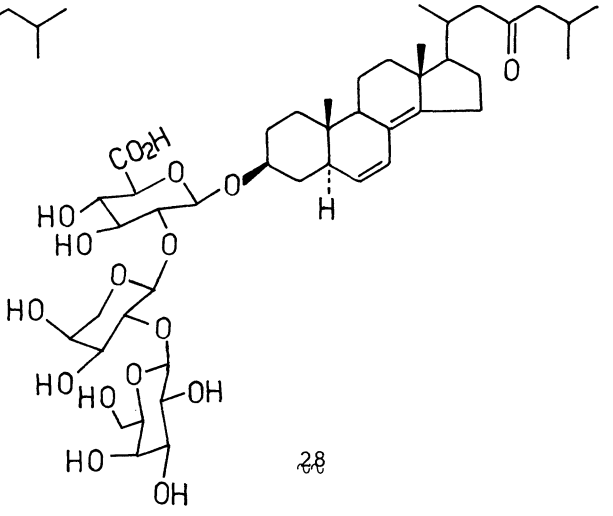

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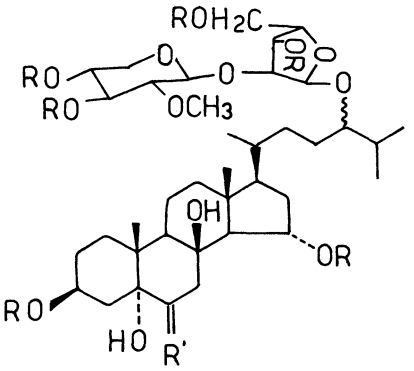
THE 24-O-GLYCOSIDATED STEROIDS

A third group of steroidal glycosides have been now discovered in two starfish species, the Pacific Protoreaster nodosus collected near Nouméa - Nouvelle Calédonie - and the Mediterranean Hacelia attenuata, collected in the bay of Naples. These compounds, which occurr in very small amounts, are composed of a polyhydroxylated (five or six hydroxyl groups) steroidal aglycone and a carbohydrate portion which is glycosidally attached at C-24 of the steroid; there is no sulphate group. The oligosaccharide portion includes α-L-arabino furanosyl or 2-O-methyl-β-D-xylopyranosyl-(1→ 2)-α-L-arabinofuranosyl residues. Both species also contain the sulphated "asterosaponins".

Methanol extraction of lyophilized specimens of the starfish P.nodosus yielded, after SiO_2 short column chromatography followed by preparative LC on SiO_2 and HPLC on C_{18} μ-bondapak, the novel glycoside, nodososide, in 0.003% yield, which showed moderate cytotoxic activity. The structure determination of nodososide, 29, is described in a preliminary communication (Ref. 40) and here is briefly summarized. Characterization of nodososide, $C_{38}H_{66}O_{14}$, pointed to an hexahydroxylated saturated sterol linked to a disaccharide residue. Acid methanolysis followed by benzoylation with p-bromobenzoyl chloride and pyridine of the reaction mixture yielded 2-O-methyl-3,4-di-O-(p-bromobenzoyl)-β-D-xylopyranoside, CD : 236/253, Δε + 12/ - 38, A = - 50 and methyl 2,3,4-tri-O-(p-bromobenzoyl)-α-L-arabinopyranoside, CD : 236/253, Δε - 30/ + 95, A = + 125. The signs and amplitudes of the exciton-split CD curves accompayning the two structures, established that the xyloside belong to the D-series and the arabinoside to the L-series (Ref. 41). An analysis of the richly detailed 500 MHz high-resolution ^1H NMR spectrum of 29 established the configuration of the glycoside linkages and that the arabinose is in its furanose form. (Table 3). Analysis of the ^{13}C NMR spectrum provided corroborative evidence. Acetylation of 29 produced an hexaacetate 30 showing in the ^1H NMR spectrum the 2-O-Me-xyl 2-H and the arab 2-H signals essentially unshifted, thus establishing



29 , R = H , R' = 

30 , R = Ac , R' = 

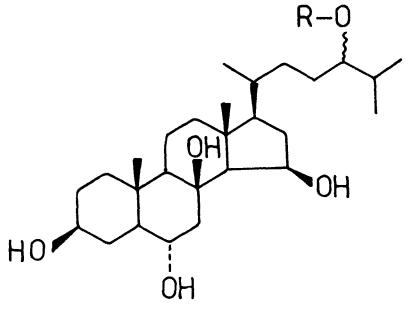
31 , R = Ac , R' = O

TABLE 3. 500 MHz ^{1}H NMR Data in δ (Hz) of nodososide (29)

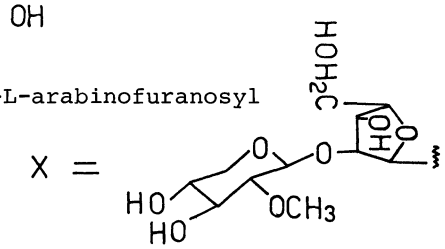
	Proton					
	1	2	3	4	5	
2-O-Me-β-D-xylopyranosyl	4.537 d (7.7)	3.036 dd (7.7, 9.0)	3.472 dd (9.0, 9.0)	3.631 ddd (10.3,9.0,5.6)	3.261 dd (11.6,10.3)	- 3.903 dd (11.6,5.6)
α-L-arabino-furanosyl	5.146 brs	4.153 d (3.8)	4.129 dd (7.2, 3.8)	4.034 ddd (7.2, 4.6,3.5)	3.761 dd (12.5, 4.6)	- 3.835 dd (12.5,3.5)

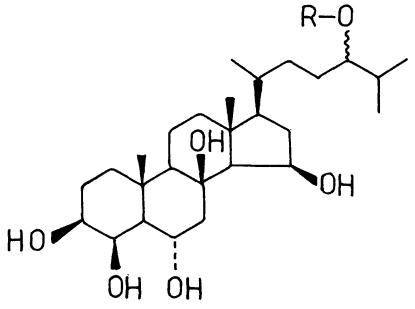
both the sequence and the interglycosidic linkage as shown. The 5α-cholestane-3β,5,6β,8,15α,24-hexol formulation for the aglycone component was based mainly on ^{1}H and ^{13}C NMR studies. Assignments of carbon signals have been made by using 5α-cholestane-3β,5,6β-triol and 24-hydroxycholesterol as model structures (Refs. 42 and 43) and the substituent effects that have been published for hydroxylated steroids (Refs. 44, 45). Rutine chemical transformations and the related spectral properties provided additional evidence confirming the proposed formulation 29. Oxidation of the hexaacetate 30 produced a monoketone 31, whose ^{1}H NMR showed the 19-H signal at upfield position, δ 1.00, relative to 30, δ 1.337, thus giving evidence for the removal of a 1,3-diaxial methyl-hydroxyl interaction in the conversion 30 → 31, consistent with 6β-OH assignment in 30 (and 29). Furthermore the acetate 30 formed a phenylboronate; since the ketone 31 did not react with phenylboronic anhydride, the formation of the boronate ester, which involves the 6β-OH, requires one tert-hydroxyl be situated at the 8β-position.

The Mediterranean Hacelia attenutata yielded five further related glycosides 32 - 36, all with the carbohydrate moiety at C-24. The disaccharides 32 and 34 are the major components in the glycosides mixture, and have been obtained in 0.01% and 0.005% (yield based on dry weight of the animals), respectively.

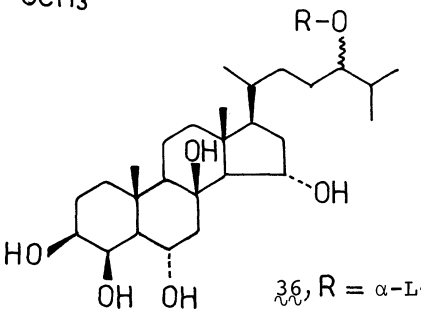


32, R = X
33, R = α-L-arabinofuranosyl





34, R = X
35, R = α-L-arabinofuranosyl



36, R = α-L-arabinofuranosyl

TABLE 4. ^{13}C NMR data of the starfish-derived 24-O-glycosidated steroids

Carbons		29	32	34	35	36
1		34.3	39.2	39.6	39.4	39.5
2		31.8	32.2	26.8	26.6	26.7
3		67.3	71.4	73.1	73.0	72.9
4		42.4	33.2	68.9	68.7	68.7
5		75.7	54.0	57.4	57.2	57.0
6		77.9	66.5	63.9	63.6	63.6
7		41.8	50.0	50.6	50.3	51.4
8		76.7	76.7	76.5	76.5	75.3
9		48.7	56.8	57.8	57.6	57.7
10		39.1	37.4	37.6	37.5	37.4
11		19.4	19.3	18.8	18.7	18.7
12		42.4	42.6	42.6	42.4	42.1
13		44.8	43.7	43.8	43.6	44.6
14		66.3	61.8	62.0	61.7	66.9
15		69.2	70.2	70.2	70.0	68.9
16		40.9	42.2	42.2	42.1	41.7
17		55.0	57.2	57.2	57.0	54.8
18		15.6	16.6	16.6	16.4	15.5
19		18.2	14.3	17.3	17.2	17.4
20		35.4	35.6	35.6	35.5	35.2
21		18.9	18.9	18.9	18.6	18.6
22		31.9	32.1	32.2	32.0	31.7
23		27.9	28.1	28.1	28.2	28.1
24		83.5	83.3	83.3	83.3	83.5
25		30.6	30.8	30.8	30.9	30.8
26		18.2	18.1	18.1	18.0	18.0
27		18.2	18.2	18.2	18.1	18.2
arab	1'	107.6	107.5	107.4	109.4	109.6
	2'	93.1	92.8	92.8	83.8	83.8
	3'	77.6	77.6	77.6	78.6	78.6
	4'	85.0	85.0	85.0	85.3	85.2
	5'	62.5	62.7	62.6	62.6	62.7
2-O-Me-xyl	1"	105.2	105.1	105.1		
	2"	84.1	84.3	84.3		
	3"	77.8	77.8	77.8		
	4"	71.0	77.1	77.1		
	5"	67.1	67.1	67.1		
OCH_3		60.7	60.6	60.6		

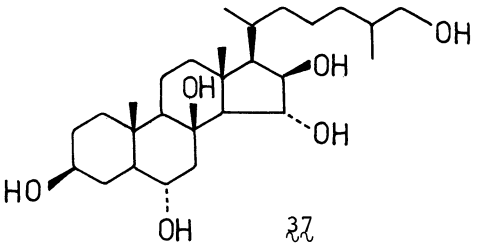
Spectra were recorded in pyridine-d_5 solution.

The arabinosides have been isolated in 0.0005% (34), 0.0014% (35) and 0.001% (36) yield. The isolation required repeated chromatographic steps including preparative LC on SiO_2, Sephadex LH-20 and HPLC on C_{18} μ-bondapack. The structures of these compounds followed from the FD-mass spectral data and analysis of the ^1H and ^{13}C NMR spectra.(Table 4).
The configurations of the sugar units were established by using the same procedure used with nodososide (29).

POLYHYDROXYLATED STEROIDS

The starfishes Protoreaster nodosus and Hacelia attenuata also yielded four related polyhydroxylated sterols. Polyhydroxysteroids are not uncommon to marine species and they have been isolated from alcyonarians (Ref. 13 and 46). The greatest number of marine polyoxygenated steroids have been aglycone constituents of starfishes, but they have never been encountered before as non-conjugated molecules in this class of marine animals.

The methanol extract of Protoreaster nodosus was a complex mixture from which, in addition to the previous nodososide (29), three related polyhydroxylated sterols 37, 38, 39 were isolated by several chromatographic steps. All of them, obtained in 0.035%, 0.002% and 0.0045% yield (dry weight basis), respectively, showed moderate cytotoxic activity. The structure determination is described in Ref. 47 based upon ^1H and ^{13}C NMR studies and chemical transformations. For



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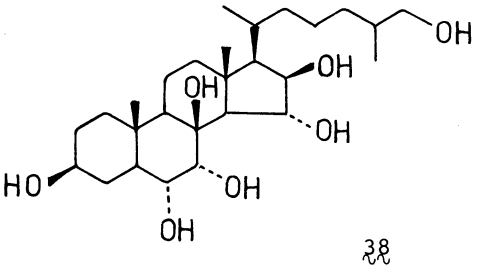

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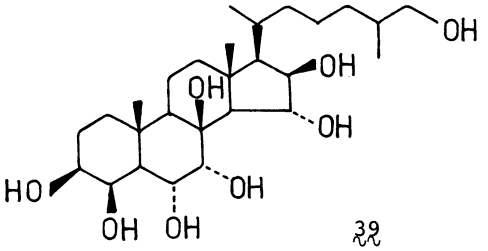

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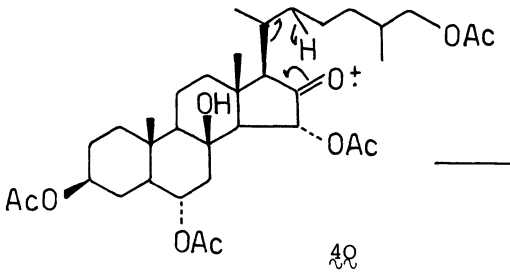
example, treatment with excess acetic anhydride of the hexol 37 produced the 3,6,15,26-tetraacetate, which, on oxidation with Jones reagent, was converted into the monoketone 40, whose EI mass spectrum displayed two diagnostically important ions at m/z 464 and 449 (base peak), typical of 16-ketosteroid. The transformation of the tetraacetate into the ketone 40 was accompained by the following changes in the ^1H NMR: the double doublet (J = 12.0 and 2.5 Hz) at δ 4.69 (15β-H) in the spectrum of the acetate was semplified into a doublet (J = 13.5 Hz) and has moved downfield to δ 5.13; decoupling experiments illustrated that 15β-H is coupled with 14α-H, δ 1.85 (d, J = 13.5 Hz); the resonance frequency of the 21-protons was shifted in the spectrum of the ketone (δ 0.90⟶ 0.99), thus giving support to the 26-OH assignment in 37. Perhaps the most significant feature of the ^1H NMR was the small change in the resonance frequency of the 18-protons on passing from the tetraacetate to the ketone 40 (δ 1.19⟶ 1.15), which is only compatible with 16β-oriented hydroxyl group in the acetate (and in 37).

The heptol 38 is related to the previous hexol 37 by introduction of the seventh hydroxyl group at 7α-position. Routine acetylation at room temperature of 38 led to the introduction of four acetate groups at 3,6,15 and 26-positions and oxidation with Jones reagent of the tetraacetate produced a monoketone 41, whose EI mass spectrum displayed two ions at m/z 480 and 465 indicative of a 16-ketosteroid. The transformation of the tetraacetate into the ketone 41 was



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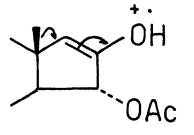
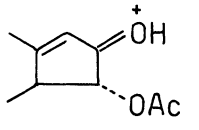
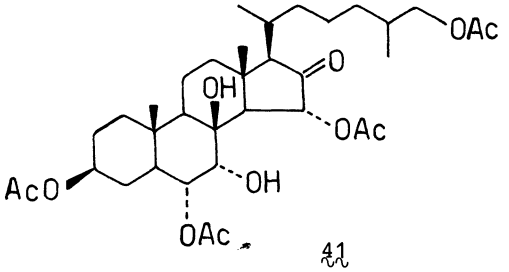


m/z 464



m/z 449 (100 %)



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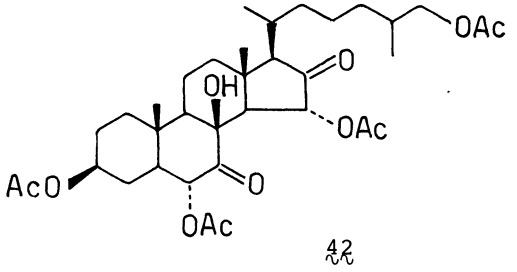

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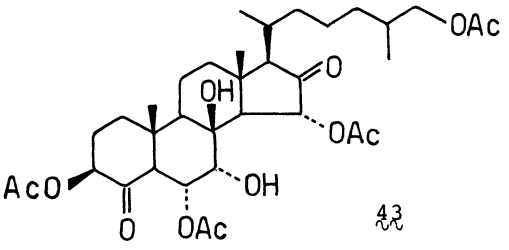
accompained by changes in the ^{1}H NMR similar to those observed with the ketone 40, but significantly in the spectrum of 41 the 14-H signal has moved downfield to δ 2.32, in agreement with the 7α-OH assignment in 38. Treatment of 41 with dimethylsulfoxide-trifluoroacetic anhydride led to the oxidation of the 7α-hydroxyl group giving rise to the formation of the diketone 42. The ^{1}H NMR of the diketone 42 was similar to that of the monoketone 41, except that the double doublet at δ 5.18 due to 6β-H is replaced by a doublet (J = 12 Hz) resonating downfield at δ 5.61, and the hydroxymethine signal is absent.

The third polyhydroxylated sterol 39 from P.nodosus contains one more hydroxyl group relative to the heptol 38 and the comparison of the ^{13}C NMR spectra of 38 and 39 immediately indicated that the novel sterol 39 was related to 38 by introduction of the eighth hydroxyl group at 4β-position. Similar transformation as before (oxidation with pyridinium dichromate of the derived tetraacetate) gave the diketone 43, which, by virtue of the C-4 and C-16 oxo functionalities, provided for an apparent first-order ^{1}H NMR spectrum in the downfield region. In particular the oxidation of the tetraacetate to the diketone 43 produced in the ^{1}H NMR the downfield shift of the 3α-H signal from δ 4.75 to δ 5.20 (dd, J = 11.0 and 7.0 Hz), the disappearance of the 4α-H signal and the change of the chemical shift of the 19-protons from δ 1.30 to δ 1.01, in agreement with the oxo function at C-4.

One more polyhydroxylated sterol, 44, was isolated from the methanol-chloroform extract of the starfish Hacelia attenuata. It lacks the 8β-hydroxyl group, which is a common feature in starfish-derived 24-O-glycosidated steroids and polyhydroxysterols from Protoreaster nodosus. The hydroxylation at C-8 is uncommon to steroids and has been encountered before only in one marine sterol isolated from the soft coral Litophyton viridis (Ref. 44). The 15α,16β,26-triol pattern seems a common element of starfish-derived polyhydroxylated sterols.

Finally, we would note that the octol 38 constitues, as far as we know, the more highly hydroxylated sterol isolated from a natural source.



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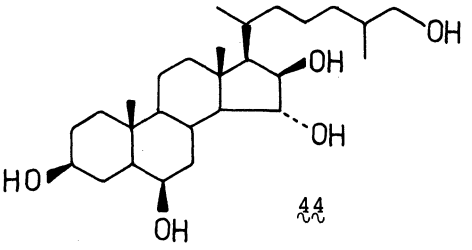

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