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STARFISH SAPONINS VI - UNIQUE 22,23-EPOXYSTEROIDAL CYCLIC GLYCOSIDES, MINOR CONSTITUENTS FROM *Echinaster sepositus*+

by

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Abstract - *On the basis of comparative ^1H- and ^{13}C-n.m.r. data, the structures of the minor saponins from the starfish Echinaster sepositus have been elucidated to be 2 - 4. They closely resemble 1, the major saponin from the same starfish, and, in addition to the cyclic trisaccharide moiety bridging C-3 and C-6 of the steroid, include epoxide functionalities in the steroidal side-chains.*

Recently[1] we have elucidated the structure of the major saponin from *Echinaster sepositus* to be *1*. On prolonged acid hydrolysis it gave the 3β-hydroxy-5α-cholesta-8,14-dien-23-one (*5*)[2]. Along with major amount of *5* (*ca.* 80% of the total sapogenins mixture) the HCl hydrolysis of the total saponin mixture also yielded three minor genins, *6* - *8*[3]. The origin of these chlorohydrins from their corresponding epoxides during acid hydrolysis was confirmed by the formation of the bromohydrins, when the hydrolysis was carried out with HBr. We now describe the structure elucidation of the minor saponins based on 22,23-epoxysteroidal aglycones.

Extraction and fractionation of the saponins mixture from *E. sepositus* have previously been described[1]; the last step of the fractionation consisted of reversed phase h.p.l.c., which gave three fractions in the ratio 0.6 : 8 : 1.4 and eluted in that order. The central fraction corresponded to the major saponin *1*. The two minor fractions, after a further h.p.l.c. step, afforded h.p.l.c. homogeneous materials. The more polar one *2*, $[\alpha]_D = -56.5°$ (c, 1, H_2O), analyzed for $C_{44}H_{67}O_{18}Na$ and gave, on hydrolysis with HCl, the C_{26} chlorohydrin *6*, while the less polar material, $[\alpha]_D - 71°$ (c, 0.4, H_2O), was a mixture of two isomeric components, 3 and 4, analyzed for $C_{45}H_{69}O_{18}Na$ and gave, on hydrolysis with HCl, the C_{27} chlorohydrins *7* and *8* in the approximately ratio of 2 : 1. Both materials, in analogy with *1*, also gave glucose, galactose and glucuronic acid, and did not show u.v. absorptions beyond 210 nm. In the ^1H-n.m.r. (DMSO) both materials showed (*a*) a broad signal ($W_{\frac{1}{2}}$ = 12Hz) at δ 5.46 corresponding to the olefinic proton at C-7, (*b*) two methyl singlets at δ 0.82 and 0.52 corresponding to CH_3-19 and CH_3-18, respectively, and three one-proton doublets at δ 5.03

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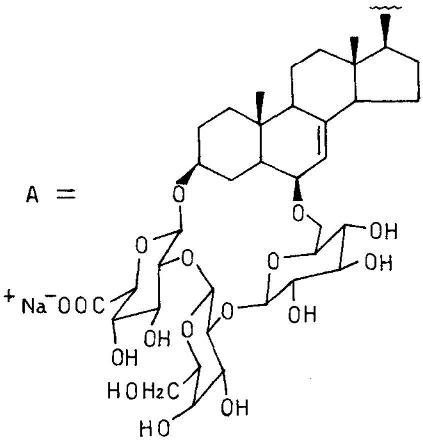
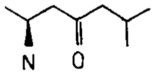
A =





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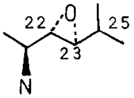

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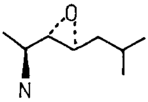
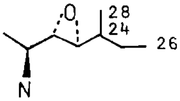
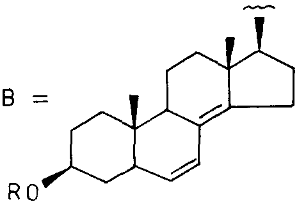


3, N=A ; 11, N=B



4, N=A ; 12, N=B

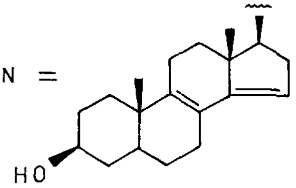
B =

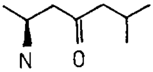


R = β-D-Gluc(1→2)-β-D-

-Gal-(1→2)-β-D-glucur.

N =





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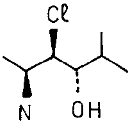

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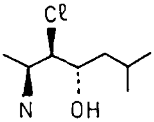

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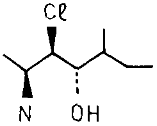

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(J = 7Hz), 4.55 (J = 7.5Hz) and 4.45 (J = 8Hz) corresponding to the anomeric protons. The same signals were also observed in the spectrum of *1* with small variations for H-7 and CH_3-18, which in *1* resonated at δ 5.43 and 0.55, respectively. Otherwise significant differences were observed for the resonances of the side-chain methyl protons; in *1* CH_3-21 protons resonated at δ 0.84 (d, J = 7Hz) and the CH_3-26,27 protons at δ 0.82 (6H, d, J = 7Hz); in *2* the CH_3-21 doublet appeared downfield shifted at δ 0.94 and the isopropyl methyl signal was split into two doublets at δ 0.87 and 0.85; in the spectrum of the mixture of *3* and *4* the CH_3-21 protons resonated at δ 0.92, while the remaining side-chain methyl protons formed a complex absorption centred at 0.90 ppm. Similarly both materials resembled *1* in their ^{13}C-n.m.r. spectra; indeed all resonances associated with the steroidal tetracyclic nucleus carbons as well as with the sugar carbon atoms had identical δ's (± 0.1 ppm) in all spectra except C-16 which

TABLE I - ^{13}C-n.m.r. shifts of the opened minor saponins *10*,*11* and *12* in CD_3OD and of the models *13*,*14* and *15* in $CDCl_3$. Pertinent shifts discussed in the text are shown in italics.

Compound	17	20	21	22	23	24	25	26	27	28	29
10[a]	*57.7*	*39.3*	*16.8*	63.9[d]	63.4[d]	-	31.9	19.5[e]	18.8[e]	-	-
11[a]	*57.8*	*39.3*	*16.8*	65.2	57.2	42.6	28.5	23.0[f]	23.1[f]	-	-
12[a]	*57.8*	*39.2*	*16.7*	63.6[g]	63.0[g]	38.7	28.6	11.9	-	15.9	-
13[b]	*56.3*	*38.9*	*16.3*	62.9	58.7	49.0	29.6	19.5	19.5	21.2	12.4
14[b]	*53.8*	*38.8*	*16.1*	62.1[h]	61.9[h]	49.0	29.4	19.8[i]	20.2[i]	21.3	12.4
15[c]	*53.9*	*33.1*	*22.5*	62.0	58.2	38.1	27.0	22.6	22.6	-	-

a) Remaining carbon resonances in *10*, *11* and *12* have identical δ's (± 0.1 ppm) and are similar (± 0.1 ppm) to those described for *9*[1] except for C-15 and C-16 which in the spectra of the epoxysaponins resonated at 26.2 and 27.9 ppm; signals assigned to *11* and *12* have been extracted from the spectrum taken on their *ca.* 2 : 1 mixture; the 22- and 23-methine signals in *11* and *12* were distinguished by substituent chemical shift considerations, and comparison with *13* as reference compound.

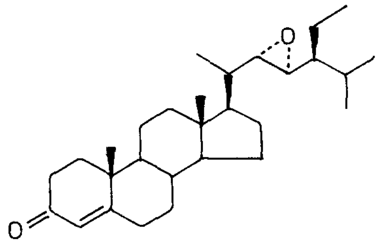
b) Remaining carbon resonances in *13* and *14* are similar (± 0.2 ppm) to those described for cholest-4-en-3-one[4].

c) Remaining carbon resonances in *15* are similar (± 0.1 ppm) to those described for ergosteryl acetate[5].

d-i Assignments can be reversed.

in the spectrum *2* and in that of the mixture of *3* and *4* was observed at 29.4 and 29.5 ppm, respectively (the shifts have previously been reported for *1*[1]). Both materials resembled *1* also in the chemical behaviour. On very mild acid treatment they gave the corresponding opened glycosides, u.v. active [λ_{max} 259 (s) 251 (ε, 18.200), 244 (s)], $[\alpha]_D$ - 34.7° for *10* and - 37.9° for the mixture of *11* and *12*. Direct comparison of their spectral data, ^{1}H-n.m.r. [0.96 s, 0.66 s, 5.3 d (J = 10Hz), 6.12 dd (J = 10, 3Hz) for CH_3-18, CH_3-19, H-6 and H-7 in the spectra of both materials] and ^{13}C-n.m.r. (all resonances associated with the steroidal tetracyclic nucleus carbons as well with sugar carbon atoms are virtually superimposable in the spectra of *9*, *10* and the mixture of *11* and *12*) with those of the opened glycoside *9*[1] established the steroid -3O-β-D-glucopyranosyl-(1 → 2)-β-O-galactopyranosyl-(1 → 2)-β-D-glucuronopyranoside structures for both materials.

Further, the comparison of the ^{13}C-n.m.r. shifts of the side-chain carbon atoms with those of the models, *13*, *14* and *15* (Table I), has confirmed the 22α,23α-epoxy-*trans*-stereochemistry of the minor *E. sepositus* saponins. The synthesis of *13* and *14* have already been described[3]; the 22,23-*cis*-model *15* has been prepared as follows. Ergosteryl acetate was converted into the cyclic adduct by reaction with 4-phenyl-1,2,4-triazolidine-3,5-dione and then ozonized to afford the hexanor-aldehyde *16*, according to Barton *et al.*[6]. Wittig reaction of *16* with the phosphorane derived from isopentyltriphenylphosphonium bromide in THF at room temp. overnight[6] furnished, after reacetylation, a *cis-trans* (*ca.*9 : 1) mixture of the protected 3-acetoxyl-cholesta-5,7,22-diene. The Δ^{22}-*cis*-isomer, m.p. 116-117°, ^{13}C-n.m.r., C-20 : 33.8 ppm (expected for a Δ^{22}-*trans*-isomer : C-20 : 40.5) was then epoxidized with 50% of *m*-Cl perbenzoic acid at 0°C to give the corresponding 22,23-epoxyderivative (only one isomer), m.p. 100-1°C, $[\alpha]_D$ - 10.1°. Retro-1,4-cycloaddition by heating the adduct at 120° in DMSO with K_2CO_3[7] eventually yielded the 22,23(*cis*)-epoxy-cholesta-5,7-dien-3β-yl acetate (*15*), m.p. 78-80°C, $[\alpha]_D$ - 51°, m/e 380 (M^+ - AcOH), δ 0.63 s (CH_3-18), 0.96 s (CH_3-19), 1.01 d (CH_3-26,-27), 1.14 d (CH_3-21), 2.6 m and 3.04 m (H-22, H-23). The high field resonance of C-21 (16.8-16.7 ppm) and the low field resonance of C-20 (39.3 - 39.2 ppm) in the natural epoxides clearly excluded a *cis*-stereochemistry (C-21 : 22.5, C-20 :



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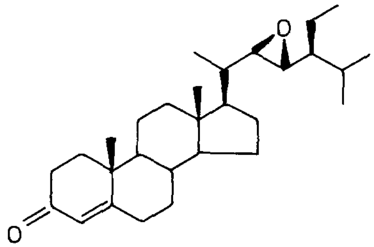

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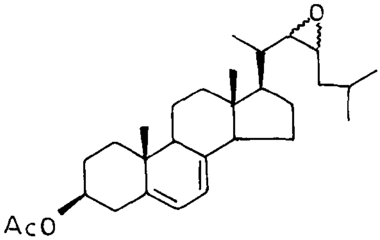

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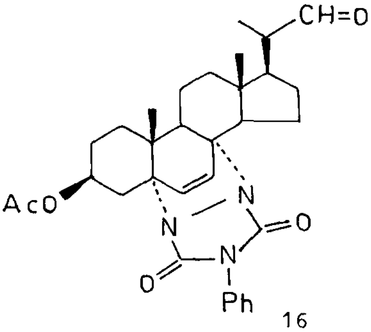

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33.1 ppm in *15*) for them, while the resonance of C-17, which is significantly high field shifted in the model β-*trans*-epoxide *14*, was the clear indicator of the 22α,23α-*trans*-stereochemistry for the natural saponins.

Thus the structures of the opened minor *E. sepositus* saponins are established to be *10*, *11* and *12* and accordingly the cyclic structures *2*, *3*, *4* follow for the intact saponins. They combine the uniqueness of a macrocyclic ring structure made up by the sugar moieties which bridge C-3 and C-6 of the steroid with an unique epoxide functionality at C-22 and C-23 in the side-chains.

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