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STARFISH SAPONINS II. 22,23-EPOXYSTEROIDS, MINOR GENINS FROM THE STARFISH *ECHINASTER SEPOSITUS*[*]

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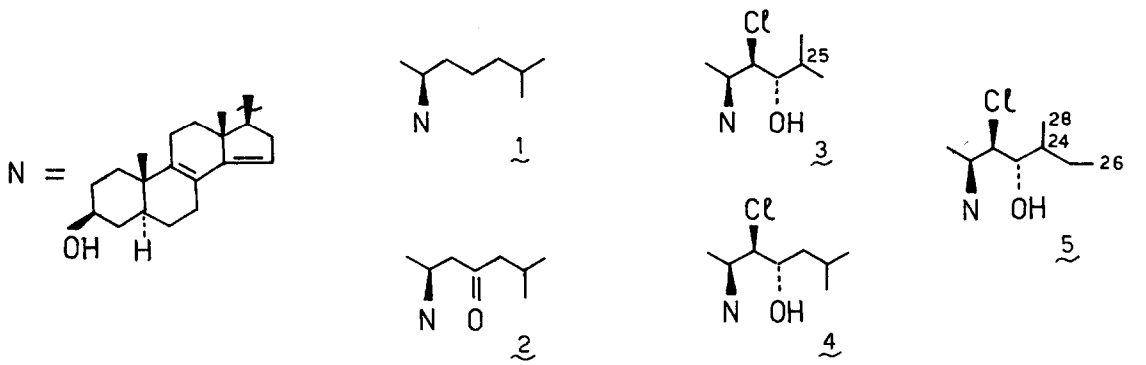
Recently[1] we reported the isolation of the novel steroid 3β-hydroxy-5α-cholesta-8,14-dien-23-one (*2*) from the hydrolyzate with HCl of the *Echinaster sepositus* saponins mixture. We now describe the isolation from the same source and the structure determination of three minor genins: 24-nor-22(R)-chloro-5α-cholesta-8,14-diene-3β,23(S)-diol (*3*), 22(R)-chloro-5α-cholesta-8,14-diene-3β, 23(S)-diol (*4*) and 27-nor-24ξ-methyl-22(R)-chloro-5α-cholesta-8,14-diene-3β,23(S)-diol (*5*).



The likely origin of these chlorohydrins from their corresponding 22,23-epoxides during HCl hydrolysis of the saponins has been confirmed by formation of the corresponding bromohydrins, when the hydrolysis was carried out with HBr. 22,23-Epoxysteroids are of interest because of their unique natural occurrence and probable role in the biosynthesis of marine 23-oxo-[2] and 22,25-oxidosteroids[3]. In this connection it is important to note that recently Berti *et al.*[3] reported the easy transformation of 22,23-epoxysteroids into 22,25-oxidosteroids by using BF_3-ether complex.

Compounds *3*, *4* and *5*, obtained as a single fraction after chromatography on silica gel of the crude asterosapogenins[1], were separated by reverse phase h.p.l.c. (C-18 μ-bondapack, CH_3CN : H_2O, 4 : 1) and eluted in that order in the approximately ratio of 1 : 2 : 1, respectively. High resolution mass spectrometry established the molecular formulas, $C_{26}H_{41}O_2Cl$ (measured 420.2755; calculated for $C_{26}H_{41}O_2{}^{35}Cl$ 420.2795) for *3*, oil $[\alpha]_D$ - 12.3°, and $C_{27}H_{43}O_2Cl$ (measured 434.2934, *4* and 434.2941, *5*; calculated for $C_{27}H_{43}O_2{}^{35}Cl$ 434.2951) for both *4*, oil, $[\alpha]_D$ - 41.5° and *5*, oil, $[\alpha]_D$ -28.0°. The presence of the nucleus of 5α-cholesta-8,14-dien-3β-ol in all three compounds was established in a straight forward manner from spectral data, u.v. (λ_{max} 248 nm), m.s. (peaks at 269-271 due to the loss of the side-chain), ^{1}H-n.m.r. [H_3C-18, 0.84 ± 0.01; H_3C-19, 0.98 ± 0.01; H-3, 3.60 ± 0.02 (bm); H-15, 5.36 ±

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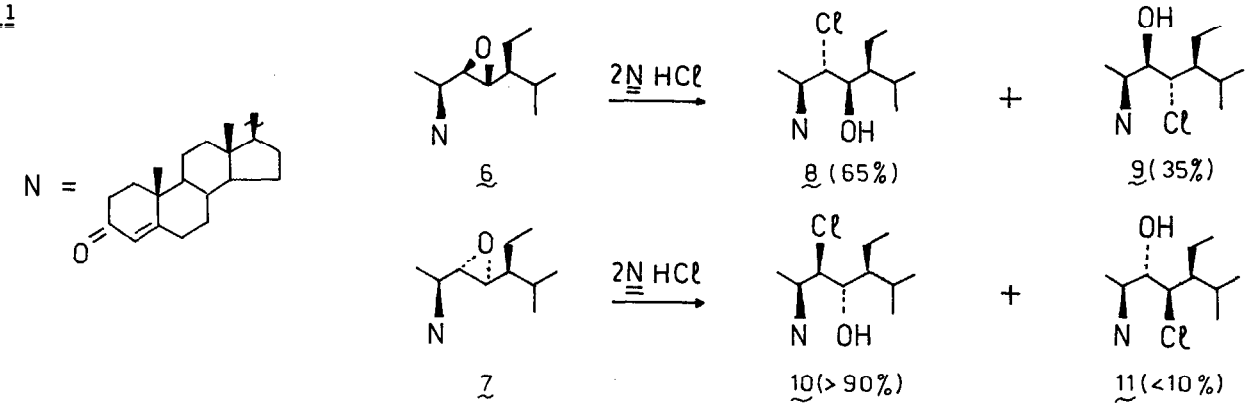
CHART 1



Epoxide 6 H-22 or H-23: 2.74 (dd, 2.2, 7.5 Hz); C-22 & C-23: 62.1 & 62.9; C-17: 53.8; m/e 426 (M^+, 5%), 341 (100%); m.p. 144-146°; $[\alpha]_D$ + 73.2°; more polar.

Epoxide 7 H-22 and H-23: 2.5 br; C-22: 62.9; C-23: 58.7; C-17: 55.8; m/e 426 (M^+ <1%), 341 (100%); m.p. 116-117°; $[\alpha]_D$ + 47.2°; less polar.

Chlorohydrin 8 H_3C-18: 0.72; m/e 462-464 (M^+), 348-350 (22,23-cleavage); m.p. 214-216°; less polar.

Chlorohydrin 9 H_3C-18: 0.77; m/e 426 (M^+-HCl), 329 (22,23-cleavage); m.p.230-232°; more polar.

Chlorohydrin 10 H_3C-18: 0.77; m/e 326 (M^+-HCl), 348-350 (22,23-cleavage); m.p. 217-221° (dec.); less polar.

Chlorohydrin 11 H_3C-18: 0.73; m/e 329 (22,23-cleavage); m.p. 194-195°; more polar.

Note: Epoxide *6* and *7* were obtained in a 6:4 ratio by treatment of (22E)-stigmast-4,22-dien-3-one with m-chloroperbenzoic acid and separated by h.p.l.c. (C-18 μ-bondapack); *8*, *9*, and *10*, *11* were separated by p.l.c. on SiO .

TABLE II. - 270 MHz ^{1}H-n.m.r. ($CDCl_3$) data in δ (Hz)[a]

Proton	*3*	*4*[b]	*5*	*8*	*9*	*10*	*11*
H-22	3.98 (d,9.5)	3.80 br	3.98 (d,9.5)	4.04 (d,10)	3.90 (d,10)	4.08 (d,10)	3.78 (d,9)
H-23	3.62 (dd,9.5,3.5)	3.80 br	3.58 (dd,9.5,3.5)	3.97 (d,10)	4.02 (dd, 10,3)	3.85 (dd,10,3)	4.28 (d,9)

(a) >CHCl and >CHOH were differentiated upon acetylation: >CHO-protons were observed at 5.16-5.30 ppm while >CHCl protons were only slightly affected.

(b) H-22 and H-23 signals were resolved in the spectrum of the acetate; 4.01 (d, 9.5) and 5.16 (bt, 9.5) ppm.

0.01 ($W_{\frac{1}{2}}$ 6Hz) ppm] and especially ^{13}C-n.m.r. (Table I), and comparison with those of *1* and *2*. In addition to H_3C-18 and H_3C-19 singlets the methyl region in the 270 MHz ^{1}H-n.m.r. spectra of *3* and *4* showed three 3H doublets (H_3C-21, H_3C-26 and H_3C-27) at 1.04, 1.00 and 0.88 ppm and 1.05, 0.98 and 0.95 ppm, respectively, while the spectrum of *5* exhibited two 3H doublets (H_3C-21 and H_3C-28) at 1.04 and 0.92 ppm and one partially overlapped 3H triplet at 0.93 ppm attributed to the presence of an ethyl on the side-chain. In agreement with this assignment is the ^{13}C-n.m.r. frequency for C-26 (11.5, q). These data are indicative for the presence of a 24-nor side-chain, a conventional C_8 side-chain and a C_8 side-chain of the 27-norergostane type in *3*, *4* and *5*, respectively. Naturally accurring C_{26} sterols with the missing carbon originating from the side-chain (24-nor) and C_{27} sterols having a 27-norergostane type side-chain have been recently encountered among marine sources[4].

TABLE I. - ^{13}C-n.m.r. ($CDCl_3$) chemical shifts[a] of *1* - *5* and *8* - *10* in δ (TMS = 0)

Compound	15	17	18	20	21	22	23	24	25	26	27	28	29
1	117.4	57.3	15.8	34.1	19.0	36.2	23.8	39.6	28.1	22.7	22.8	-	-
2	116.7	57.1	15.7	30.8	20.0	50.3	210.0	52.6	24.5	22.6	22.6	-	-
3	116.9	54.4	16.2	35.1	13.0	67.8	76.2	-	29.3	20.3	14.4	-	-
4	116.8	54.2	16.1	35.1	13.1	71.0[b]	71.3[b]	44.3	24.9	21.6	21.6	-	-
5	116.8	54.0	16.0	35.1	13.0	67.9	76.9	36.1	29.7	11.5	-	16.0	
8	24.5	54.2	11.9	39.8	14.9	67.3	73.5	47.4	29.2	19.4	18.6	21.2	13.5
9	23.8	53.0	12.0[c]	37.2	12.1[c]	73.7	64.8	46.4	27.9	20.0	19.2	21.3	12.5[c]
10	24.1	53.4	12.5[d]	36.7	12.6[d]	67.7	73.5	46.1	26.5	19.2	19.0	21.3	12.9[d]

(a) - Remaining carbon resonances have almost identical δ's (± 0.1 ppm) in *1-5* and have already been described for *1* and *2* in the previous paper[1]. Chemical shifts of carbons other than those cited above in *8*, *9* and *10* were only slightly affected by the structure change in the side chain, and are similar (± 0.2 ppm) to those described for cholest-4-en-3-one[5].

(b,c,d) - Assignments can be reversed.

The 270 MHz ^{1}H-n.m.r. spectra of *3* and *5* are completed by a 1H d (J 9.5 Hz) at 3.98 ppm (>CHCl) coupled with a dd (J 9.5, 3.5 Hz) emerging from the 3α-H broad multiplet at *ca.* 3.60 ppm and due to an additional hydroxy methine proton; in *4* these signals overlap at 3.80 (2H) ppm, but in the spectrum of its diacetate *4a* they became clearly separated and resonated at 4.01 (d, J 9.5 Hz, >CHCl) and 5.16 (bt, J 9.5 Hz, >CHOAc) ppm. The pattern of these two protons in *4a* is only compatable with the oxygenated function at C-23, while the ^{13}C-n.m.r. data of *4* (Table I) require the chlorine to be located at C-22. Using the C-24 chemical shift of *1* and chlorine and hydroxyl substituent parameters given in ref. *6*, the predicted chemical shift for the C-24 carbon of structure *4* is 43.6 (exp. 44.3); similar calculations predict a C-22 chemical shift of 40.2 in a 23-hydroxy-24-chlorostructure. This assignment is strengthned by the high field frequency of the H_3C-21 carbon (γ-steric interaction). The very closely comparable ^{1}H- (Table II) and ^{13}C-n.m.r. (Table I) data of *4* with those of *3* and *5* suggest also for these genins a 22-chloro-23-hydroxystructure. This assignment receives additional confirmation from the C-22 and C-23 ^{13}C-n.m.r. frequencies when compared with those of the models 22-chloro-23-hydroxy- (*8*,*10*) and 22-hydroxy-23-chloro- (*9*) -chlorohydrins; in a 22-hydroxy-23-chlorostructure the bearing hydroxyl carbon would resonate at higher field. The 22R, 23S stereochemistry in all three compounds *3-5* is suggested from the comparison of H-22 and H-23 ^{1}H-n.m.r. signals pattern with those of the -22(R)-chloro-23(S)-hydroxystigmast-4-en-3-one (*10*) and its [22S, 23R]-diasteroisomer *8* (see

Table II) and from the 22-Cl and 23-OH substituent effects on C-17, C-20 and C-21 ^{13}C-n.m.r. carbon shifts in the two sets of compounds (*3-5* and *8,10*) (see Table III). This stereochemical assignment is supported by the fact that [22S, 23S]-epoxide *7*, when treated with HCl in the same conditions used for the hydrolysis of the *E. sepositus* saponins mixture, gave almost exclusively one chlorohydrin, *10*, in agreement with the results of the acid hydrolysis of the saponins mixture, which gave only one isomer of each chlorohydrin (no significant amount of any different isomer was detectable)

TABLE III. - Differences in chemical shifts between the chlorohydrins *3-5* and the model *1*, and between the chlorohydrins *8*, *10* and the model β-sitosterol *12*[a]

Carbon	*3 - 1*	*4 - 1*	*5 - 1*	*8 - 12*	*10 - 12*
17	-2.9	-3.1	-3.3	-2.1	-2.9
20	+1.0	+1.0	+1.0	+3.6	+0.5
21	-6.0	-5.9	-6.0	-4.0	-6.0~-6.4

(a) ^{13}C-n.m.r. chemical shifts of the β-sitosterol side-chain: C-17:56.3; C.20:36.2; C-21:18.9; C-22:34.2; C-23:26.9; C-24:46.1; C-25:29.0; C-26 & C-27:19.8 & 19.2; C-28:23.3; C-24:12.1.

The model chlorohydrins *8-11* were obtained as summarized in Chart 1; the stereochemistry of the epoxides *6* and *7*, as well the structures and stereochemistries of the derived chlorohydrins were assigned according to Nakane *et al*[7].

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