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TWO NEW BILE ALCOHOLS, 3-EPIMYXINOL AND 3-EPI-16-DEOXYMYXINOL FROM THE HAGFISH, Heptatretus burgeri*

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Only three bile alcohols are known which have the β-oriented 3-hydroxyl group rather than α-oriented as in the large number of naturally occurring bile acids and bile alcohols. The 3β-hydroxylated bile alcohols are myxinol and 16-deoxymyxinol which occur as their disulfates in biles of two hagfish species, Eptatretus stoutii and Myxine glutinosa.[1,2] and latimerol which is the principal bile alcohol of the coelacanth, Latimeria chalunae.[3] While a second bile alcohol of the coelacanth is the 3α-epimer of latimerol, 5α-cyprinol, no 3α-hydroxylated bile alcohols have as yet been found in the hagfishes. The present investigation was undertaken to ascertain whether the hagfishes, like the coelacanth, contain the 3α-epimers of their principal bile alcohols.

Gall-bladder bile of the hagfish, Heptatretus burgeri, was extracted with ethanol to yield crude bile salts which on TLC (Silica Gel G, $CHCl_3$-MeOH-AcOH-H_2O, 13:4:2:1) gave two spots (R_f values, 0.06 and 0.10, taurocholate=0.28) corresponding to the disulfate esters of the myxinols. Acid hydrolysis of the bile salts (180 mg from 2 gall-bladders) by the method of Palmer[4] afforded the desulfated product (59.5 mg), GLC analysis (Fig. 1) of which indicated the presence of four different bile alcohols: I (2 % of the total bile alcohol); II (10 %); III (28 %); IV (60 %). The desulfated product was chromatographed on a column of silica gel using a system of ethyl acetate graded into benzene to get three fractions.

The fastest eluted fraction was homogeneous by TLC, but GLC analysis revealed that the fraction contained two components, I and II, in the ratio of 1:5. The structures of these bile alcohols were deduced from the comparison of their GC-MS data with those of synthetic samples. The bile alcohol II had identical gas chromatographic retention time and mass spectral properties as those of 5α-cholestane-3β,7α,26-triol (16-deoxymyxinol) prepared and kindly given to us by Professor Elliott.[5] The bile alcohol I had a retention time of 0.92 and 0.88 relative to 16-deoxymyxinol on OV-17 and QF-1 columns, respectively. These ratios were in

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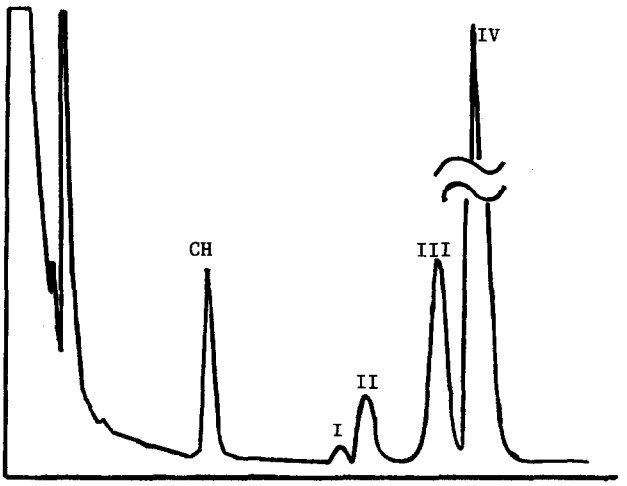
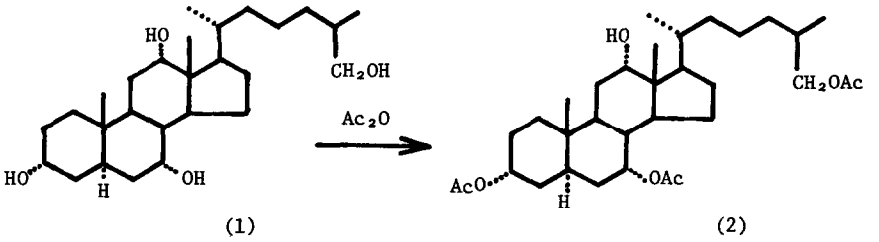


Fig. 1.

Gas Chromatographic Pattern of Bile Alcohols from Heptatretus burgeri.

The sample was analyzed as the trimethylsilyl derivative.
Column: 3 % OV-17.

CH: Cholesterol.
I: 3-Epi-16-deoxymyxinol.
II: 16-Deoxymyxinol.
III: 3-Epimyxinol.
IV: Myxinol.



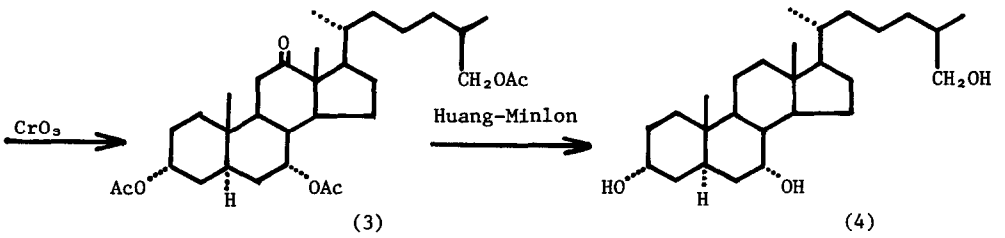


Fig. 2. Synthesis of 3-Epi-16-deoxymyxinol

good agreement with the separating factors found between 3α,7α-dihydroxy-5α-cholanoate and its 3β-epimer[6] (Table I). The MS of I showed a similar fragmentation pattern to that of 16-deoxymyxinol. These data suggested that the difference between I and 16-deoxymyxinol is a stereochemical one, most likely at C-3. Confirmation of this structural assignment was attempted by synthesis (Fig. 2). Anhydro-5α-cyprinol, an artifact of 5α-cyprinol sulfate by alkaline hydrolysis, was reduced with $LiAlH_4$ in tetrahydrofuran to provide 5α-cholestane-3α,7α,12α,26-tetrol (1).[7] The tetrol (1) was treated with a mixture (1:1:4) of acetic anhydride, pyridine, and benzene at room temperature to yield the 3,7,26-triacetate (2), mp 120-122°, IR (KBr, cm^{-1}):

Table I. Relative Retention Times of 3α- and 3β-Hydroxylated 5α-Steroids*

Compounds	3 % QF-1	Ratio 3α/3β	3 % OV-17	Ratio 3α/3β	0.2 % Poly I-110	Ratio 3α/3β
Methyl 3α,7α-dihydroxy-5α-cholanoate**	1.00	0.88	0.92	0.92		
Methyl 3β,7α-dihydroxy-5α-cholanoate**	1.14		1.00			
I (3-Epi-16-deoxymyxinol)	0.95	0.90	1.17	0.94	2.16	0.85
II (16-Deoxymyxinol)	1.05		1.25		2.54	
III (3-Epimyxinol)	1.28	0.90	1.49	0.91	2.69	0.85
IV (Myxinol)	1.42		1.64		3.15	

* The samples were analyzed as the trimethylsilyl derivatives. Relative retention times referred to the trimethylsilyl derivative of methyl deoxycholate as 1.00.

** Data from reference 6.

Table II. Proton Resonances of Bile Alcohols*

Compounds	Chemical shifts				
Protons at--	C-3α	C-3β	C-7β	C-16β	C-26
Synthetic 5α-cholestane-3α,7α,26-triol	——	4.36	4.12	——	3.75
III (3-Epimyxinol)	——	4.34	4.11	4.34	3.75
IV (Myxinol)	3.90	——	4.08	4.36	3.78

* The spectra were taken in pyridine-d_5 solutions on JEOL JNM-PS-100 spectrometer at 100 MHz. The chemical shifts are expressed as δ ppm from internal tetramethylsilane.

3560 (OH), 1715 (OAc). Chromic acid oxidation of the acetate (2) afforded 3α,7α,26-triacetoxy-5α-cholestan-12-one (3), IR (KBr, cm^{-1}): no OH, 1720 (OAc and C=O). Huang-Minlon reduction of the 12-keto compound (3) gave the desired 5α-cholestane-3α,7α,26-triol (4), mp 187-188°, $C_{27}H_{48}O_3$ (M^+ 420.3574), IR (KBr, cm^{-1}): 3370 (OH). Gas chromatographic retention time and mass spectral properties of the synthetic 3-epi-16-deoxymyxinol (4) was completely identical with those of the natural bile alcohol I.

The second eluted fraction contained only the bile alcohol IV. Crystallization from ethyl acetate gave crystals, mp 204-205°, $C_{27}H_{48}O_4$ (M^+ 436.3622), IR (KBr, cm^{-1}): 3380 (OH), which was identified as myxinol by comparison of the spectral properties with the reported data.[1,2]

The latest eluted fraction was homogeneous by TLC and GLC. Crystallization from ethyl acetate gave pure sample of the bile alcohol III, mp 178°, $C_{27}H_{48}O_4$ (M^+ 436.3553), IR (KBr, cm^{-1}): 3380 (OH). The MS of III showed a similar fragmentation pattern to that of myxinol. A comparison of gas chromatographic retention times of III and 3-epi-16-deoxymyxinol with

those of myxinol and 16-deoxymyxinol showed a constant ratio on each of the three phase (Table I). Chromic acid oxidation of III afforded an acid in good yield, which was identical in TLC and GC-MS with an authentic sample of 3,7,16-trioxo-5α-cholestan-26-oic acid prepared from myxinol by chromic acid oxidation. The PMR spectrum of III lacked the C-3αH signal, but in addition to the C-7βH, C-16βH, and C-26H_2 signals, the signal due to the C-3βH was seen (Table II). Based on these findings, III has been clarified as 3-epimyxinol.

The present finding of the 3α-hydroxylated bile alcohols, 3-epi-16-deoxymyxinol and 3-epi myxinol, in the hagfish is very interesting as a positive proof for the biogenetic assumption that the 3β-hydroxylated bile alcohols, myxinol and 16-deoxymyxinol, in the myxinids arise from cholesterol by the route which involves 3-oxo intermediates rather than by the route which maintains the 3β-hydroxyl group of cholesterol throughout.[8,9]

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NOMENCLATURE

The following IUPAC names apply to the steroids discussed in this manuscript: myxinol=5α-cholestane-3β,7α,16α,26-tetrol; 16-deoxymyxinol=5α-cholestane-3β,7α,26-triol; 3-epimyxinol=5α-cholestane-3α,7α,16α,26-tetrol; 3-epi-16-deoxymyxinol=5α-cholestane-3α,7α,26-triol; latimerol=5α-cholestane-3β,7α,12α,26,27-pentol; 5α-cyprinol=5α-cholestane-3α,7α,12α,26,27-pentol; taurocholate=3α,7α,12α-trihydroxy-5β-cholanolyl taurine; deoxycholic acid=3α,12α-dihydroxy-5β-cholanoic acid; anhydro-5α-cyprinol=26,27-epoxy-5α-cholestane-3α,7α,12α-triol; cholesterol=cholest-5-en-3β-ol.

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