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NEW BILE ALCOHOLS, 5α- AND 5β-DERMOPHOLS FROM AMPHIBIANS[1)]

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In our attempt to better understand the biosynthesis and molecular evolution of bile acids, we have continued our search for biogenetically significant bile acids and bile alcohols which are present in lower vertebrates such as fishes and amphibians. Here, we wish to report the presence of new bile alcohols named 5α- and 5β-dermophols in some amphibians.

Gall-bladder bile of the caecilian, Dermophis mexicanus, was extracted with ethanol to yield bile salts which on TLC (silica gel G, n-butanol : acetic acid : water = 17: 2 :1) gave a single spot with a mobility slightly greater than that of scymnol sulfate and much greater than that of taurocholate. Its IR spectrum (KBr disk) showed broard bands at 1230 cm^{-1}(sulfate) and 3400 cm^{-1}(hydroxyl). Treatment of the bile salts with CCl_3COOH/dioxane[2)] afforded the neutral product, the TMS derivative of which on GLC gave a single peak with a retention time (2.73 and 4.00 relative to the TMS ether of methyl cholate (1.00) on 3% OV-17 and 1.5% OV-1 columns, respectively) different from that of any hitherto known bile alcohol. The IR spectrum of desulfated product showed a characteristic pattern in region between 880 and 1075 cm^{-1}assignable to the cholic acid type nucleus[3)]. These results were interpreted in that the caecilian bile salts contain as principal or sole constituent a sulfate of a previously unknown bile alcohol having the 5β-configuration and a 3α,7α,12α-pattern of hydroxyl substitution.

The mass spectrum of the new bile alcohol TMS ether obtained by a combined GC-MS technique (Shimadzu-LKB 9000 instrument, 3% OV-17 column) exhibited a series of peaks at m/e (relative intensity): 797 (5), 707 (7), 617 (48), 527 (37), 437 (47) and 347 (49). In addition fragments were found at m/e 343 (34) and 253 (100), typical of steroids having three nuclear hydroxyl groups[4)]. The same type of the mass fragmentation pattern was seen in the spectrum of the TMS ether of 5β-bufol (2), which also exhibited a series of peaks at m/e: 709 (39), 619 (15), 529 (79), 439 (97) and 349 (89) originated from scission between C-25 and C-26 followed by subsequent elimination of TMS-OH molecules, and peaks at m/e 343 (26) and 253 (100). The only difference

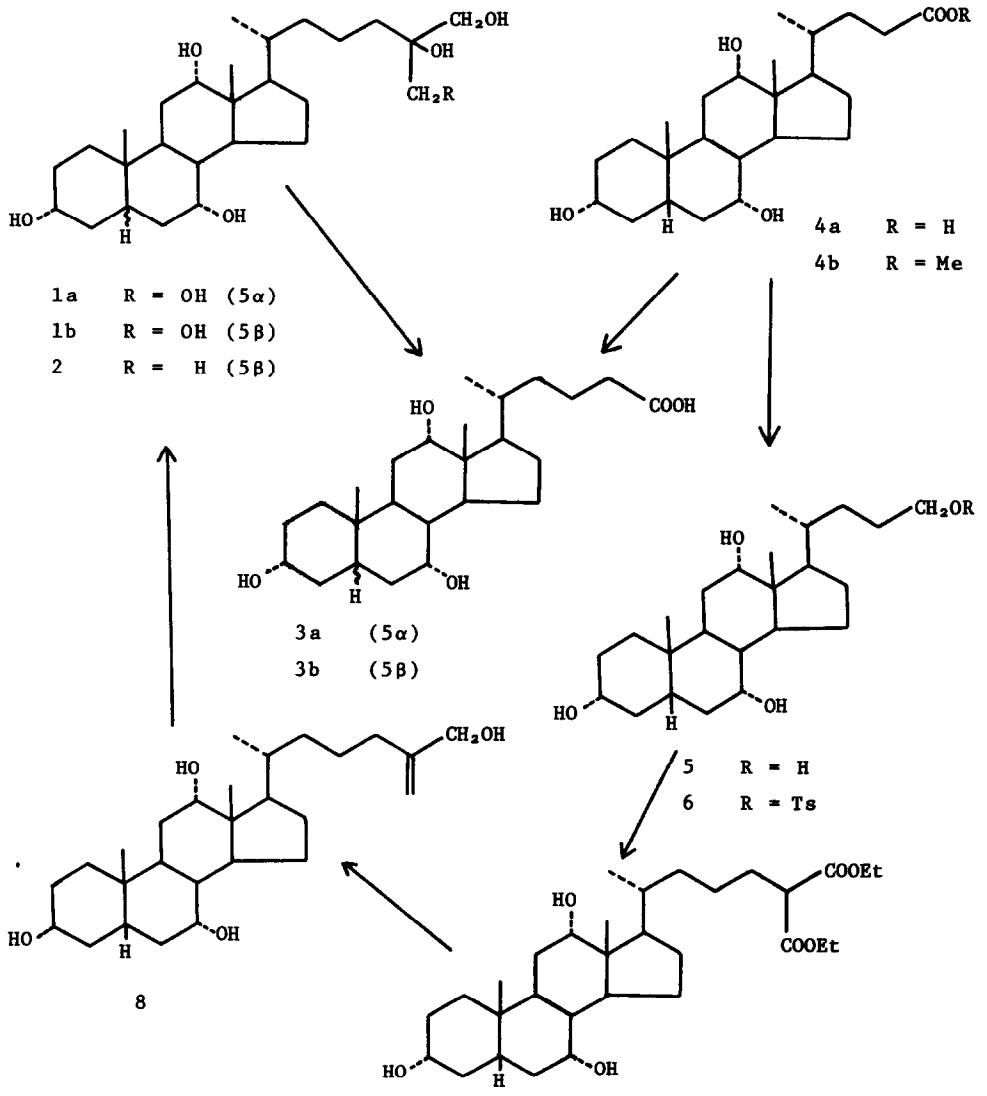
was that in the region above m/e 400 the peaks in the spectrum of the new bile alcohol TMS ether were at m/e ratios consistently higher by 88 mass units than their counterparts in that of 5β-bufol TMS ether, suggesting that the caecilian bile alcohol is a derivative of 5β-bufol (2) with an extra hydroxyl group on the side chain.

A portion of the desulfated product was treated with $Pb(CH_3COO)_4$ to afford a steroidal acid in good yield, which was characterized as 25-homocholic acid (3b) by direct comparison of the chromatographic properties and mass spectrum with an authentic sample prepared from cholic acid (4a) by Arndt-Eistert synthesis[5]. Hence, the new bile alcohol, 5β-dermophol, has the structure 1b (5β-cholestane-3α,7α,12α,25,26,27-hexol). This was fully confirmed by partial synthesis. Methyl cholate (4b) was treated with $LiAlH_4$ to convert into 5β-cholane-3α,7α,12α,24-tetrol (5), which via 24-monotosylate (6) was treated with sodio diethylmalonate in refluxing ethanol to yield the malonic ester (7). Reduction of the ester (7) with $LiAlH_4$ in the presence of NaH in dimethoxyethane gave the unsaturated tetrol (8), which with OsO_4 gave the desired hexol (1b), mp 166 — 169° (from acetone), M^+ 468.3452 (calcd. for $C_{27}H_{48}O_6$ = 468.3451), NMR (δ) in pyridine-d_5: 0.78 (s, 3H, 18-CH_3), 0.93 (s, 3H, 19-CH_3), 1.14 (d, 3H, 21-CH_3), 3.2 — 4.1 (m, 3H, 3-CH, 7-CH, 12-CH and s, 4H, 26-CH_2, 27-CH_2), IR (KBr disk) 3400 cm^{-1}(hydroxyl). The synthetic hexol was completely identical in TLC and GC-MS with the natural 5β-dermophol.

5β-Dermophol was also found as minor consistuent in the bile of the toad, Bufo b. formosus. A combined GC-MS examination as the TMS derivative of the hydrolyzed toad bile salts revealed the presence of four different bile alcohols, one of which was identified with 5β-dermophol (3% of the total bile alcohol) by comparison of GLC retention data and mass spectrum with the authentic sample. The others were known bile alcohols, 5α- and 5β-cholestane-3α,7α,12α,26-tetrols and 5β-bufol (2, 2 and 90%, respectively).

The availability of the authentic 5β-dermophol enabled us to identify another new bile alcohol in three species of amphibians. Analytical gas chromatography of the bile alcohol TMS derivatives obtained from the newt, Diemyctylus pyrrhogaster, showed three peaks, two of which were known bile alcohols, 5α-bufol (60% of the total bile alcohol) and 5α-cyprinol (8%), and the third (28%) had a retention time of 0.95 and 0.88 relative to the TMS ether of 5β-dermophol on OV-17 and OV-1 columns, respectively. These ratios were in good agreement with constant separating factors found between the pair of 5α-bile alcohols and 5β-counterparts under the employed conditions[2]. The mass spectrum obtained by the scanning of the third peak was similar to that of 5β-dermophol TMS ether. It has known that no appreciable differences appear between the spectra of 5β-bile alcohol TMS ethers and their 5α-counterparts[4]. Reversed phase partition column chromatography[6] of the bile alcohol mixture obtained from the newt furnished the third bile alcohol in pure state. Its IR spectrum was typical of a bile alcohol containing the allocholic acid type nucleus[3]. Treatment with $Pb(CH_3COO)_4$ of the 5α-bile alcohol gave an acid, whose TLC and GC-MS suggested to be 5α-isomer (3a) of the 25-homocholic acid (3b). All the evidence suggests that the third bile alcohol of the newt has the structure 1a (named as 5α-dermophol).

5α-Dermophol was also found in the biles of the congo eel, Amphiuma means, and the giant salamander, Megalobactrachus japonicus. The former contained 5α-bufol (93% of the total bile alcohol) and 5α-dermophol (6%), and the latter 5α-cyprinol (64%) and 5α-dermophol (16%).



The presence of dermophol in amphibian bile might be expected, since a further hydroxylation of either bufol (25,26-OH) or cyprinol (26,27-OH), the chief bile constituent in almost all amphibians, leads to dermophol (25,26,27-OH).

Since bile acids were not found in the caecilian, the 5β-dermophol might be the end product of cholesterol metabolism.

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NOMENCLATURE

The following IUPAC names apply to the substances discussed in this manuscript.

cholic acid	= 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid
allocholic acid	= 3α,7α,12α-trihydroxy-5α-cholan-24-oic acid
25-homocholic acid	= 3α,7α,12α-trihydroxy-25-homo-5β-cholan-25-oic acid
scymnol	= 5β-cholestane-3α,7α,12α,24,26,27-hexol
5α- and 5β-bufols	= 5α- and 5β-cholestane-3α,7α,12α,25,26-pentols
5α-cyprinol	= 5α-cholestane-3α,7α,12α,26,27-pentol

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