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# OCCURRENCE OF POLYHYDROXY ALCOHOLS IN THE GALL BLADDER BILE OF A MUD-PUPPY, *SALAMANDER NECIURUS*

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## ABSTRACT

Bile obtained from the gall bladder of *Salamander necturus* was investigated for the presence of bile alcohols by a combined technique of Thin Layer Chromatography (TLC), Gas Liquid Chromatography (CIE) and Gas Chromatography-Mass Spectrometry (GC-MS). Dried bile salts were hydrolysed with 25N KOH in an autoclave at 121°C Unsaponifiable matter was extracted with ethyl acetate, three times, washed with distilled water, dried with anhydrous sodium sulphate, evaporated to dryness and analysed for its bile alcohols.

Analytical GLC revealed the presence of at least thirteen bile alcohols in the mixture. Gm major bile alcohols, i.e. 5α-anhydtuyprinol and 5α-cyprinol constituted about 73% of the total unsaponifiable matter. Others were identified as 3α, 7α, 12α, trihydroxy-5β-$\Delta^{23}$-homocholene,3α,7α,12α-trihydroxy-5α-Δ-nor-27-cholestene, 3α-7α,12α, 24,25-pentahydroxy-5α-cholestane, 5α-bufol and 5α-dermophol.

## Introduction

It has been observed that the composition of the gall bladder bile differs from lower to higher animals. Higher animals lack the presence of bile alcohols in their gall bladder bile.

Recent studies on bile acids and alcohols have gained immense interest because of their varied nature specifically in lower animals. Such studies also attracted attention because of their relation to cholesterol which is considered to be implicated in cardiovascular disease and gall stone formation. In higher animals cholesterol is normally catabolized to hydroxy derivatives of cholanic acids. However, several species of fishes, amphibians and reptiles have been reported to produce both bile acids and bile alcohols. In our previous reports (Ali, 1975, 1990) the occurrence of bile acids of 5a-cholestane series has been described in *Salamander necturus*. The present paper describes the occurrence of several bile alcohols in the gall bladder bile of *Salamander necturus.*

## Materials and Methods

### *Reference compounds*

5α-Cyprinol and 5α-anhydrocyprinol were isolated from the carp bile (Hoshita *et al.,* 1963) and used as a standard. 3α,7α,12α,24-Tetrahydroxy-5β-cholane

was a gift from Professor W.H. Elliott, Saint Louis University, USA. All the solvents and reagents were of Analar grade.

### *Isolation of bile alcohols*

The bile (3.7 ml) of Salamander necturus was taken in 20 ml of ethyl alcohol and left for several days under nitrogen. Precipitated proteins were filtered and the remaining ethyl alcohol extract was evaporated to dryness by slow stream of nitrogen to provide 255 mg of dried bile salts. The bile salts after hydrolysing with 25 nil of 25N KOH in an autoclave at 121°C for 14 hours was diluted with an equal volume of distilled water. The unsaponifiable matter was then extracted three times with ethyl acetate, dried with anhydrous sodium sulphate and evaporated to dryness under nitrogen to yield 29 mg. The whole powdered material was stored at 4°C till further analysis.

### *Chromatographic analysis of unsaponifiable matter*

Bile alcohols were separated on a silica gel thin layer chromatographic glass plate using solvent system: ethyl acetate: acetic acid: water (85:10:5 v/v). The spots were developed by spraying with sulphuric add or visualizing under iodine vapour. Bile alcohol mixture was further separated into their individual entities as trimethyl silyl ether derivatives by analytical GLC using 1% SE-30 column. The GC-MS of trimethyl silyl ethers of bile alcohols was carried out using 1% OV-17 column in a LKB 9000 gas chromatograp-mass spectrometer (LKB instrument), Rockville, M.D.) with an ion$^{i}$zing energy, 20 eV, and accelerating voltage ranging from 2.1- 35 Kv.

## Results

Figure 1 shows the separation of bile alcohols and standard hydroxy compounds on a thin layer chromatogram The distribution of bile alcohols of Salamander necturus in the di-,tri-,and pentahydroxy regions were prominent. Figure 2gives the GLC separation of TMS ethers of bile alcohols

### *Identification of bile alcohols by GC-MS*

The bile alcohol fraction contained as many as 20 constituents in the total unsaponifable matter. Only 13 were marked on the GLC chromatogram (Figure 2), others being present in minor quantities recognizable between peak HI and IV. Attempts were also made to identify some of the unmarked minor bile alcohols. The major components (GLC peak V and IX) constituted about three-fourth (73%) of the total unsaponifiable matter. Peaks V (RRT 2.73) and XI (RRT 4.28) were identified as 5a-anhydrocyprinol and 5a-cyprinol respectively and compared well with 5a-anhydrocyprinol and 5a-cyprinol isolated from carp bile.

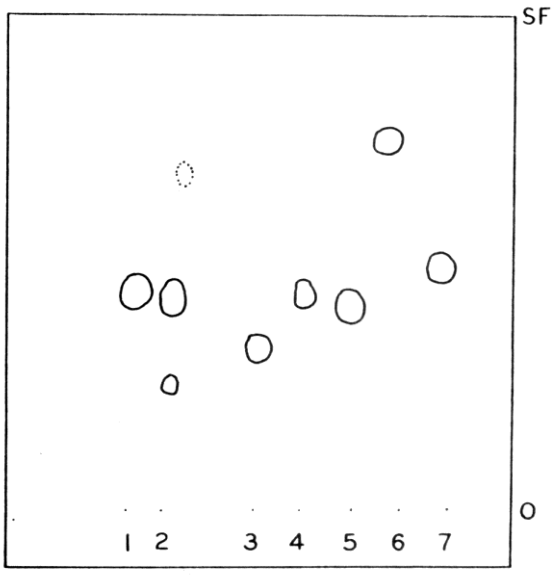



Figure 1. Thin layer chromatogram of bile alcohols of *Salamander necturus*. Solvent system (EAW): ethyl acetate: acetic add water (85:10:5 v/v). The number refers to the following compounds: 1 and 5: 5α-anhydro-cyprinol 2: Salamander bile alcohols; 3: 3α, 7α, 12α, 2-tetrandroxy-5β-cholene; 4: 5β-anhydrocyprinol; 6: 3α,7α,12α,-trihydroxy-5β-cholestane; 7: 5α -cyprinol; SF.: solvent front; 0: origin.

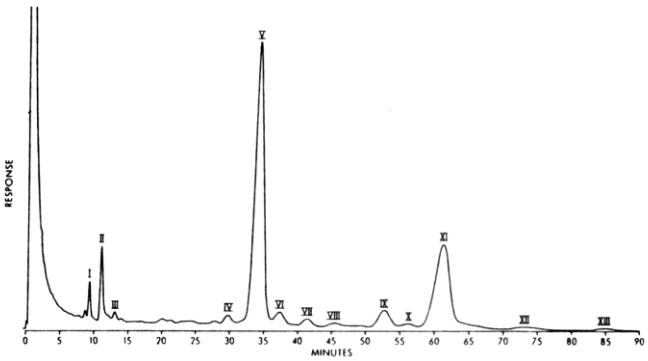


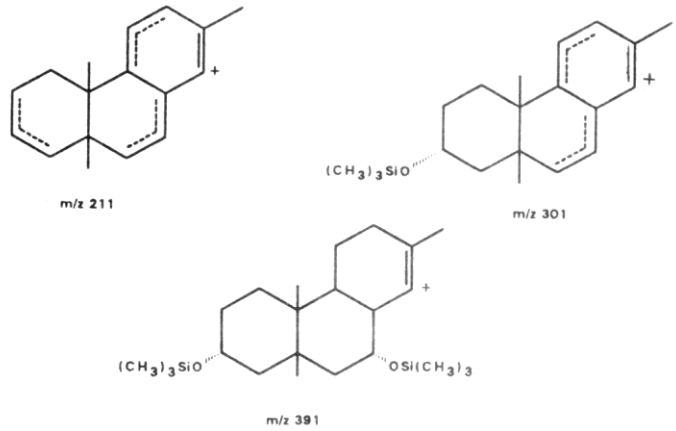

Figure 2. Gas - liquid chromatogram of bile alcohols as trimethyl silyl ethers on 1% SE-30 at 230°C. The identities of the peaks are provided in the text.

In most of the bile alcohols, the stereochemistry at C-5 was established by comparing the intensity of the fragment ions at m/z 343 and 253 respectively. The intensity of fragment ion at m/z 343 is larger than that of m/z 253 in the spectra of 3α,7α,12α-trihydroxy-5α-cholestane derivatives and of the TMSi ethers of methyl esters of comparable 5α -bile acids; the reverse is true for the 5β-derivatives (Elliott, 1972; Cronholm and Johanson,1970; Ali *et al.*, 1976).

The important fragmentation ions of trimethylsilyl ethers of 5a-anhydrocyprinol were seen at m/z 650 $[M]^+$, 0.8%; 635 $[M-15]^+$, 13%; 560 $[M-90]^+$, 5.5%; 545 $[M-90+15]^+$,45%, 530 $[M-90+CH_2O]^+$, 1.6%; 470 $[M-180]^+$, 98%; 455 $[M-180+15]^+$, 11.7%; 440 $[2x90+CH_2O]^+$, 7.9%; 433 [M-90+ side chain]$^+$, 4.8%; 380 $[M-270]^+$, 24%; 365 $[M-270+15]^+$, 9%; 343 [M-180+ side chain]$^+$, 100%, a base peak; and 253 [M-270+side chain]$^+$, 44%. Several other ions produced due to side chain and/or nuclear cleavages were also seen at m/z 481 (4%); 391 (6%); 301 (3.4%); 295 (1.6%); 281 (9%); 273 (44%); 226 (6.5%), 211 (13.6%) and 199 (11.75) respectively.

Peak XI (RRT 4.88) was the second major alcohol in the fraction and provided the mass spectrum similar to 5a-cyprinoL The fragment ions appeared at m/z 812, $[M]^+$,0.4%; 797 $[M-15]^+$, 03%, 722 $[M-90]^+$, 2.7%; 632 $[M-180]^+$, 71% 619 $[M-90+103]^+$, 3.6%; 617 [M-180+15] 68%; 542 [64-270] 12.7%; 527 $[M-270+15]^+$, 35%; 461 [M-90+Cn]+, 1.4%; 453 $[M-270+89]^+$ 3%; 363 $[M-360+891^+$, 25%; 343 [M-180 + side chain]$^+$, 100% , a base peak; and 253 [M-270 + side chain]$^+$, 60.7%. Other ions were observed at m/z 406 $[M-90+B]^+$, 15%; 381 (43%); 316 (3.1%); 301 (2.3%); 295 (12%); 281 (3.3%); 226 (4%); 211 (8.7%) and 199 (83%). The relative intensities of the ions at m/z 343 and 253 are in agreement of a 5α-configuration in the molecule.

The residual 27% of the alcoholic fraction possessed varieties of alcohols in minor amounts. Peak I (RRT 0.73) gave a molecular ion at m/z 606 (0.8%). Other fragments appeared at m/z 591 $[M-15]^+$, 0.5%; 551 $[M-C_{22}]^+$, 0.2%; 516 $[M-90]^+$, 53%; 501 $[M-90+15]^+$, 1.7%; 461 $[M-90+C_{22}]$+, 1.5%; 426 $[M-180]^+$, 40%; 411 $[M-180+15]^+$, 63%; 385 $[M-180+C_{23}]^+$, 1.3%; 371 $[M-180+C_{22}]^+$ 4.1%; 343 [M-180+ side chain]$^+$, 23.4%; 336 $[M-270]^+$, 57%; 321 $[M-270+15]^+$, 11%; 295 $[M- 270+C_{23}]^+$, 5%; 281 $[M-270+C_{22}]^+$, 11.8% and 253 [M-270+side chain]$^+$, 100%, a base, peak indicated the structure of this molecule as 3α,7α,12α-trihydroxy-5β-$\Delta^{23}$-homocholene. There were other fragments at m/z 211 (11%), 226 (16%); 301(5%) and 391 (2%). They presumably represent the following fragments:




This alcohol is the only constitute which bears the 5β-configuration as judged from the relative intensities of ions at m/z 343 and 253.

Peak II (RRT 0.86) provided a spectrum which has a distinct molecular ion at m/z 620 (3.8%). The predominant ions were seen at m/z 530 $[M-90]^+$, 133%; 515 $[M-90+15]^+$, 33%; 440 $[M-180]^+$,100%, base peak; 433 [M-90+side chain], 4%; 425 $[M-180+15]^+$, 10%; 371 [M- 180+Czz]$^+$, 6%; 350 [M-270] 13%; 343 [M-180+side chain]$^+$, 81%; 335 $[M-270+15]^+$, 8.4%; 295 $[M-270+C_{23}]^+$, 2%; 281 $[M-270+C_{22}]$ 2.2% and 253 [M-270-side chain], 43%. Other ions at m/e 391 (6%); 301 (2.7%); 226 (5%); 211 (88%); and 199 (8%) were due to the cleavage of the steroid ring and the side chain. On the bail of chromatographic behaviour and fragmentation pattern of the molecule, the structure of this peak is assigned as 3α, 7α, 12α,trihydroxy-5α-$\Delta^{24}$-nor-27-cholestene.

Peak III (RRT 1.01) provided fragments which account for the structure as 3α, 7α, 12α-trihydroxy-5-cholest-$\Delta^{24}$-ene. This fraction was contaminated with minor amounts of cholesterol. The ions belonging to - $\Delta^{24}$-trihydroxy cholesterol derivative were at m/z 634 $[M]^+$, 1.4%; 554 $[M-90]^+$, 53%; 461 $[M-90+C_{22}]^+$, 17%; 454, [M-180], 9%; 371 $[M-180+C_{22}]^+$, 37%; 343 [M-180+side chain]$^+$, 31%; 301 (42%), 295 $[M-270+C_{23}]^+$, 6%; 281 $[M-270+C_{22}]^+$ , 11%; 253 [M-270+ side chain]$^+$, 28%; 226 (5.1%), 211 (10%); and 199 (13%). In addition to these ions, the peak exhibited ions which are diagnostic to cholesterol molecule, *i.e.* the ions at m/z 458 $[M]^+$, 103%; 429 (5%); 368 [M- 90]$^+$, 34%; 329 (46%); 275 (6%); 255 (19%); 129 (100%), base peak; 228 (4%) and 201(8%). It is therefore suggested that 3α, 7α, 12α trihydroxy-5α- $\Delta^{24}$-cholestene is associated with traces of cholesterol in Peak III.

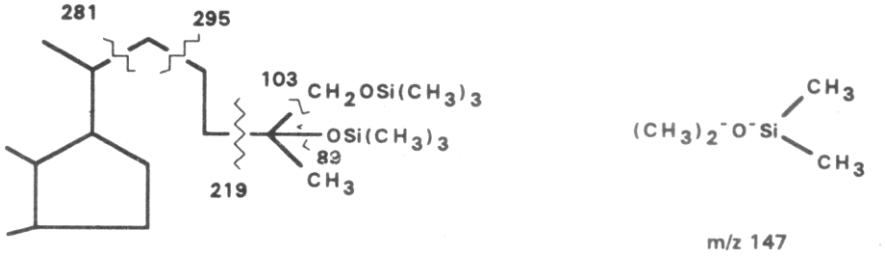
Peak IV (RAT 236) gave a molecular ion at m/z 648 (8%) which is two hydrogen lest than anhydrocyprinol or any other pentahydroxy cholestane derivative. There were abundant fragments due to cleavage at $C_{23}$ and $C_{22}$ positions of the side

chain structure indicating a double bound at $\Delta^{23}$ or $\Delta^{24}$. All the ions are in favour of the structure as $\Delta^{23}$ -5β/5α-anhydrodermophol which might have been produced by dehydration *of* hexol, i.e. 5α-dermophol The dominant ions were seen, at m/z 633 [M-15]$^+$, 3%; 618 [M-$CH_2$]$^+$, 6%; 558 [M-90]$^+$, 53%; 528 [M-90]$^+$, 8%; 475 [M-90+$C_{23}$]$^+$, 1.4%; 468 [m-180] 35%; 46 [M-90+$C_{22}$]. 14%; 453 [M-180+15]$^+$, 7%; 438 [M-180+$CH_2O$]$^+$, 5%; 433 [M-90+side chain]$^+$, 11%; 371 [M-180+$C_{22}$]$^+$, 20%; 343 [M-180+ side chain]$^+$, 91%; 316 (7%); 301 (5%); 295 (83%); 281 (12%); 271 (26%) and 253 [M-270+ side chain]$^+$, 100%, a base peak. Additional peaks at 226 (8%); 211 (19%) and 199 (19%) were also seen. Since the relative proportions *of* ions at m/z 343 and 253 are more or less equal, the configuration at 5 positions was not decided positively. However, the occurrence of 5α-dermophol, a hexol in the bile suggest that this anhydrounsaturated compound more likely represents 5α-isomer.

Peak VI (RRT 2.95) appears to be a tetrol having homocholane skeleton. The molecular ion peak at mix 696 was not prominent. Other ions suggesting this structure were at ns/z 681 [M-15]$^+$, 4%; 591 [M-90+ 15]$^+$, 5%; 501 [M-180+15]$^+$, 20%; 41 [M-270+15]$^+$, 23.9%; 343 [M-180+side chain]$^+$, 93%; 321 [M-360+15]$^+$, 20%; 316 (10%), 301 (4%); 295 (3.4%); 281(55%); 253 (67%); 226 (8%); 21 (18%) and 199 (15%). The pattern of eliminating -$CH_3$ group along with TMS; OH is very unusual. It is therefore suggested that this compound has possibly either an extremely labile methyl gorup or is an isomer of a pentol bearing the structure as described for peak VH since fragments at m/z 591, 501, 411, and 321 were common in both the compounds. The exact structure of this peak is yet to be established.

Peaks VII, VIII, IX, and X in the chromatogram (Figure 6) appear to be isomeric to each other since identical spectra were obtained. Under the GC system both 24a- and 240-hydroxy isomers are seen to be separated. The fragmentation pattern of peak VII and VIII combined in GC-MS and taken as one peak warmed the structure of this peak as 3α,7α,12α -24,25- pcntahydroxy-5α -cholestane. The specific ions were observed at m/z 812 [M]$^+$, 0.3%; 797 [M-90]$^+$, 0.2%; 681 [M-131]$^+$, 0.6%; 666 [M-131+15]$^+$, 0.3%; 591 [M-90+131]$^+$, 1%; 501 [M-180+131]$^+$, 2.3%; 411 [M-270+131]$^+$, 2.9%; 321 [M-360+131]$^+$, 2.8%; 343 [M-180+side chain]$^+$, 14.8%; 253 [M-270+ side chain]$^+$, 13%; 295 (1.6%); 281 (32%); 273 (43%); 231(43%) and 131(100%), a base peak. The stereochemistry at $C_{24}$ and $C_{25}$ is not determined.

Peak IX (RRT 4.19) is also a pentol providing fragment ions at m/z 812 [M]$^+$, 1%; 797 [M-15]$^+$, 1.7%; 722 [M-90]$^+$, 5%; 708 [M-103]$^+$, 40%; 632 [M-1801$^+$, 14%; 617 [M-180+15]$^+$, 63%; 481(43%); 371 [M-180+$C_{22}$]$^+$, 5%; 343 [M-180 + side chain]$^+$, 46%; 295 (4%); 281 (6%); 253 (353%); 226 (5%); 219 (81%); 211 (12.1%); 199 (13%) and 147 (100%), a base peak. A series of fragments appeared where elimination of mass 89 unit was common, i.e. ions at m/z 619 [M-89+103]$^+$, 20%; 529 [M-89+90+103]$^+$, 72%; 439 [M-180+89+103]$^+$, 26% and 349 [M-270+89-103]$^+$, 26%. These fragments and the dominant ion at 219 suggest the following cleavage of the side chain and the base peak at 147. The structure of this compound is therefore suggested as 3α,7α,12α,25,26-pcntahydroxy-5α-cholestane (5α -bufol).




Fragmentation of the side chain (Peak IX)

Peaks XII and XIII are polar material eluted at RRT 5.81 and 6.70 respectively. Only peak XII was identified as 5α-hexahydroxy-5α-cholestane. The positions of the hydroxyl groups on the side chain were determined from the fragmentation pattern. Molecular ion peak at m/z 902 was not seen. Other ions were observed at m/z 812 $[M-90]^+$ 4%; 797 $[M-90+ 15]^+$, 29%; 707 $[M-180+15]^+$, 25%; 633 $[M- 180+89]^+$, 7%; 618 $[M-180+89]^+$, 82%; 527 $[M-270+151^+$, 19%; 437 $[M-360+15]^+$, 30%; 347 $[M-450+15]^+$, 19%; 343 [M-180+side chain]$^+$, 39%; 253 [M-270+side chain]$^+$, 30%; 301 (5%); 295 (4.7%); 281 (18%); 226 (29%); 211 (11%); 199 (10%) and 147 (100%). A base peak ion at m/z 147 is believed to be due to the condensation of two trimethyl silylether fragments attached to two neighboring carbons in the molecule The structure is therefore suggested to be 3α,7α,12α, 25, 26,27-hexayhydroxy-5α -cholestane (5α-dermophol).

Attempts were also made to assess the minor components eluted on the GLC (Figure 6) between peaks III and IV. This region contains seven or eight minor constituents. Mass spectrometeric findings of the minor peak emerging just after Peak III gave ions corresponding to tetrahydroxy-5a-homochalene. The molecular ion peak at 6% was not observed. The specific ions suggesting this structure were at m/z 606 $[M-90]^+$, 3.8%: 591 $[M-90+15]^+$, 27%; 561 [M-180] 58%; 503 $[M-180+103]^+$, 9%; 481 $[M-90+C_{21}]$+, 7%; 371 $[M-180+C_{22}]^+$. 16%; 343 [M-180+side chain], 100%, base peak; 336 $[M-360]^+$, 9%; 316 (11%); 295 $[M-27- + C_{23}]^+$, 10%; 281 $[M-270 + C_{22}]^+$, 11%; 253 [M-270+side chain]$^+$, 70%; 226 (12%); 211 (19%) and 199 (16%). The chromatographic behaviour and the fragmentation pattern suggest that the compound bears the structure as 3α,7α,12α -25-tetrahydroxy-5α-homocholane.

The twin peak in the central region between the peaks III and IV gave an impure spectrum. The major component gave ions at m/z 620 $[M-90]^+$, 4%; 605 [M-90+15]+, 2%; 530 $[M-180]^+$, 64%; 515 $[M-180+15]^+$, 4%; 425 (5%); 391(7%); 371 $[M-180+C_{22}]^+$, 11%; 343 [M-180+side drain]$^+$, 80%; 316 (10%); 301 (5%); 295

(10%); 281 (2%); 253 (57%); 226 (7%); 211 (14%) and 199 (5%). The fragments are in accord with the structure 3α, 7α, 12α; 25-tctrahydroxy-5α-nor-27-cholestane.

The minor peak appearing just before peak IV gave the fragmentation pattern of 3α, 7α, 12α,-25-tetrahydroxy-5α-cholestane. It has a feeble molecular ion at m/z 724 (03%), and prominant ions at m/z 634, $[M-90]^+$, 1.8%; 544 $[M-180]^+$, 18%; 529 $[M-180+15]^+$, *3*%; 454 $[M-270]^+$, 6%; 433 [M-90+ side chain]$^+$, 4%; 391 (3%); 371 $[M-270+C_{22}]^+$, 18%; 364 $[M-3601]^+$, 1.6%; 343 [M-180+side chain]$^+$, 53%; 316 (5%); 301 (0.6%); 295 (6%); 281 (3%); 253 [M-270+side chain]$^+$, 32%; 226 (1%); 211 (10%); 199 (7%) and 131 (100%), a base peak. The stericochemistry at position $C_{25}$ was not determined.

## Discussion

The major bile alcohols in the hydrolzyed bile of Salamander necturus were 5α-anhydrocyprinol and 5α-cyprinol. 5α-anhydroyprinol is presumably an artifact formed during hydrolysis from 5α-cyprinol. Giant salamander (Amimoto, 1966) and Diemycrylus pynhogaster contain 5a-cyprinol in their bile (Kihara et al, 1977). 5α-cyprinol appears not to be metabolized in the giant salamander. Intraperitoneal injection of 5α-Cyprinol-12β-$^3$H into a giant Salamander yielded 5α-cyprinol and 5α-anhydrocyprinol. Small activity was found to be associated with allocholic acid and an other unidentified tetrahydroxy-5α-cholestanic add (Amimoto, 1966). On the other hand rat (Yamada, 1966) and guinea pig (Yukawa, 1965) can utilize 88-cyprinol efficiently for the production of cholic acid. The bile alcohols having the cyprinol side chain arc widely distributed in natural bile. 5a-Cyrpinol is a chief constituent of all the reported species of Cyprinidae (Hoshita et al, 1964), and lungfish (Haslewood, 1978, 1967). In carp, both 5a-anhydrocyprinol and 5α-cyprinol occurred in its alkali hydrolyzed bile (Haslewood, 1955; Une et al, 1980). Recently Une et al (1980) examined ten species of frog from the family, Ranidae and found that two species, Rana japonica and Rana tagoi contained appreciable amount of 5α-cyprinol along with other polyhydric alcohols.

The relative proportions of other pentols, i.e. 3α 7α,12α,24, 26 pentahydroxy-5α-cholestane and 3α,7α,12α,26-penaahydroxy-5α-cholestane (5α-bufol) in Salamander necturus were low as compared to 5α-cyprinol and Sa-anhydrocyprinol Rana erythrea (Une *et al*, 1980) contained 5α-bufol to the extent of 68% of the total bile alcohol in the sulfated form. Amphiuma means and Diemyctylus pynhogaster (Kihara et al., 1977) constituted major proportion of bile alcohol as 5α-bufol 5α-bufol was also present in the bile of Hylarana albolabries and ptyhadena taenioscelis (Anderson et al., 1974). A small amount of another pentol, 3α,7α,12α, 24,26-pentahydroxy-5α-cholestane (5α-chimaerol) was also indicated in the bile of Necturus. Bussjaeger (1963) reported 5a-chimaerol sulfate as a major bile salt in five species of catostomidae ranging from 49-87%. In our study, analytical GLC and mass spectral data suggest that there are at least two hexahydroxy bile alcohols in the mud puppy, one of them is 5α-dermophol (3α,7α,12α,25,26, 27-tetrahydroxy-5α-

cholestane). Recently, the structure of this alcohol has been established. 5a-Dermophol has been identified in species of Salamandriade (Kihara *et al*, 1977), whereas Amphiuma means contained this alcohol to the extent of 6% only (Kihara *et al*, 1977).

The occurrence of 3α,7α,12α,25-tetrahydroxy-5α-cholestane has not been reportd to occur in the natural bile. However, this compound was indicated to be formed in minor quantities when tritium labelled 3α,7α-dihydroxy-α-cholestane was incubated with rabbit liver microsomes (Ali et al, 1976). The 5β and 5α analogue of 3α,7α,12α,26-tetrahydroxy-cholestane have been reported to occur in the bile of Toad, lung fish and carp ( Anderson *et al*, 1974; Amos et al, 1977; Hoshita *et al*, 1965). It is assumed that the presence of 3α,7α,12α, -trihydroxy-$\Delta^{23}$-ene, 3α,7α, 12α, trihydroxy-5α-cholest-$\Delta^{23}$ nor-27-one and 3α,7α,12α,-trihydroxy-5α-$\Delta^{24}$-nor-27-cholestene were produced in minor amounts in slamander bile during the alkaline hydrolysis and represented artifacts of polyhydric alcohols. Similar observations have been recorded in the bile of a bull frog, Rana catesbiana. From the gall bladder bile of bull frog, Nakagawa (1963) isolated a new bile sterol, 3α,7α,12α,-trihydroxy-$\Delta^{24}$-bishomocholene from bull frog bile while Masui (1963) indicated the presence of 3α,7α,12α-trihydroxy-$\Delta^{24}$-homo-5α-cholene in the bile of the same amphibian. Salamander necturus has been found to be a good source of allocholic compounds specially allocholic and 3α,7α,12α,-trihydroxy-5α-cholestaoic acid (Ate, 1990). Allocholic acid was also demonstrated to be present to the extent of 80-90% in the gall bladder bile of Uromastix hardwickii (Ali *et al*, 1976). It appears that there is some biochemical link between Salamander and lizards. Even between the two lizards studied previously, *Varanus* monitor contains 513 bile acids as compared to Uromastix in which major bile acids belonged to 5α-series (Ali et al 1976, 1982).

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