Bile acids. LXVII. The major bile acids of *Varanus monitor*[1]

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Abstract The major bile acids of gall bladder bile of *Varanus monitor* have been separated by thin-layer chromatography and shown to be derivatives of taurine. After alkaline hydrolysis, the free acids were separated by thin-layer and partition chromatography. Identification or characterization of the free acids was facilitated by gas–liquid chromatography and gas–liquid chromatography–mass spectrometry of the methyl esters or methyl ester-trimethylsilyl ethers. About 13% of the total bile acids was represented by the C_{24} acids cholic, deoxycholic, allocholic, chenodeoxycholic, and 12-oxo-3α-hydroxy-5β-cholanic acids, of which cholic acid constituted about 50%. The remainder of the bile acids consisted of eight C_{27} acids of which varanic acid was the major constituent; an isomer of varanic acid and 3α,7α,12α-trihydroxy-5β-cholestanoic acid were also identified. By chromatographic behavior and mass spectral fragmentation, the structures of four C_{27} acids with unsaturated side chains were elucidated as follows: 3α,7α-dihydroxy-5β-cholest-23-enoic, 3α,7α-dihydroxy-5β-cholest-24-enoic, 3α,7α,12α-trihydroxy-5β-cholest-23-enoic, and 3α,7α,12α-trihydroxy-5β-cholest-24-enoic acids. Similarly, the structure of the 12-deoxy analog of varanic acid, 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid, was suggested for the component that constituted 7% of the total.—**Ali, S. S., E. Stephenson, and W. H. Elliott.** Bile acids. LXVII. The major bile acids of *Varanus monitor*. *J. Lipid Res.* 1982. **23:** 947–954.

Supplementary key words gas–liquid chromatography–mass spectrometry • varanic acid • 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid • Δ^{23}-cholestenoic acids • Δ^{24}-cholestenoic acids • unsaturated C_{27} bile acids

In conjunction with an interest in the origin and metabolism of allo bile acids (2, 3) we have searched for natural sources of these materials. The bile acid fraction from bile of a lizard native to Pakistan, *Uromastix hardwickii*, was shown (4) to contain about 90% allocholic acid. In continuation of these studies, this report considers the bile acid composition of *Varanus monitor* (species Varanidae). Haslewood and Wooton (5) first reported the presence of the taurine conjugate of varanic acid (3α,7α,12α,24ξ-tetrahydroxy-5β-cholestanoic acid) in bile of this species obtained from the vicinity of the Nile river (*Varanus niloticus*). A second major acidic constituent was also obtained from this source, but was not identified. Varanic acid has also been isolated from bile of *Varanus salvator* (6), *Bombina orientalis* (7), and children with Zellweger syndrome (7, 8). This study reports the major bile acids of *Varanus*, including the taurine conjugates of varanic acid and 3α,7α,12α-trihydroxy-5β-cholestanoic acid, and demonstrates the presence of taurine conjugates of 12-deoxy varanic acid and of four C_{27} bile acids with unsaturation in the side chain.

METHODS AND MATERIALS

General

TLC of conjugated bile acids with authentic standards was performed on a plastic chromatoplate (Eastman chromatogram sheet 301R) coated with silica gel G. The plate was developed two times in a solvent system of isopropanol–chloroform–ammonium hydroxide 3:16:1 (v/v). TLC of free bile acids was carried out in a solvent system of chloroform–methanol–acetic acid 80:20:3 (v/v); standards were also chromatographed with the bile acid mixture.

GLC was carried out with columns of 3% OV-17 or QF-1 on 100–120 mesh Gas Chrom Q (9). All retention times are RRT related to methyl deoxycholate (1.00) or its TMS derivative (1.00) (9). Bile acids were methylated with freshly prepared diazomethane; a portion of the methyl esters was analyzed by GLC, and another portion was converted to the corresponding Me-TMS and analyzed by GLC.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; GLC-MS, gas–liquid chromatography–mass spectrometry; MS, mass spectrum; TMS, trimethylsilyl; Me-TMS, methyl ester trimethylsilyl ether; Me, methyl ester; RRT, relative retention time.

[1] A portion of this work was presented at the 59th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, NJ, April 13–18, 1975; an abstract has been published (1).

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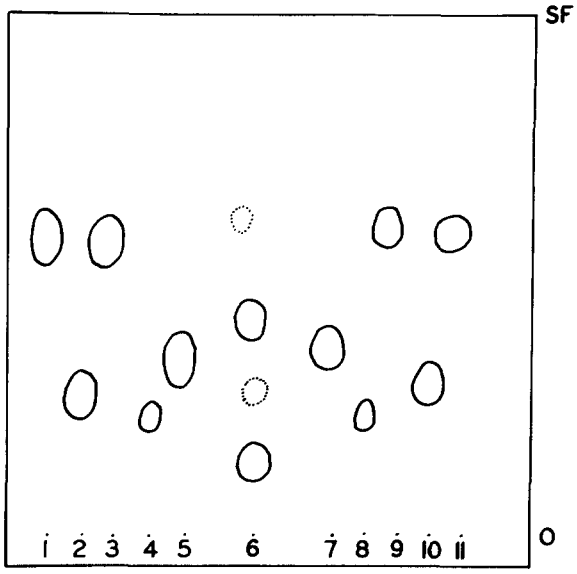

Fig. 1. TLC separation of free bile acids from *Varanus monitor* after alkaline hydrolysis of bile. Solvent system: chloroform–methanol–acetic acid 80:20:3 (v/v). Lane 6 contains material from hydrolyzed *Varanus* bile. Standards in the other lanes are: lanes 1 and 11, chenodeoxycholic acid; 2 and 10, cholic acid; 3 and 9, allochenodeoxycholic acid; 4 and 8, allocholic acid; 5 and 7, 3α,7α,12α-trihydroxy-5α-cholestanoic acid.

GLC–MS of Me and Me-TMS derivatives of bile acids was carried out (4) with an LKB Model 9000 gas chromatograph-mass spectrometer fitted with a glass column (6 ft × 4 mm o.d.) packed with 1% OV-17 (column temp, 260°C; ion source, 270°C; ionization energy 70 ev, and accelerating voltage ranging from 2.1 to 3.5 Kv).

Reference compounds

Analytical samples of deoxycholic, cholic, chenodeoxycholic, 12-oxo-3α-hydroxy-5β-cholanic, allocholic, and allochenodeoxycholic acids were available from previous studies (9). A sample of 3α,7α,12α-trihydroxy-5β-cholestanoic acid was a gift from Dr. Russell F. Hanson, University of Minnesota Medical School, Minneapolis, MN; 3α,7α,12α-trihydroxy-5α-cholestanoic acid was derived from carp bile (10).

Isolation of *Varanus* bile acids

Bile from the gallbladders of five *Varanus monitor* captured in October was collected in 95% ethanol and left for several days at 4°C. Precipitated protein was removed by filtration, the filtrate was lyophilized, and the dried bile (508 mg) was stored at 4°C. A portion was monitored for conjugated bile acid by TLC. The remaining portion was hydrolyzed with 2.5 N KOH for 14 hr in an autoclave at 15 psi and 120°C. After dilution of the hydrolysate with an equal volume of water and extraction with ethyl acetate, 42.8 mg of unsaponifiable material was obtained. The composition of this material will be reported in a subsequent paper. The aqueous phase was acidified with 6 N HCl to pH 1, and extracted with ethyl acetate to provide 206 mg of dried acidic material. Aliquots were removed for study by TLC and for GLC after treatment with diazomethane.

Fractionation of bile acid mixture

Bile acids (100 mg) were separated by acetic acid partition chromatography (4, 11). Fractions of eluted material were designated according to the percentage of benzene in hexane; e.g., fraction 20-2 represents the second fraction of eluent containing 20% benzene in hexane. Each fraction was monitored by TLC and GLC.

RESULTS

TLC of conjugated bile acids showed only the presence of taurine derivatives, whereas after hydrolysis (**Fig. 1**) two major spots appeared, one less polar than authentic 3α,7α,12α-trihydroxy-5β-cholestanoic acid, and the other more polar than cholic or allocholic acids. The R_f's of minor spots were comparable to those of known di- and trihydroxy cholanic acids. By GLC of the Me-TMS derivatives, eleven numbered peaks were distinguished (**Fig. 2**) and shown by GLC-MS to contain a number of bile acids. To simplify interpretation of GLC-MS data, further fractionation of free bile acids was undertaken by means of acetic acid partition chromatography (**Fig. 3**).

The residues of appropriate fractions were combined and designated as follows: fractions 0-1 through 0-3 afforded fraction A (4.4 mg) which showed no evidence of bile acids by TLC or GLC-MS; fraction B (2.0 mg) consisted of fractions 0-4 through 20-2; fraction C (4.8 mg) from fractions 20-3 and 20-4; fraction D (27.1 mg) from fractions 40-1 through 40-4; fraction E (6.7 mg) from fractions 60-1 and 60-2; fraction F (12.7 mg) from fractions 60-3 through 100-1; fraction G (42.8 mg) from fractions 100-2 through 100-4 and the methanol wash. Material in these fractions was analyzed by TLC, GLC on two columns as methyl esters and as Me-TMS, and by GLC-MS.

Fraction B

One spot (R_f 0.76) was seen in TLC, but two distinct peaks were observed on GLC as Me and Me-TMS derivatives (**Table 1**). In the mass spectrum (**Fig. 4**) of the Me-TMS of material from the first peak (RRT 1.76 on OV-17), the fragment ions m/z 590, 500, 410, 283, and 255 (**Table 2,** No. 7) establish the presence of two nuclear hydroxyl groups and a double bond in the side chain of a C_{27} acid. The ions m/z 373 and 283 result

from allylic cleavage at C-20–C-22, and place the double bond at C-23. The ratio of abundances of the ions m/z 345 and 255 (9:60) confirms the 5β-configuration; the small fragment ion m/z 243 (2.8%) locates the hydroxyl groups at positions 3 and 7 (12). The structure suggested for this acid is 3α,7α-dihydroxy-5β-cholest-23-enoic acid (Δ^{23}-DHC, Table 1). The MS of the methyl ester supports this structure; m/z 446, [M^+] (10%); 428 [M-18] (9%); 410 [M-(2 × 18)] (4%); 301 [M-(18 + 127)] (56%); 283 [M-(2 × 18 + 127)] (100%); and 255 [M-(2 × 18 + 155)] (20%). The major ions m/z 301 and 283 confirm the presence of the double bond at C-23.

The mass spectrum (**Fig. 5**) of the Me-TMS of the component in the second peak (RRT 2.44 on OV-17) (Table 1) indicates that this acid is isomeric with the above material. Although base peak is the ion m/z 255, the abundances of the ions m/z 463, 373, and 283 (all resulting from loss of mass 127) are correspondingly smaller (Table 2, No. 6). A cluster of peaks around the ion m/z 283 (m/z 285, 13%; 284, 14%; 283, 23%; 282, 9%; 281, 13%) suggests (13) that this substance is the Δ^{24}-derivative, e.g., 3α,7α-dihydroxy-5β-cholest-24-enoic acid (Δ^{24}-DHC, Table 1). The spectrum of the methyl ester supports this conclusion; m/z 446, [M^+] (14%); 428, [M-18] (33%); 410, [M-(2 × 18)] (27%); 289, [M-157] (19%); 273, [M-(18 + side chain)] (39%); 271, [M-(18 + 157)] (41%); 255 [M-(2 × 18 + 155)] (100%) and 253, [M-(2 × 18 + 157)] (43%).

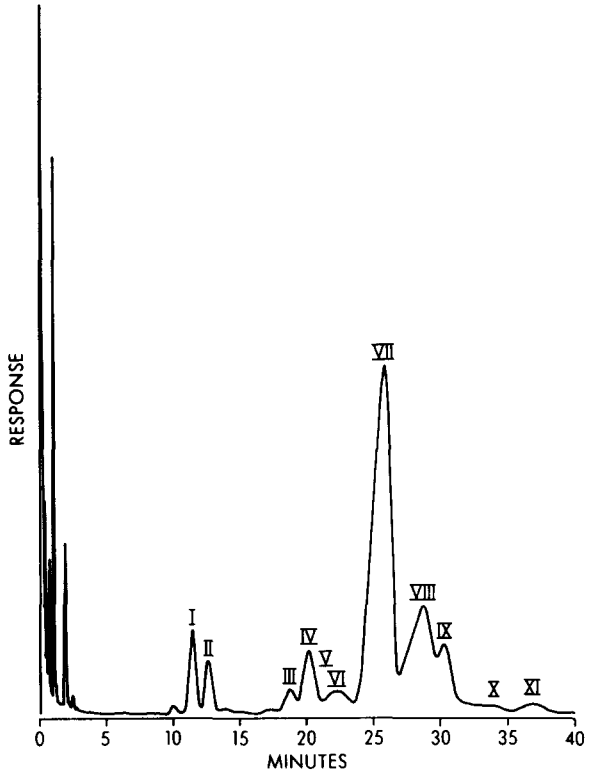

Fig. 2. Gas chromatogram of methyl ester-trimethylsilyl ethers of free acids from *Varanus* bile after alkaline hydrolysis. Conditions: a silanized glass column (6 ft × 0.25 in o.d.) packed with 3% OV-17 on Gas Chrom Q (100/120 mesh) was operated at 260°C; flow rate of carrier gas (helium) was 60 ml/min. at 40 p.s.i. Identified constituents of labeled peaks are listed in Table 1.

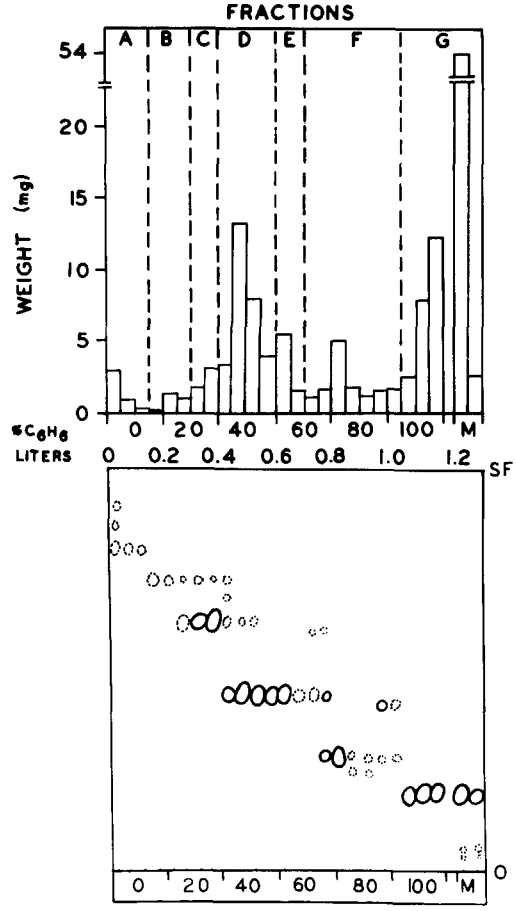

Fig. 3. Acetic acid partition chromatography of free bile acids from *Varanus*. Upper panel: weight in mg of material eluted in various fractions from column initially containing 100 mg of free bile acids. Column base was washed Celite 545 packed with 70% aqueous acetic acid; column was eluted with four 25-ml portions of a mixture of increasing amounts of benzene in hexane. M = methanol wash. Lower panel: thin-layer chromatogram of aliquots from above fractions. Solvent mixture: chloroform–methanol–acetic acid 80:12:3 (v/v) O = origin; SF = solvent front.

Fraction C

Two spots on TLC (R_f 0.63 and 0.79) were seen. Deoxycholic (DC), and chenodeoxycholic (CDC) acids were identified by GLC of the Me ester and as Me-TMS, and by GLC-MS (Table 1). From RRT and GLC-MS, methyl 12-oxo-3α-hydroxy-5β-cholanate (Table 1) was also identified. Major ions were: m/z 404, [M^+]; 386, [M-18]; 368, [M-(2 × 18)]; 355, [M-(18 + 31)]; 353, [M-(2 × 18 + 15)]; 331, [M-71]; 313, [M-(18 + 73)]; 289, [M-115]; 271, [M-(115 + 18)]; 262, [M-(115 + 27)];

TABLE 1. Bile acids from *Varanus monitor*

Bile Acids		% of Total	R_f[a]	RRT of Methyl Ester on QF-1 Std.	RRT of Methyl Ester on QF-1 Sample	RRT of Methyl Ester on OV-17 Std.	RRT of Methyl Ester on OV-17 Sample	RRT of Me-TMS QF-1 Std.	RRT of Me-TMS QF-1 Sample	RRT of Me-TMS OV-17 Std.	RRT of Me-TMS OV-17 Sample	Peak in Fig. 2	Derived from Fraction
A. C_{24} Acids													
Allocholic[b]	(AC)	0.8	0.24	2.66	2.66	2.51	2.51	1.00	1.00	0.82	0.82	Before I	F
Cholic	(CA)	5.8	0.26	2.29	2.27	2.25	2.25	1.05	1.05	0.90	0.90	I	F
Deoxycholic	(DC)	3.8	0.63	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	II	C
Chenodeoxycholic	(CDC)	<0.5	0.63	1.16	1.16	1.14	1.31	1.08	1.08	1.00	1.00	II	C
12-Oxo-3α-(OH)-cholanic		<0.5	0.79	1.61[c]	1.55	1.12[c]	1.13	3.19[c]	2.93	1.85	1.85	VI	C
B. C_{27} Acids													
3α,7α-(OH)$_2$-cholest-Δ^{23}-enoic	(Δ^{23}-DHC)	1.8	0.76		1.39		1.45		1.55		1.76	VI	B
3α,7α-(OH)$_2$-cholest-Δ^{24}-enoic	(Δ^{24}-DHC)	0.8	0.76		2.24		3.17		2.25		2.44	IX	B
3α,7α,12α-(OH)$_3$-cholestanoic	(THC)	1.6	0.46					1.46[d]	1.43	1.49[d]	1.50	III	D
3α,7α,12α-(OH)$_3$-cholest-Δ^{23}-enoic	(Δ^{23}-THC)	6.4	0.46						1.63		1.61	IV	D
3α,7α-12α-(OH)$_3$-cholest-Δ^{24}-enoic	(Δ^{24}-THC)	16.7	0.46						2.33		2.16	VII	D
3α,7α,24-(OH)$_3$-cholestanoic	(DV)	6.0	0.42						2.19		2.30	VIII	E
3α,7α,12α,24-(OH)$_4$-cholestanoic	(VA)	37.1	0.16					2.11[e]	2.14	2.01[e]	2.01	VII	G
3α,7α,12α,24-(OH)$_4$-cholestanoic	(i-VA)	4.0	0.16						2.30		2.24	VIII	G

[a] Solvent system: chloroform–methanol–acetic acid 80:12:3 (v/v).

[b] All other bile acids listed are members of the 5β-series.

[c] Data reported in ref. 9.

[d] Calculated from ref. 27.

[e] Calculated from ref. 28.

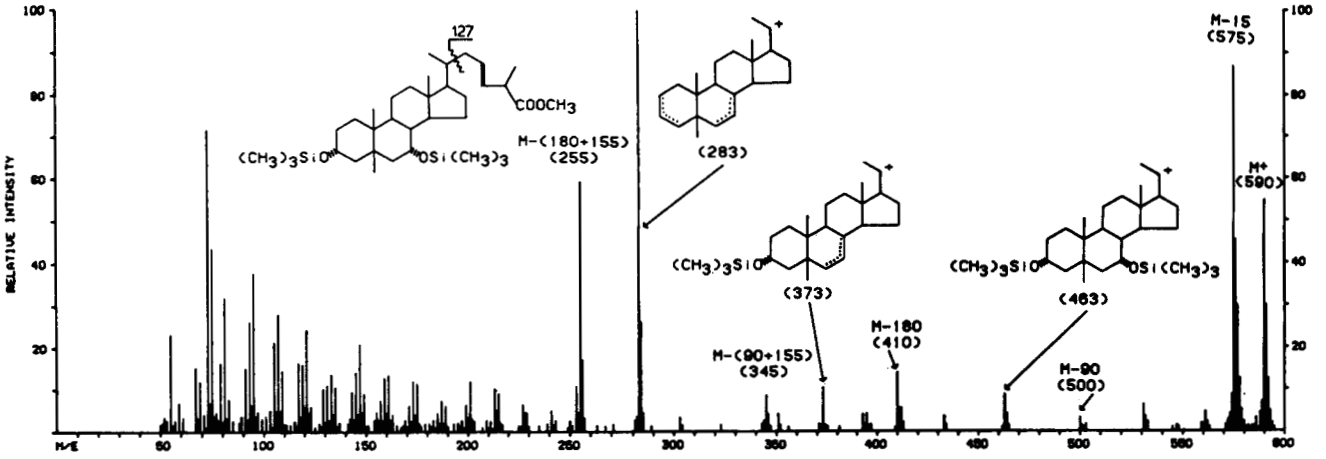

Fig. 4. Mass spectrum of methyl ester-TMS ether of dihydroxy acid of RRT 1.76 on OV-17. Important fragment ions are noted. Compound tentatively identified as 3α,7α-dihydroxy-5β-cholest-23-enoic acid.

253, [M-(115 + 2 × 18)]; 231, [M-(115 + 18 + 40)] (base peak). Three other minor components will be considered at a later time.

Fraction D

On TLC, one major spot (R_f 0.46) was associated with three minor areas (R_f 0.63, 0.73, and 0.80). By GLC on OV-17 of the Me-TMS, two major peaks (RRT 1.61 and 2.16, Table 1) and three minor peaks (RRT 1.50, 1.80, and 2.39) were obtained. GLC-MS of the constituents of the major peaks showed that they are structurally related.

The MS of the Me ester of the constituent of RRT 1.61 showed the following: m/z 462, [M^+] (2%); 444, [M-18] (7%); 426, [M-(2 × 18)] (13%); 411, [M-(2 × 18 + 15)] (9%); 408, [M-(3 × 18)] (11%); 393, [M-(3 × 18 + 15)] (46%); 299, [M-(2 × 18 + 127)] (100%); 281, [M-(3 × 18 + 127)] (71%); 271, [M-(2 × 18 + side chain)] (83%); and 253, [M-(3 × 18 + side chain)] (69%). These data considered with those from the spectrum of the Me-TMS (Table 2, No. 5) indicate that this C_{27} trihydroxy acid is also a Δ^{23}-derivative (an analog of No. 7, Table 2) and has the structure 3α,7α,12α-trihydroxy-5β-cholest-23-enoic acid (Δ^{23}-THC, Table 1).

The spectrum of the methyl ester of the second relatively more polar compound was similar to that of the above Δ^{23}-acid, but differed considerably in the abundance of characteristic ions. The fragment ions m/z 462, [M^+] (3%); 444, [M-18] (12%); 426, [M-(2 × 18)] (42%); 305, [M-157] (4%); 299, [M-(2 × 18 + 127)] (9%); 289, [M-side chain] (19%); and 287, [M-(18 + 157)] (18%) are each 16 amu larger than comparable

TABLE 2. Relative abundances of important fragment ions of C_{27} bile acids of *Varanus* as methyl ester-TMS ethers[a]

Fragment Ion	m/z	1	m/z	2	m/z	3	m/z	4	5	m/z	6	7
[M]	768	1	680	2	680	2	678	17	10	590	34	55
[M-15]	753	29	665	100	665	13	663	100	27	575	81	87
[M-90]	678	5	590	16	590	7	588	83	45	500	26	3
[M-(90 + 15)]	663	3	575	11	575	5	573	35	7	485	3	1
[M-127]	641		553				551	2	13	463	1	9
[M-(2 × 90)]	588	100	500	6	500	25	498	37	11	410	42	14
[M-(90 + 127)]	551		463				461	1	9	373	4	10
[M-(3 × 90)]	498	13	410	4	410	49	408	56	10			
[M-(2 × 90 + 127)]	461		373	1			371	3	25	283	23	100
[M-(90 + S.C.)]	433	1	345	5	433	3	433	2		345	11	9
[M-(90 + S.C. + 2H)]	431		343	2			431	2	1	343	10	2
[M-(3 × 90 + 127)]	371	1					281	15	100			
[M-(2 × 90 + S.C.)]	343	14	255	28	343	37	343	26	14	255	100	60
[M-(2 × 90 + S.C. + 2H)]	341		253	7	341		341	2	4	253	30	11
[M-(3 × 90 + S.C.)]	253	27			253	100	253	99	42			
[M-(3 × 90 + S.C. + 2H)]	251	4			251		251	13	9			

[a] The C_{27} bile acids are: 1, varanic acid; 2, 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid; 3, 3α,7α,12α-trihydroxy-5β-cholestanoic acid; 4, 3α,7α,12α-trihydroxy-5β-cholest-24-enoic acid; 5, 3α,7α,12α-trihydroxy-5β-cholest-23-enoic acid; 6, 3α,7α-dihydroxy-5β-cholest-24-enoic acid; 7, 3α,7α-dihydroxy-5β-cholest-23-enoic acid. S.C. = side chain.

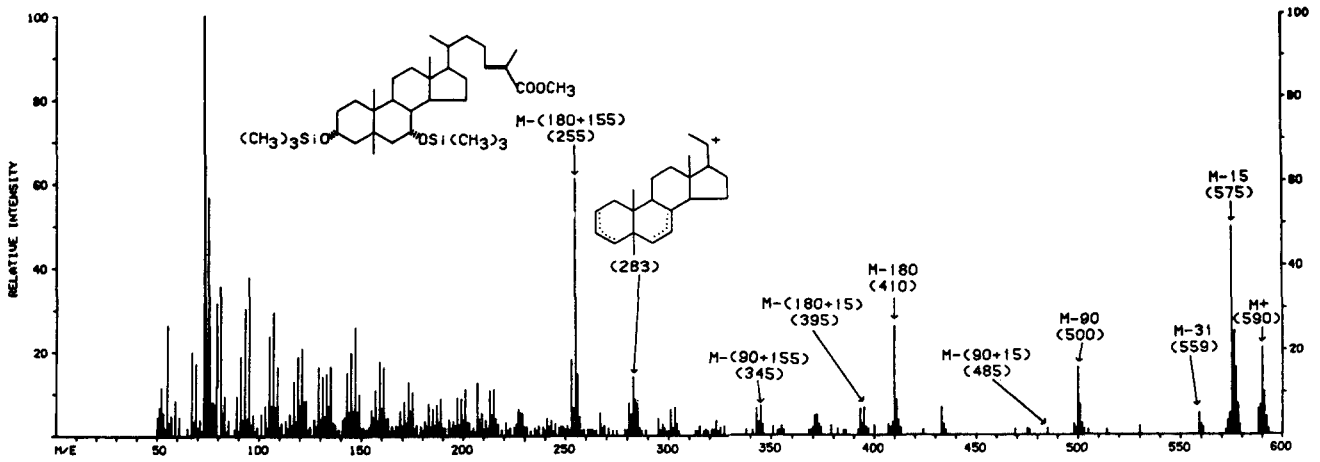

Fig. 5. Mass spectrum of methyl ester-TMS ether of isomeric dihydroxy acid of RRT 2.44 on OV-17. Compound tentatively identified as 3α,7α-dihydroxy-5β-cholest-24-enoic acid.

ions derived from the Δ^{24}-acid in Fraction B. The ions m/z 271 [M-(2 × 18 + side chain)] (71%), 269 [M-(2 + 18 × 157)] (49%), and 253 [M-(3 × 18 + side chain)] (100%) also suggest the presence of a trihydroxy acid. Comparable fragment ions in the spectrum of the Me-TMS (Table 2, No. 4) and the cluster of ions around the ion m/z 281 (m/z 283, 3%; 282, 8%; 281, 15%; 280, 5%; 279, 6%) (13) support the structure, 3α,7α,12α-trihydroxy-5β-cholest-24-enoic acid (Δ^{24}-THC, Table 1) for this product.

The minor component with RRT 1.50 was identified via the Me-TMS as 3α,7α,12α-trihydroxy-5β-cholestanoic acid by comparison of RRTs (THC, Table 1) and the MS (Table 2, No. 3) with an authentic sample. The relative proportion of ions m/z 253 and 353 signifies the presence of the 5β-configuration. The minor components (RRT 1.80 and 2.39) appear to be unsaturated acids corresponding to the 24-methyl analogs of the Δ^{23}- and Δ^{24}-trihydroxy acids just discussed; they will be considered in a subsequent paper.

Fraction E

On TLC only one spot (R_f 0.42) was seen. GLC on OV-17 of the Me-TMS provided one major peak (RRT 2.30 comparable to peak VII, Fig. 2) and several very minor peaks similar to those reported in Fraction D. The MS of the Me-TMS (**Fig. 6**) and the chromatographic behavior of the major constituent suggest strongly that this material is 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid (12-deoxyvaranic acid, DV) (Table 2, No. 2). Additional support for this structure is obtained from comparison of the retention time ratio, RTR (10, 14) of the Me-TMS with comparable compounds. The RTRs of the Me-TMS of DV and of VA on QF-1 and on OV-17, respectively, are 1.02 and 1.14, comparable to 1.03 and 1.11 for CDC and CA, 1.00 and 1.06 for 5α-CDC and AC, 1.06 and 1.19 for 5α-DHC and 5α-THC (10), 0.96 and 1.13 for Δ^{24}-DHC and Δ^{24}-THC, 0.95 and 1.09 for Δ^{23}-DHC and Δ^{23}-THC, and 1.03 and 1.11 for THC and DHC.[5]

Fraction F

Analysis by GLC, TLC, and GLC-MS confirmed the presence of cholic (CA) and allocholic (AC) acids, with the former in appreciably greater amounts (Table 1). The ratio of abundances of the ions m/z 343 and 253 from cholate Me-TMS was 0.3:1 compared to 2:1 for allocholate; the abundance of the ion m/z 261 from allocholate was 2 to 10 times that from cholate (12). Three very minor constituents with longer retention times are isomeric with these trihydroxy C_{24} acids and will be reported later.

Fraction G

On TLC only one spot was observed (R_f 0.16). GLC of the Me-TMS on OV-17 gave one major peak (RRT 2.01, about 93% of this fraction) and four minor peaks (RRT 1.72, 2.24, 2.62, and 4.43). The major component was identified by GLC-MS as varanic acid (VA) (3α,7α,12α,24ξ-trihydroxy-5β-cholestanoic acid) (Table 2, No. 1), and agreement of RRT with reported values for the Me-TMS on OV-17 and QF-1 (Table 1). The MS of the component of RRT 2.24 was exactly the same as varanic acid, suggesting that this is probably the 24β-isomer of varanic acid, (i-VA). The nature of material in the other minor peaks will be described at a later time.

[5] The RRTs for Me-TMS of DHC (3α,7α-dihydroxy-5β-cholestanoic acid) on OV-17 or QF-1 have not been reported. The values 1.67 (OV-17) and 1.47 (QF-1) were calculated from reported RRTs with the following equation: $RRT_{DHC} = RRT_{THC} \times RRT_{CDC}/RRT_{CA}$. See Table 1 for letter abbreviations of bile acids.

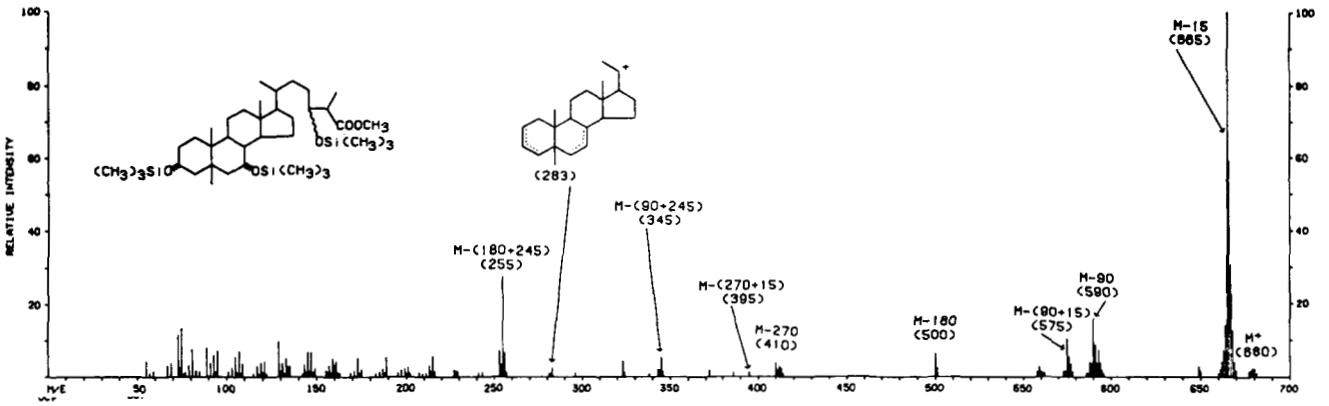

Fig. 6. Mass spectrum of methyl ester-TMS ether of trihydroxy acid of RRT 2.30 on OV-17. Compound tentatively identified as 3α,7α,24ξ-trihydroxy-5β-cholestanoic acid.

DISCUSSION

These studies demonstrate the presence in bile of *Varanus monitor* of at least 13 bile acids, including varanic acid (5), which were identified by GLC-MS. Allocholic acid, the only 5α-bile acid found, was present in such a small amount (0.8%) as to suggest that it may be a metabolite of cholic acid due to the action of intestinal bacteria. The ratio of deoxycholate to cholate (1:1.5), and the identification of 12-oxo-3α-hydroxy-5β-cholanate support this proposal.

From Fractions B and D, two pairs of isomeric unsaturated C_{27} acids were separated and identified by GLC-MS via their Me-TMS as dihydroxy and trihydroxy Δ^{23}- and Δ^{24}-acids, respectively. Allylic cleavage (13) of the side chain (Fig. 4) was clearly of major importance for the Δ^{23}-acids with the formation of the ions m/z 283 (100%) for the Me-TMS of Δ^{23}-DHC and of m/z 281 (100%) for Δ^{23}-THC (Table 2, No. 7 and 5), respectively. The Δ^{24}-acids showed little evidence of loss of the mass fragment of 127 (Table 2, No. 4 and 6), but favored loss of the entire side chain. Although cleavage of the 17-20 bond was accompanied by the migration of two nuclear hydrogens to provide the ions m/z 253 for the diol or 251 for the triol, the abundance of these ions was considerably less than reported with unsubstituted sterenes (13); the proximity of the methyl ester to the 24-25 double bond may be responsible for this difference. The cluster of ions about m/z 283 and 281 provided additional evidence that each of these acids was unsaturated at C_{24}. Based on these data and current knowledge of the biosynthesis of C_{24} bile acids (17, 18), we suggest that these acids are 3α,7α-dihydroxy- or 3α,7α,12α-trihydroxy-5β-cholest-Δ^{23}- and Δ^{24}-enoic acids.

Reports on a "Δ^{23}-3α,7α,12α-trihydroxycoprostanic acid" (19), 3α,7α-dihydroxy-5β-cholest-24-en-26-oic acid (20), 3α,7α,12α-trihydroxy-5β-cholest-24-en-26-oic acid (21, 22), and 3α,7α,12α,24ξ-tetrahydroxy-5β-cholestanoic acid (iVA, Table 1) (23, 24) have appeared, but no data are available on behavior in GLC or GLC-MS. Hence, establishment of structures of these acids is deferred pending their preparation and characterization.

To ascertain whether the Δ^{23}- and Δ^{24}-trihydroxy C_{27} acids are artifacts of biliary varanate produced during alkaline hydrolysis, a sample of isolated varanic acid was treated with 2.5 N KOH in an autoclave at 15 psi and 120°C for 18 hr. After GLC of the Me-TMS of the isolated products, the only peak seen corresponded to that of varanate.[6] Parmentier et al. (7) reported that unconjugated varanic acid, was "largely degraded" when heated with 20% KOH at 215°C for 15 min or with 4.5 M KOH at 130°C. Until other evidence is available we conclude that the dihydroxy- and trihydroxy-Δ^{23}- and Δ^{24}-5β-cholestenoic acids are authentic metabolites in this species.

Finally, this may be the first report of the natural occurrence of 12-deoxyvaranic acid (DV), the dihydroxy analog of varanic acid which should be an intermediate in the biosynthesis of chenodeoxycholate (25). In a study of the in vitro metabolism of 3α,7α-dihydroxy-5β-cholestanoic acid, Gustafsson (26) reported that fragment ions in GLC-MS from the Me-TMS of material similar to 3α,7α,24-trihydroxy-5β-cholestanoic acid were m/z 665, 500, 410, 345, and 255; abundances were similar to corresponding ions for the Me-TMS of varanic acid. Since no RRT's were reported, no comparison can be made with the DV obtained from *Varanus*. Full characterization of this and other C_{27} bile acids from *Varanus* is an objective of this laboratory.

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[6] Ali, S. S. Unpublished data.

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