Bile acids. LI. Formation of 12α-hydroxyl derivatives and companions from 5α-sterols by rabbit liver microsomes

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Abstract A comparison of the activity of rabbit liver microsomes fortified with 0.1 mM NADPH to promote 12α-hydroxylation over 60 minutes with appropriate sterols provided the following relative order of activities: 5α-cholestane-3α,7α-diol, 100; 7α-hydroxy-5α-cholestane-3-one, 76; 7α-hydroxycholest-4-en-3-one, 64; 5α-cholestane-3β,7α-diol, 24; allochenodeoxycholate, 22. If the more polar products are included, the first three sterols are equivalent in product formation. Investigation by gas–liquid chromatography–mass spectrometry of the nature of these more polar products derived from 5α-cholestane-3α,7α-diol showed that a series of tetrols was formed; i.e., 5α-cholestane-3α,7α,12α,25-tetrol as the major product and lesser amounts of 5α-cholestane-3α,7α,12α,24- and 3α,7α,12α,23-tetrols. No significant amount of 26-hydroxylated product was formed.

Supplementary key words 5α-cholestane-3α,7α,12α,25-tetrol · tetrols from 5α-cholestane-3α,7α-diol · 5α-cholestane-3α,7α-diol · 7α-hydroxycholest-4-en-3-one · Gas–liquid chromatography–mass spectrometry · 12α-hydroxylation · microsomal hydroxylation · sterol side chain hydroxylation

To delineate a mechanism for the 12α-hydroxylation of allochenodeoxycholate to allocholate observed in the rat (1), experiments with liver microsomal preparations fortified with NADPH were undertaken with allochenodeoxycholate and precursor sterols, 5α-cholestane-3α,7α-diol and the 26-hydroxy and 26-carboxyl derivatives of this diol (2). The requirements for the microsomal system were found to be similar to those for hydroxylation of 7α-hydroxycholest-4-en-3-one (3), a precursor of cholic acid. Since molecular models show that the coplanar 5α-cholestane-3α,7α-diol is virtually superimposable upon 7α-hydroxy-cholest-4-en-3-one, it was suggested that a single enzyme system may be responsible for the facile 12α-hydroxylation of these substrates (2). Because the rabbit liver microsomal system produced substantially greater quantities of allocholate from allochenodeoxycholate (4), the properties of this preparation were investigated in greater detail with allochenodeoxycholate and 5α-cholestane-3α,7α-diol (5, 6). This paper compares the 12α-hydroxylase activity of rabbit liver microsomes fortified with NADPH with 7α-hydroxycholest-4-en-3-one (the natural precursor for cholic acid) and precursors of allocholate, namely 5α-cholestane-3α,7α-diol, 5α-cholestane-3β,7α-diol, 7α-hydroxy-5α-cholestane-3-one, and allochenodeoxycholate. The nature of additional metabolites derived from further action by rabbit liver microsomes on 5α-cholestane-3α,7α-diol is also described. A preliminary communication has been presented (7).

METHODS AND MATERIALS

Neutral sterols were separated by TLC (8), GLC (9) and acetic acid partition chromatography (10). In the latter system the fractions are designated according to the percentage of benzene in hexane; e.g., Fraction 60-4 represents the fourth fraction of eluent containing 60% benzene in hexane. Methods for mass spectrometry (11), radioassay (6), and analysis of products of incubation by TLC (6) were similar to those reported. The specific activities of the substrates were: [3β-^{3}H]allochenodeoxycholic acid, 3.9×10^8 dpm/mg; [5α,6α-$^3H_2$]5α-cholestane-3β,7α-diol, 4.07×10^7 dpm/mg; [5α,6α-$^3H_2$]5α-cholestane-3α,7α-diol, 4.11×10^7 dpm/mg; 7α-hydroxy-[5α,6α-$^3H_2$]5α-cholestan-3-one, 4.01×10^7 dpm/mg. These materials were prepared, purified and characterized as previously described (2, 6). Samples of 7α-hydroxy[6β-^{3}H]cholest-4-en-3-one (sp act 1.3

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl. Systematic names of the compounds referred to in the text are as follows: allochenodeoxycholic acid, 3α,7α-dihydroxy-5α-cholanic acid; allocholic acid, 3α,7α,12α-trihydroxy-5α-cholanic acid; cholestanol, 5α-cholestan-3β-ol.

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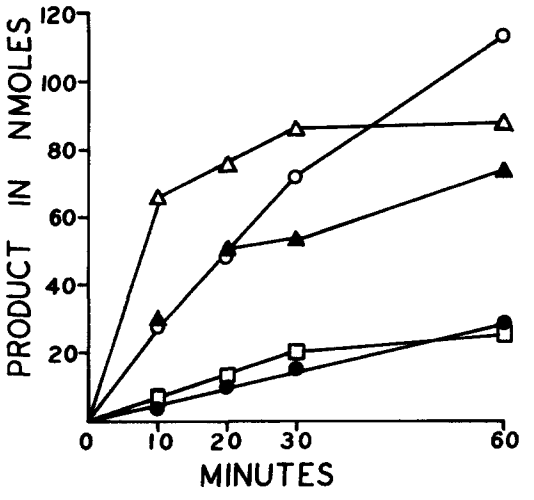

Fig. 1. Comparison of rate of 12α-hydroxylation of substituted 5α-cholestanes with allochenodeoxycholate. Substrates: ○, 5α-cholestane-3α,7α-diol; ●, 5α-cholestane-3β,7α-diol; △, 7α-hydroxy-5α-cholestan-3-one; ▲, 7α-hydroxycholest-4-en-3-one; □, allochenodeoxycholate. Incubations were carried out with 175 nmoles of sterol or 165 nmoles of allochenodeoxycholate with 3 ml of rabbit liver microsomal preparations (6 mg protein/ml).

$\times 10^7$ dpm/mg) and 7α,12α-dihydroxycholest-4-en-3-one were gifts of Dr. Henry Danielsson.

Preparation of microsomal fraction

Male rabbits weighing 3.5–4.5 kg were killed by infusion of air in the ear vein and a portion of the right lobe of the liver was excised and placed in freshly prepared Bucher's medium (0.1 M phosphate, pH 7.4) at 4°C. Liver homogenates, 20% (w/v) were prepared (3) and centrifuged at 20,000 *g* for 20 min. The microsomal fraction was obtained after centrifugation of the 20,000 *g* supernatant fluid at 100,000 *g* for 1 hr. (12). Protein determinations were made with the biuret reagent with bovine serum albumin as a standard. In most experiments the concentration of microsomal protein was adjusted to 6 mg/ml.

Incubation procedure

The substrate (175 nmoles of sterol or 165 nmoles of allochenodeoxycholic acid) was incubated at 37°C in air with shaking in 3 ml of microsomal preparation (6 mg protein/ml) in modified Bucher's medium (0.1 mM phosphate buffer, pH 7.4) with 3 μmoles of NADPH as previously reported (6). A microsomal preparation heated at 100°C for 15 min prior to incubation with the substrate was used as a control in each case. Incubations were carried out in duplicate (1, 6). Reactions were quenched with 10 volumes of 95% ethanol and the mixtures were left to stand several hours prior to removal of protein by filtration. The precipitates were washed with three 5-ml volumes of ethanol, and the filtrate was evaporated under nitrogen. Recoveries of incubated radioactivity were 95% or greater.

RESULTS

The results of incubation of four radioactive sterols and allochenodeoxycholate for 10, 20, 30, and 60 min with rabbit liver microsomes are shown in **Fig. 1**. The formation of 12α-hydroxylated product was linear up to 30 min with the 3α,7α- and 3β,7α-diols, and allochenodeoxycholate; linearity for 20 min was shown by 7α-hydroxycholest-4-en-3-one, whereas the reaction with 7α-hydroxy-5α-cholestan-3-one was linear between 10 and 30 min. Based on the amount of 12α-hydroxylated product formed in 60 min in comparable incubations, the sterols were ranked in descending order on a scale of 100 as follows: the 3α,7α-diol, 100; 7α-hydroxy-5α-cholestan-3-one, 76; 7α-hydroxycholest-4-en-3-one, 64; the 3β,7α-diol, 24; allochenodeoxycholate, 22. However, as reported earlier (6), 8–12% of the radioactivity detected on the thin-layer plate from incubation of 5α-cholestane-3α,7α-diol appeared in regions more polar than 5α-cholestane-3α,7α,12α-triol. Similarly, substantial amounts of more polar products were found after incubation of 7α-hydroxy-5α-cholestan-3-one and 7α-hydroxycholest-4-en-3-one. If the activity in these more polar regions is included in the total of products formed from 5α-cholestane-3α,7α-diol, 7α-hydroxy-5α-cholestan-3-one, and 7α-hydroxycholest-4-ene-3-one, the comparative values are then 100, 110, and 103, respectively. **Fig. 2** shows the location of radioactive material on the chromatograms following incubation for 20 min of each of the five substrates.

Because of our interest in the nature of these materials more polar than 5α-cholestane-3α,7α,12α-triol, derived from the 3α,7α-diol in relation to the biosynthesis of allocholate, the parameters of time of incubation, and concentrations of substrate, protein, and added NADPH were determined (**Fig. 3**) similar to those investigated for the conversion of the 3α,7α-diol to the 3α,7α,12α-triol (6). Generally, the results were comparable to those for 12α-hydroxylation; the reaction was linear from 10–60 min; maximum amount of substrate approximated 225 nmoles; protein concentration approached 10 mg/ml; and the optimal concentration of NADPH was 0.1 mM.

To concentrate those metabolites more polar than 5α-cholestane-3α,7α,12α-triol, the mixtures from two

60 min incubations were combined (total activity, 2.9×10^7 dpm) and the components were separated by acetic acid partition chromatography. The 3α,7α,12α-triol appeared with the 3α,7α-diol in Fractions 0-1 through 0-4. Radioactivity in Fractions 40-1 through 40-3 (4.3×10^5 dpm) was mixed with 48.6 mg of 3α,7α,12α-trihydroxy-5α-cholestanoic acid, (13), a C_{27} acid eluted in this region (14). After four successive crystallizations from aqueous–methanol, methanol, and methanol–acetone, the specific activities were 8.7 (calculated), 4.4, 1.4, 1.08, and 1.18×10^3 dpm/mg. The sp act had dropped to 13% of the calculated value and corresponded to less than 0.2% of the total chromatographed radioactivity. Similarly, the radioactivity contained in Fractions 60-1 through 60-4 (2.5×10^6 dpm) was diluted with 50.1 mg of authentic 5α-cholestane-3α,7α,12α,26-tetrol and chromatographed. Most of the radioactivity was eluted in Fractions 60-1 and 60-2, whereas the mass of added tetrol spread from Fractions 40-4 through 80-4 (32.5 mg). Fractions 40-4 through 80-4 were pooled, 22.8 mg of additional authentic tetrol was added (total mass 50.2 mg, calcd. sp act 2.25×10^4 dpm/mg) and the product was crystallized three times from methanol–acetone and methanol with the following sp act: 5.7, 2.78, and 1.53×10^2 dpm/mg. The specific activity of the material in the first mother liquor showed 4.86×10^4 dpm/mg. Thus, the radioactivity in 5α-cholestane-3α,7α,12α,26-tetrol could account for no more than 0.64% of the total radioactivity in Fractions 40-4 through 80-4.

Identification of metabolites more polar than 5α-cholestane-3α,7α,12α-triol

Mixtures from 53 incubations of [5α,6α-$^3H_2$]5α-cholestane-3α,7α-diol were pooled, diluted with methanol, filtered, and the filtrate concentrated under nitrogen. The residue (6.6×10^8 dpm) contained a significant quantity of solid soluble in methanol and chloroform, perhaps from the organic constituents of Bucher's medium used in the incubations. The radioactive material was fractionated by acetic acid partition chromatography (**Fig. 4**). About 90% of the tritium was eluted in Fractions 0-1 through 20-1, containing unchanged 3α,7α-diol (Fractions 0-1 and 0-2) and the 3α,7α,12α-triol (Fractions 0-3 through 20-1). Approximately 10% of the tritium spread from Fractions 40-1 through 100-2 was pooled and the contents were partitioned between ethyl acetate and water. The organic phase (2.27×10^7 cpm) was washed with water and extracted with 5% potassium hydroxide to remove traces of organic acid.

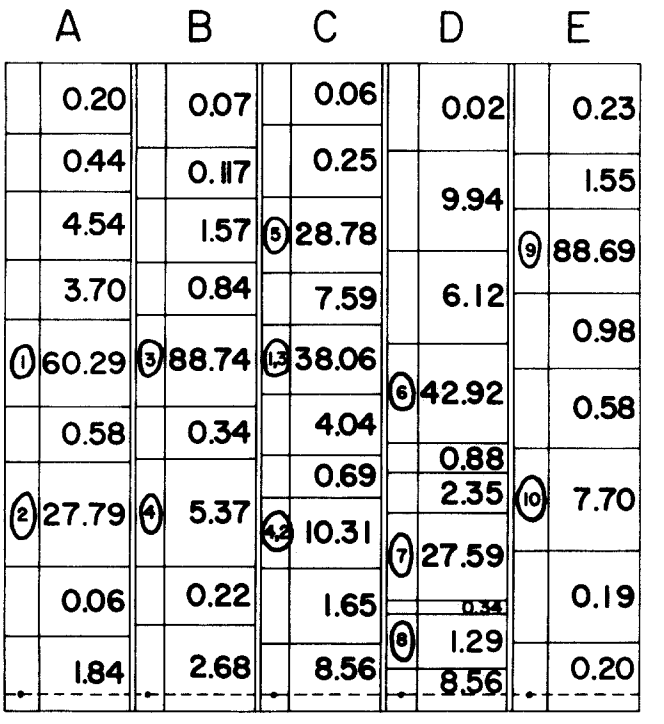

Fig. 2. Thin-layer chromatograms of extracts of incubations of (A) [5α,6α-$^3H_2$]5α-cholestane-3α,7α-diol, (B) [5α,6α-$^3H_2$]5α-cholestane-3β,7α-diol, (C) 7α-hydroxy-[5α,6α-$^3H_2$]5α-cholestan-3-one, (D) 7α-hydroxy-[6β-^{3}H]cholest-4-en-3-one, and (E) [3β-^{3}H]allochenodeoxycholic acid. Incubations were carried out for 20 min with 175 nmoles of sterol or 165 nmoles of allochenodeoxycholate. The numbers on the chromatograms represent the percent of chromatographed tritium found in each area. The reference compounds were: (1) 5α-cholestane-3α,7α-diol; (2) 5α-cholestane-3α,7α,12α-triol; (3) 5α-cholestane-3β,7α-diol; (4) 5α-cholestane-3β,7α,12α-triol; (5) 7α-hydroxy-5α-cholestan-3-one; (6) 7α-hydroxycholest-4-en-3-one; (7) 7α,12α-dihydroxycholest-4-en-3-one; (8) 5β-cholestane-3α,7α,12α-triol; (9) allochenodeoxycholic acid; (10) allocholic acid. The solvent systems were acetone–benzene 1:1 (A,B,C); benzene–ethyl acetate 3:7 (D), and chloroform–methanol–acetic acid 80:12:3 (E). The origin is represented by the dotted line near the bottom.

An aliquot of the residue of the neutral fraction was converted (9) to the TMSi ethers, and the product was subjected to gas–liquid chromatography (**Fig. 5**) on 1% SE-30(15). The dotted line (VI) in Fig. 5 represents elution of the TMSi derivative of added authentic 5α-cholestane-3α,7α,12α,26-tetrol. The peaks of interest (I thru V) were studied by mass spectrometry (**Table 1**) and the components were identified as: V, 5α-cholestane-3α,7α,12α, 25-tetrol; IV and III, 5α-cholestane-3α,7α,12α,24-tetrols (24α and 24β); II, 5α-cholestane-3α,7α,12α,23-tetrol; and I appears to be a mixture of cholesterol and 5α-cholestane-3α,7α,12α,22-tetrol. The relative percentage of material in peaks V, IV and III, and II were 56, 17, and 26% respectively. No significant 26-hydroxylation was found, as indicated by the absence of a peak (solid line under the dotted line

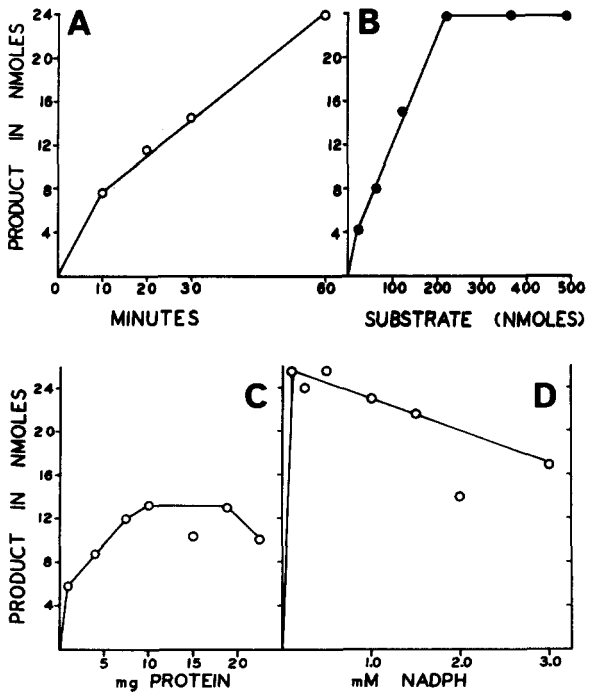

Fig. 3. Relation of yield of polyhydroxy-5α-cholestanes derived from [5α,6α-$^3H_2$]5α-cholestane-3α,7α-diol to time (A), concentration of substrate (B), protein (C), and NADPH (D). In (A) and (D) 175 nmoles of substrate were incubated with 18 mg of microsomal protein for various periods of time with 0.1 mM NADPH or for 60 min with various amounts of NADPH, respectively. Incubations (B) and (C) were carried out for 60 min with 18 mg of microsomal protein or 175 nmoles of substrate, respectively.

VI, Fig. 5), and in agreement with the previous result of isotopic dilution.

DISCUSSION

A study of the properties of hepatic steroid 12α-hydroxylase would be greatly facilitated with tissue from a species that exhibits an enhanced active enzyme system. The major biliary acids in rabbit bile are cholic and deoxycholic acids (16). Chenodeoxycholic acid has not conclusively been shown to be a constituent of normal rabbit bile (17); exogenous chenodeoxycholate is not metabolized by the rabbit to cholate (4), but is converted to 7-oxolithocholate (17), ursodeoxycholate (17), and lithocholate (17). Allodeoxycholate, a normal constituent of rabbit bile and gallstones (18, 19), is formed by the action of intestinal bacteria on allocholate (20), which is derived from cholestanol (19, 21) or 7α-hydroxycholesterol (21) in the rabbit. Alternately, exogenous allochenodeoxycholate is converted to allocholate (4, 6, Figs. 1 and 2) and in amounts 3–6 times greater than those produced by the rat under similar circumstances, which may explain in part the absence of allochenodeoxycholate in rabbit bile. Thus, rabbit liver is an attractive source of the enzyme 12α-hydroxylase.

With regard to the pathway from cholestanol to allocholic acid, Björkhem and Gustafsson (22) have compared the quantity of 12α-hydroxylated product derived from rat liver microsomes incubated with added NADPH and cholestanol (A), 5α-cholestane-3β,7α-diol (B), 7α-hydroxy-5α-cholestan-3-one (C), or 5α-cholestane-3α,7α-diol (D), and concluded that the major pathway for the formation of allocholate involved 7α-hydroxylation of cholestanol (A) to the diol (B), dehydrogenation to the ketone (C), and reduction to the diol (D) prior to 12α-hydroxylation; thus, on a relative scale, the substrates exhibited reactivities toward 12α-hydroxylation of 0, 6, 15, and 100 in the above order (1). In the present studies with male rabbit liver microsomes this order of reactivity is also found, although cholestanol was replaced in these studies with allochenodeoxycholic acid as an internal control, since we have reported on the ability of rabbit liver microsomes to promote 12α-hydroxylation of this allo bile acid (4, 5). In a comparison of 5α-cholestane-3α,7α-diol with 7α-hydroxycholest-4-en-3-one, the substrate that is naturally converted to cholic acid after 12α-hydroxylation, (Fig. 1) the yield of 12α-hydroxylated product would appear to be less for the unsaturated sterol. The slope of the curves (Fig. 1) for several of the sterols suggests product inhibition and/or the occurrence of other events in addition to 12α-hydroxylation. This latter probability is supported by the large number of microsomal-bound enzymes to be active on sterol substrates (23). For example,

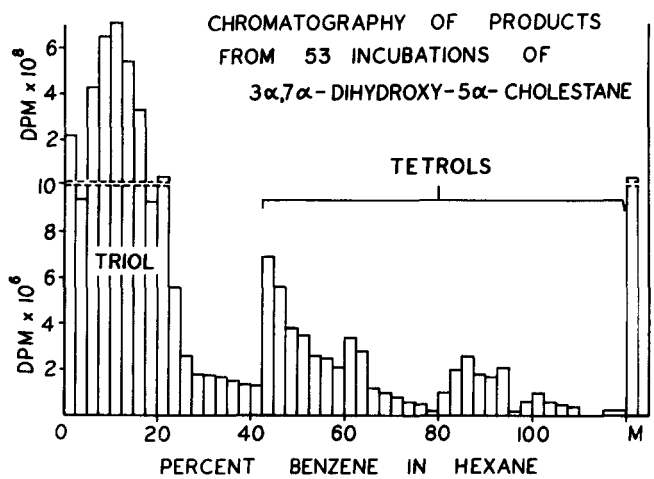

Fig. 4. Partition chromatography of residues from 53 incubations of [5α,6α-$^3H_2$]5α-cholestane-3α,7α-diol (175 nmoles) with rabbit liver microsomes.

rat liver microsomes fortified with NADPH were reported by Björkhem and Einarsson (24) to reduce 7α-hydroxy-5α-cholestan-3-one preferentially to 5α-cholestane-3β,7α-diol, and 7α-hydroxycholest-4-en-3-one to the above 7α-hydroxy-5α-cholestan-3-one. Since their products were well characterized and our major interest rested in the relative amount of 12α-hydroxylated derivative, full characterization by the usual means was not undertaken with the multiple derivatives of 7α-hydroxy-5α-cholestan-3-one or 5α-cholestane-3β,7α-diol. Similarly, Einarsson (3) reported the identification of 7α,12α-dihydroxycholest-4-en-3-one, 7α,12α-dihydroxy-5β-cholestan-3-one, 5β-cholestane-3α,7α,12α-triol and other metabolites (8.5%) following incubation of 7α-hydroxycholest-4-en-3-one with rat liver microsomes fortified with NADPH. Björkhem and Einarsson (24) further suggested the presence of 7α,12α-dihydroxy-5α-cholestan-3-one as a microsomal reduction product from 7α,12α-dihydroxy-cholest-4-en-3-one. Their solvent system (benzene–ethyl acetate 3:7, v/v) was also used in these experiments (Fig. 2) and the authentic materials migrated exactly in the same regions noted by these authors. Thus, the results shown in Fig. 1 and 2 are compatible with the concept of a single enzyme system active in 12α-hydroxylation of 5α-cholestane-3α,7α-diol or 7α-hydroxycholest-4-en-3-one. The relative quantities given for comparison of action of 12α-hydroxylase in the experiments shown in Fig. 1 may be in error to some extent, but they do show a difference; a quantitative similarity is seen in 60 min *if the more polar metabolites are also included*. The quantity

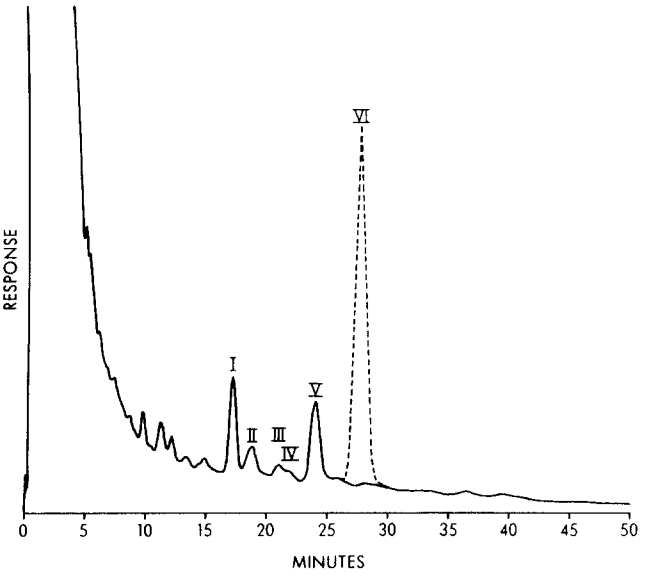

Fig. 5. Gas–liquid chromatogram of the trimethylsilyl ethers of material in the neutral fraction of polar material from multiple incubations of [5α,6α-$^3H_2$]5α-cholestane-3α,7α-diol with rabbit liver microsomes. Peak VI represents authentic 5α-cholestane-3α,7α,12α,26-tetrol added to a second sample.

(8.5%) of unidentified polar metabolites derived from 7α-hydroxycholest-4-en-3-one by Einarsson (3) compares favorably with similar materials derived from 5α-cholestane-3α,7α-diol (6).

To ascertain the nature of these metabolites more polar than 5α-cholestane-3α,7α,12α-triol, the products from two 60 min incubations were combined, purified by partition chromatography, and the appropriate portions were cocrystallized with 3α,7α,12α-

TABLE 1. Relative intensities of fragment ions in mass spectrometry of 5α-cholestane-tetrols as the TMSi ethers

m/e	Fragment Ion	Intensity of Fragment from 5α-Cholestane-tetrol			
		3α,7α,12α,25-	3α,7α,12α,24-	3α,7α,12α,23-	3α,7α,12α,26-
		Peak V	*Peaks IV and III*	*Peak II*	*Peak VI*
724	M	<1	2	1	1
709	M − 15	1			0.3
681	M − 43		9		
667	M − 57			5	
634	M − 90	2	5		29
591	M − (90 + 43)		9		
544	M − (2 × 90)	41	56	32	100
501	M − (2 × 90 + 43)		22		
454	M − (3 × 90)	9	16	17	10
411	M − (3 × 90 + 43)		21		
364	M − (4 × 90)	5	6	7	
343	M − (3 × 90 + 111)	30	48	34	61
321	M − (4 × 90 + 43)		23		
253	M − (4 × 90 + 111)	12	31	18	21
159	$[(CH_3)_3SiO = CHCH_2CH(CH_3)_2]^+$			100	
145	$[(CH_3)_3SiO = CHCH(CH_3)_2]^+$		100		
131	$[(CH_3)_3SiO = C(CH_3)_2]^+$	100			

trihydroxy-5α-cholestanoic acid or 5α-cholestane-3α,7α,12α,26-tetrol. In the former case the final specific activity of the acid indicated that less than 0.2% of the total chromatographed product could be present in this acid. The microsomal fraction was prepared in a manner similar to that used by Björkhem and Gustafsson (12) and Cronholm and Johansson (15) who determined a contamination of the microsomal preparation with mitochondria of about 1%. The presence of a very small amount of 3α,7α,12α-trihydroxy-5α-cholestanoic acid among the products of incubation may be explained by such contamination. After isotopic dilution of the appropriate material with 5α-cholestane-3α,7α,12α,26-tetrol no more than 0.64% of the metabolites from 53 incubations were concentrated, and the neutral materials were characterized by gas–liquid chromatography–mass spectrometry via their TMSi ethers as the 25-, 24α- and 24β-, and 23-hydroxylated derivatives of 5α-cholestane-3α,7α,12α-triol (Fig. 5, Table 1).

The identification of these tetrols via mass spectrometry was facilitated by earlier studies of Cronholm and Johansson (15) and Björkhem et al. (25), who observed that the most prominent fragment ions in the mass spectra of the TMSi ethers of 5β-cholestane-3α,7α,12α,25-tetrol; 3α,7α,12α,24-, and 3α,7α,12α,23-tetrols were

m/e 131 $[(CH_3)_3\,SiO{=}C(CH_3)_2]^+$, *m/e* 145 $[(CH_3)_3SiO{=}CHCH(CH_3)_2]^+$

and *m/e* 159 $[(CH_3)_3SiO{=}CHCH_2CH(CH_3)_2]^+$, respectively. Mass spectra of isomeric 5β- and 5α-sterols and bile acids are similar, i.e., the process of fragmentation is essentially the same with only occasional differences discerned in the relative intensities of selected fragment ions. Thus, the fragment ions *m/e* 131, 145, and 159 would be expected and were found (Table 1) in spectra of the TMSi ethers of the corresponding 5α-cholestane-tetrols. In the case of the TMSi ether of 5α-cholestane-3α,7α,12α,26-tetrol, base peak appeared at *m/e* 544 (Table 1); base peak for the isomeric 5β-tetrol appeared at *m/e* 253, although intense fragment ions were seen at *m/e* 544 [M − (2 × 90)] and 454 [M − (3 × 90)] (15, 25). The intensity of the fragment ion *m/e* 343 [M − (3 × 90 + 111)] is larger than that of *m/e* 253 [M − (4 × 90 + 111)] in spectra of TMSi ethers of 5α-cholestanes, of these tetrols, and of the TMSi ethers of methyl esters of comparable 5α-bile acids (11); the reverse is true for the 5β-derivatives (15, 11). Thus, the derivatives analyzed by gas–liquid chromatography–mass spectrometry (Fig. 5) are 5α-cholestanes derived from the substrate, 5α-cholestane-3α,7α-diol, and are not derived from possible existing 5β-sterols in the microsomal preparations. The absence of an appreciable peak corresponding to authentic 5α-cholestane-3α,7α,-12α,26-tetrol confirms the absence of significant 26-hydroxylation as measured by isotopic dilution.

The nature of these tetrols derived from a 5α-cholestane derivative is of particular interest in comparison with the studies of Cronholm and Johansson (15) who observed similar hydroxylation of the side chain of 5β-cholestane-3α,7α,12α-triol with rat liver microsomes. The ratio of substitution at C-25, C-24, C-23, and C-26 was 51:13:13:23; with human liver microsome hydroxylation at C-25 dominating (25), similar to the results with rabbit liver microsomes.

These results confirm the ability of rabbit liver microsomal preparations to hydroxylate 5α-cholestane-3α,7α-diol in the 12α-position and to promote hydroxylation of the side chain of the coplanar 5α-sterol in a manner similar to that effected with 7α-hydrocholest-4-en-3-one and the resultant 5β-sterol. Whether such progressive hydroxylation on the side chain is of significance in the formation of allocholic acid remains to be determined. In patients with defective bile acid synthesis (e.g., cerebrotendinous xanthomatosis) 5β-cholestane-3α,7α,12α,25-tetrol has been identified as a major biliary and fecal bile alcohol (26). Although no 5α-cholestane polyols have been reported, it would be of interest to ascertain whether the isomeric 5α-cholestane-3α,7α,12α,25-tetrol would be produced by such individuals after ingestion of 5α-cholestanol.

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