Hepatic metabolism of short-chain bile acids. Inversion of the 3-hydroxyl group of isoetianic acid (3β-hydroxy-5β-androstane-17β-carboxylic acid) by the adult rat

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Abstract The stereospecificity of mechanisms for hepatic transport of short-chain bile acids has been examined by following the hepatic metabolism and biliary secretion of 3β-hydroxy-5β-androstane-17β-carboxylic acid (isoetianic acid) administered in two different labeled forms to rats prepared with an external biliary fistula. While 93% of the administered [2,2,4,4-^{3}H]isoetianic acid was recovered in bile after 20 h, only 18% of a similar dose of [3α-^{3}H]isoetianic acid was secreted in bile over the same time period. The recovered radioactivity of the latter compound was mainly associated with bile water. With the [2,2,**4**,4-^{3}H]isoetianic acid, the bulk of the biliary isotope was determined to be in the form of two glucuronide conjugates. Spectral analysis identified these metabolites as the hydroxyl-linked (major) and carboxyl-linked (minor) β-glucuronides, not of the 3β-hydroxy compound administered, but of 3α-hydroxy-5β-androstane-17β-carboxylic acid (etianic acid), i.e., the products of hydroxyl group inversion. It is concluded that isoetianic acid is efficiently cleared from plasma and conjugated with glucuronic acid after its epimerization to etianic acid. The prevalent, but not complete, loss of the 3-tritium atom and the retention of the 2- and 4-tritium atoms probably indicates a 3-oxo-5β-androstane-17β-carboxylic acid intermediate with partial return of the label via a limited labeled pool of reduced nicotinamide cofactor. **—Little, J. M., J. S. Pyrek, A. Radominska, K. E. Shattuck, and R. Lester.** Hepatic metabolism of short-chain bile acids. Inversion of the 3-hydroxyl group of isoetianic acid (3β-hydroxy-5β-androstane-17β-carboxylic acid) by the adult rat. *J. Lipid Res.* 1991. **32:** 1949–1957.

Supplementary key words glucuronide conjugates • epimerization • biliary secretion

As a result of the analysis of mono- and dihydroxylated steroidal acids of human meconium, a unique array of previously undetected C_{20} (etianic), C_{21} (pregnan-21-oic), and C_{22} (bisnorcholestan-22-oic) short-chain bile acids has been identified (1–3). Similar C_{20} and C_{22} acids have also been found in human cholestatic serum (4). Although the metabolic origin of short-chain bile acids is apparently diverse, structural features of the C_{20} and C_{21} acids detected in human meconium suggest their direct metabolic relation to C_{21} steroids such as progesterone. Moreover, the especially abundant presence of these acids in human meconium, which correlates with the high level of progesterone during pregnancy and suggests that they are secreted in bile, attests to the physiological significance of this oxidative catabolic pathway.

The initial studies suggested that glucuronidation and/or sulfation appeared to be the most probable forms of conjugation for the short-chain bile acids. Subsequent investigations of the in vivo and in vitro metabolism of these compounds have demonstrated that glucuronidation is the major conjugation mechanism and is not limited to formation of hydroxyl-linked conjugates since carboxyl-linked glucuronides of certain acids have been identified (5–10).

In order to examine the stereospecificity of the mechanisms for hepatic transport of C_{20} bile acids, the hepatic metabolism and biliary secretion of the axial 3β-epimer of etianic acid (3β-hydroxy-5β-androstane-17β-carboxylic acid; isoetianic acid) were investigated and the results were compared with our previous studies with 3α-hydroxy-5β-androstane-17β-carboxylic acid (etianic acid) (5) and a parallel investigation of 3-oxoandrost-4-ene-17β-carboxylic acid (etienic acid) (11). The structures of these compounds are shown in **Fig. 1.** Two different species of isoetianic

Abbreviations: HPLC, high performance liquid chromatography; TLC, thin-layer chromatography; GC, gas chromatography; MS, mass spectrometry; TMS, trimethylsilyl.

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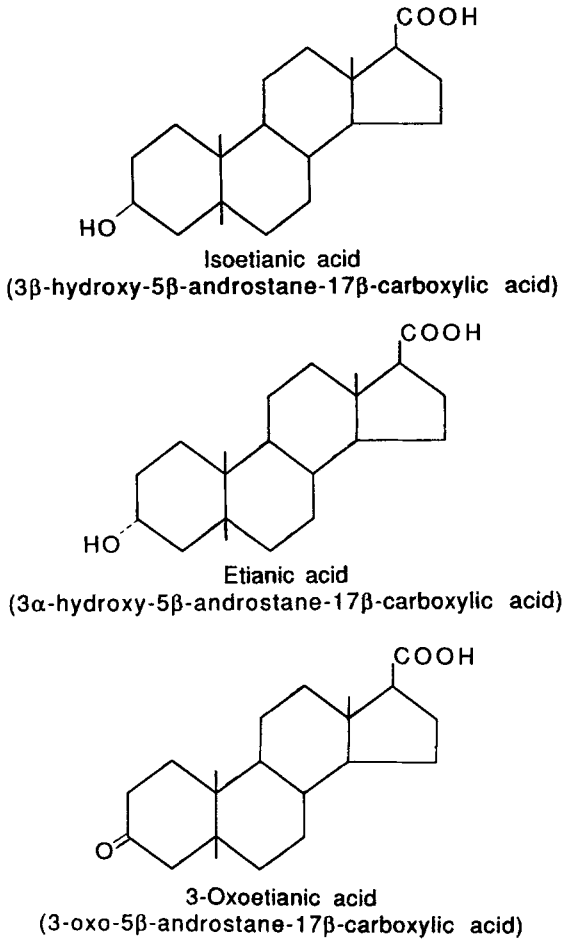

Fig. 1. Structures of short-chain (C_{20}) bile acids.

acid were used: one labeled at C-2 and C-4 permitted easy detection of isoetianic acid metabolites while another labeled only at C-3 provided insight into the inversion of the C-3 configuration as compared to the behavior of C-3 tritium-labeled etianic acid (5).

METHODS

Synthesis of [3α-^{3}H]3β-hydroxy- and [3α-^{3}H]3α-hydroxy-5β-androstane-17β-carboxylic acids

3-Oxoandrostane-17β-carboxylic acid methyl ester (4 mg; Steraloids, Wilton, NH) was dissolved in isopropanol (1.5 ml) and reduced with an excess of NaB^3H_4 (sp act 110 mCi/mmol; New England Nuclear, Boston, MA). After 30 min, 6 N HCl was added, followed by water (1 ml) and partial evaporation under nitrogen. Extraction with ethyl ether (3 × 10 ml) produced a mixture of methyl esters of [3β-^{3}H]etianic (87.4%) and [3α-^{3}H]isoetianic (12.6%) acids which were separated by HPLC (Supelcosil LC-Si, 150 × 4.6 mm, Supelco Inc., Bellefonte, PA; 0.4% (v/v) isopropanol in hexane at 1.25 ml/min; UV detection at 200 nm). The methyl esters were hydrolyzed in methanol (2 ml) and 5 N NaOH (0.2 ml) at 65°C for 18 h. The free acids recovered by ethyl ether extraction from the acidified solution were found by TLC, HPLC, and GC to be chemically and radiochemically pure (>98%).

Synthesis of [2,2,4,4-$^3H_4$]3β-hydroxy- and [2,2,4,4-$^3H_4$]3α-hydroxy-5β-androstane-17β-carboxylic acids

3-Oxo-5β-androstane-17β-carboxylic acid (3.0 mg) in ethyl ether (2.0 ml) was treated with tritiated acetic acid (50 μl; 200 μCi) prepared from acetic anhydride (0.5 ml), concentrated HCl (0.005 ml), and tritiated water (0.1 ml; 2.5 mCi; sp act 25 mCi/g; New England Nuclear). After 72 h, solvent was removed in vacuo and the tritiated ketone was reduced with unlabeled $NaBH_4$ (2 mg) as described above. The products were methylated with diazomethane and the two epimers were separated by preparative TLC in benzene–ethyl acetate 95:5. Plates were sprayed with water to visualize the bands which were eluted from the silica gel with benzene–acetone 95:5. Hydrolysis and verification of radiochemical purity were done as above.

Surgical preparation and experimental protocol

Male Sprague-Dawley rats (200–400 g) were anesthetized with ether and a polyethylene catheter (0.28 mm ID × 0.61 mm OD; Clay-Adams, Parsippany, NJ) was inserted into the common bile duct through a midline abdominal incision. The catheter was exteriorized through a stab wound in the abdominal wall and the incision was closed. All rats were maintained under light ether anesthesia for the first 2–3 h of the study. They were then allowed to regain consciousness in Bollman restraining cages in which they remained for the duration of the experiment. Labeled isoetianic or etianic acid (0.1–1.0 μCi), with or without appropriate cold carrier (5.8–32.4 mg), was dissolved in 0.1 N NaOH (0.1–0.8 ml), brought to the final injection volume (1.0–5.0 ml) with Steroid Suspending Vehicle (National Cancer Institute, Bethesda, MD), and administered intravenously via the femoral vein to a bile duct-cannulated rat.

Bile samples were collected in preweighed tubes at 10-min intervals beginning 1 h prior to injection of the dose and continuing for 1–2 h after injection. Subsequently, bile was collected at 30-min intervals for an additional 1–2 h and as a single sample thereafter, until the rats were killed at 20 h. In all studies, urine was collected during the experimental period in two fractions: 0–3 and 3–20 h. At time of killing, blood was collected from the abdominal aorta; plasma was separated and frozen. Liver, intestine, and kidneys were removed and frozen for later analysis.

Bile volume was determined by weighing samples collected in tared tubes. Liver, intestine, and kidneys were

homogenized in 2 vols distilled water and aliquots of the homogenates were extracted with 5 vols methanol followed by centrifugation of the protein precipitate. Aliquots of bile, plasma, and the methanol extracts of tissue homogenates were analyzed for tritium content in ACS scintillant (Amersham, Arlington Heights, IL) with a Tracor Mark III, model 6882, liquid scintillation system (TM Analytic, Elk Grove Village, IL). Tritiated water content of bile, urine, and plasma was determined by sublimation. An estimate of the amount of isotope distributed in body water as ^3H_2O was obtained by assuming that water constituted 65% of body weight and that ^3H_2O in plasma was representative of that in body water in general.

Total biliary 3α-hydroxy bile acid concentrations were measured with 3α-hydroxysteroid dehydrogenase as previously described (12, 13).

Isolation of labeled metabolites from bile

After the initial extraction with Bond-Elut cartridges (Varian, Harbor City, CA) of each bile sample collected for the first 60 min from two rats given 7 mg and two rats given 30 mg of isoetianic acid, aliquots were analyzed by TLC (7) to determine glucuronide conjugate composition. Whatman LK-5 plates (Pierce Chemical Co., Rockford, IL) were used for all TLC analyses. Solvent systems were as follows: System 1 (for free bile acids): isooctane–ethyl acetate–glacial acetic acid 10:10:0.25; System 2 (for bile acid conjugates): chloroform–methanol–glacial acetic acid–water 65:20:10:5; System 3 (for bile acid methyl esters): benzene–ethyl acetate 95:5; System 4 (for bile acid glucuronides): ethanol–ethyl acetate–conc. NH_4OH 45:45:15. Additional aliquots were pooled and purified further for NMR analysis as previously described (7). Proton NMR spectra of methylated-acetylated derivatives of biliary glucuronides were obtained in $CDCl_3$ at 300 MHz on a General Electric GE-300 instrument.

Gas chromatography–mass spectrometry (GC–MS)

Etianic acids secreted in bile were recovered by ether extraction after hydrolysis with β-glucuronidase as described before (5). The acid fraction was methylated with diazomethane and purified on a small silica gel column prewashed with acetone and benzene. Methyl esters were applied in benzene and eluted with benzene (10 ml) and benzene–acetone 95:5 (10 ml). Labeled products, localized in 0.5-ml fractions, were derivatized by treatment with bis-trimethylsilyltrifluoroacetamide–trimethylchlorosilane 9:1 (10 μl). The resulting methyl ester trimethylsilyl ether derivatives were analyzed by capillary GC–MS on a methyl silicone column (12 m, 0.2 mm ID; Hewlett-Packard, Avondale, PA) with 22 eV electron impact detection in a GC–MS system previously described (1–3).

For GC analysis of endogenous bile acids, bile samples were subjected to β-glucuronidase hydrolysis, followed by alkaline hydrolysis (2 N NaOH, 110–120°C, 18 h) and

TABLE 1. Experimental conditions and isotope recoveries for individual animals

Rat No.	Rat Wt.	Compound[a]	Dose	% Recovery				
				Bile[b]	Urine[b]	Body H_2O[c]	Plasma[b]	Total
	g		*mg*					
1	315	A	0.004	17.8	0.5	71.9	0.0	90.2
2	287	A	4.074	18.3	1.1	67.3	0.0	83.7
3	360	B	0.552	94.3	1.1	0.0	0.0	95.4
4	320	B	0.225	87.6	0.7	0.0	0.1	89.3
5	294	B	5.770	102.5	1.1	0.0	0.0	103.6
6	219	B	6.262	97.4	0.8	0.0	0.3	98.1
7	235	B	5.842	99.9	0.8	0.0	0.1	100.8
8	296	B	5.666	96.6	0.7	0.0	0.0	97.3
9	345	B	7.011	85.7	1.0	0.0	0.3	87.1
10	316	B	7.068	85.5	0.0	0.0	0.0	85.5
11	346	B	32.40	89.7	1.0	0.0	0.0	91.5
12	346	B	32.40	98.8	1.9	0.0	0.1	100.7
13	378	B	30.05	88.3	2.4	0.0	0.1	90.6
14	340	B	30.05	87.8	1.4	0.0	0.0	89.3
15	367	C	40.51	95.8	2.2	0.0	1.0	99.0
16	306	C	3.600	92.4	1.0	0.0	0.0	98.4
17	316	C	4.059	92.7	1.0	0.0	0.0	93.7
18	288	C	4.057	93.8	1.9	0.0	0.0	95.7
19	343	D	0.910	97.2	1.1			98.3

[a]Compounds are as follows: A. [3-^{3}H]isoetianic acid; B. [2,2,4,4-^{3}H]isoetianic acid; C. [2,2,4,4-^{3}H]etianic acid; D. [2,2,4,4-^{3}H]3-oxoetienic acid.
[b]Tritium recovered as organic material.
[c]See text for method of calculation.

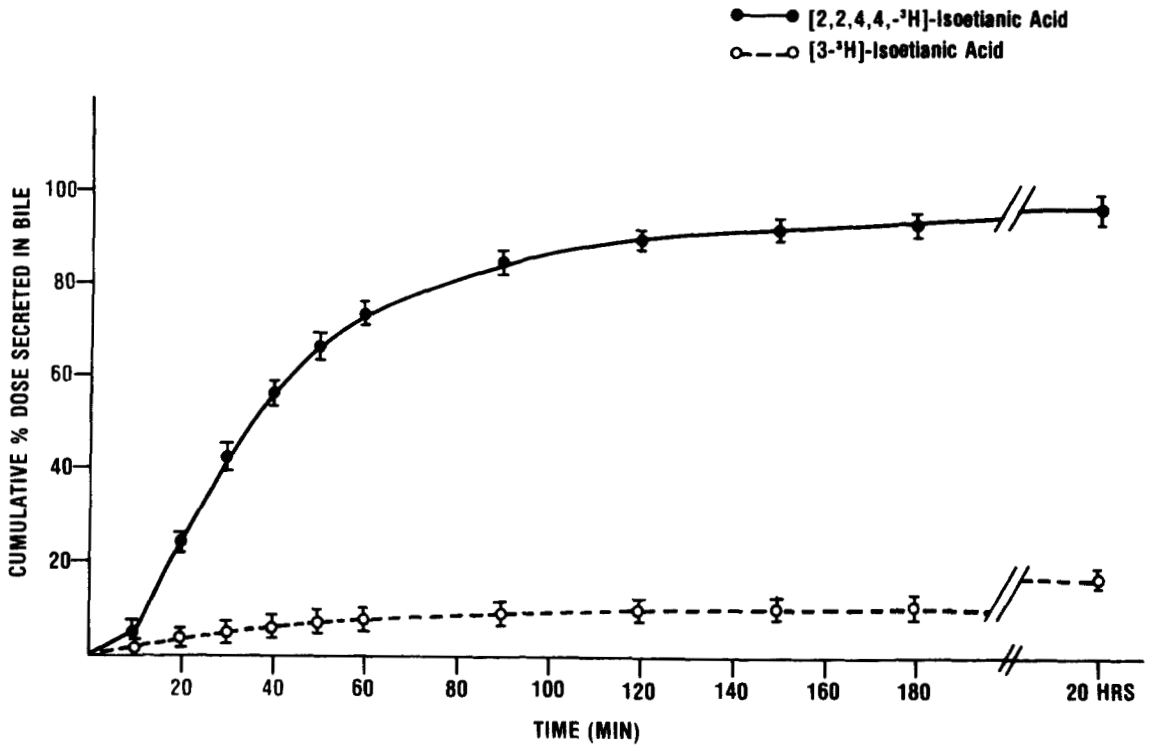

Fig. 2. Biliary secretion of administered isotope by rats given [2,2,4,4-^{3}H]isoetianic and [3α-^{3}H]isoetianic acids. Each point given for [2,2,4,4-^{3}H]isoetianic acid represents the mean of six experiments and the SEM is in brackets. [3-^{3}H]isoetianic acid points are the mean of two experiments and the brackets give the range of values.

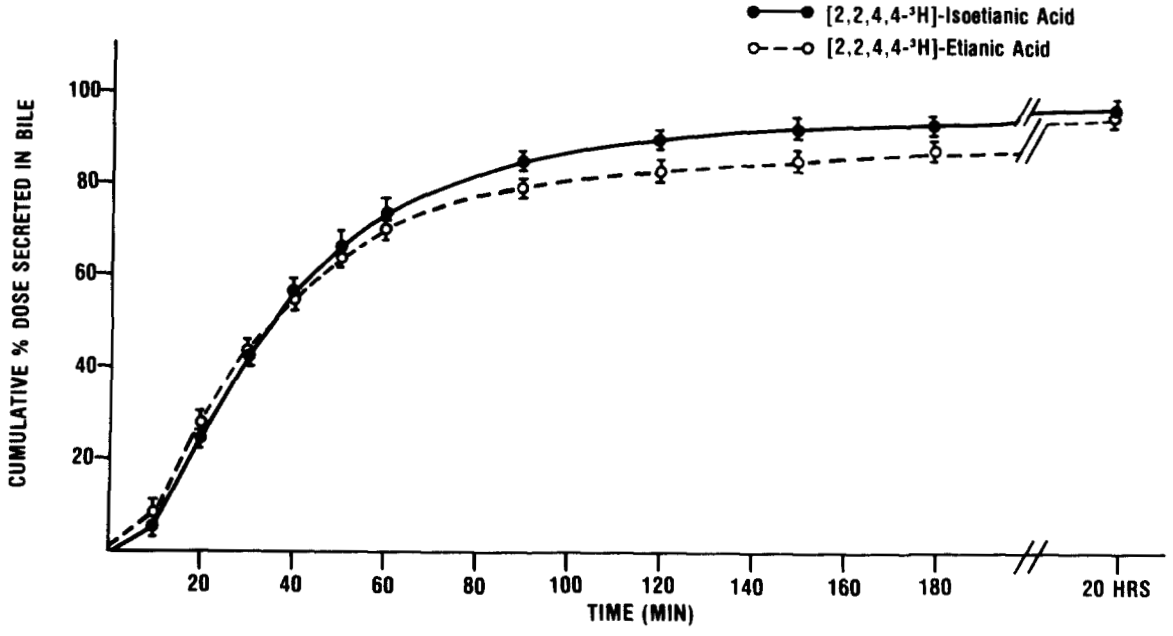

Fig. 3. Comparison of the biliary secretion of [2,2,4-4-^{3}H]isoetianic acid and [2,2,4,4-^{3}H]etianic acid. Each point is the mean of at least four experiments and the SEM is in brackets.

ethyl ether extraction. Bile acid methyl ester acetates were prepared and analyzed as previously described (2), using a Hewlett-Packard 5882 gas chromatograph.

RESULTS AND DISCUSSION

To investigate the possibility that short-chain bile acids detected in meconium and serum are secreted in bile, the adult rat was chosen as a model for the study of the hepatic metabolism and biliary secretion of etianic acid (5). This study, extending the investigation to isoetianic acid, was prompted by the fact that this 3β,5β isomer is absent from both meconium and serum and from the products of the in vivo reduction of etienic acid (1, 2, 4, 11).

Administration of [2,2,4,4-^{3}H]isoetianic acid (**Table 1**, nos. 3–8) resulted in the rapid biliary secretion of the label (**Fig. 2**). Within the first hour, 73.6 ± 2.8% of the administered dose was recovered in bile; total biliary secretion after 20 h was 96.3 ± 2.4% of the dose. Only small amounts of tritium were detected in urine and plasma (1.0 ± 0.1 and 0.15 ± 0.08% of the dose, respectively) and none in the tissue extracts. The overall recovery of the dose was 97.4 ± 4.9% and there was no detectable tritiated water in any samples. The biliary secretion of [2,2,4,4-^{3}H]etianic acid (Table 1, nos. 15–18) was nearly identical (**Fig. 3**) with 69.6 ± 2.2% of the dose secreted in the first hour and a total of 94.9 ± 1.0% secreted after 20 h. [2,2,4,4-^{3}H]3-Oxoetianic, which was investigated for comparison, was also efficiently eliminated in bile, with 79.5% of the dose secreted in the first hour (Table 1, no. 19).

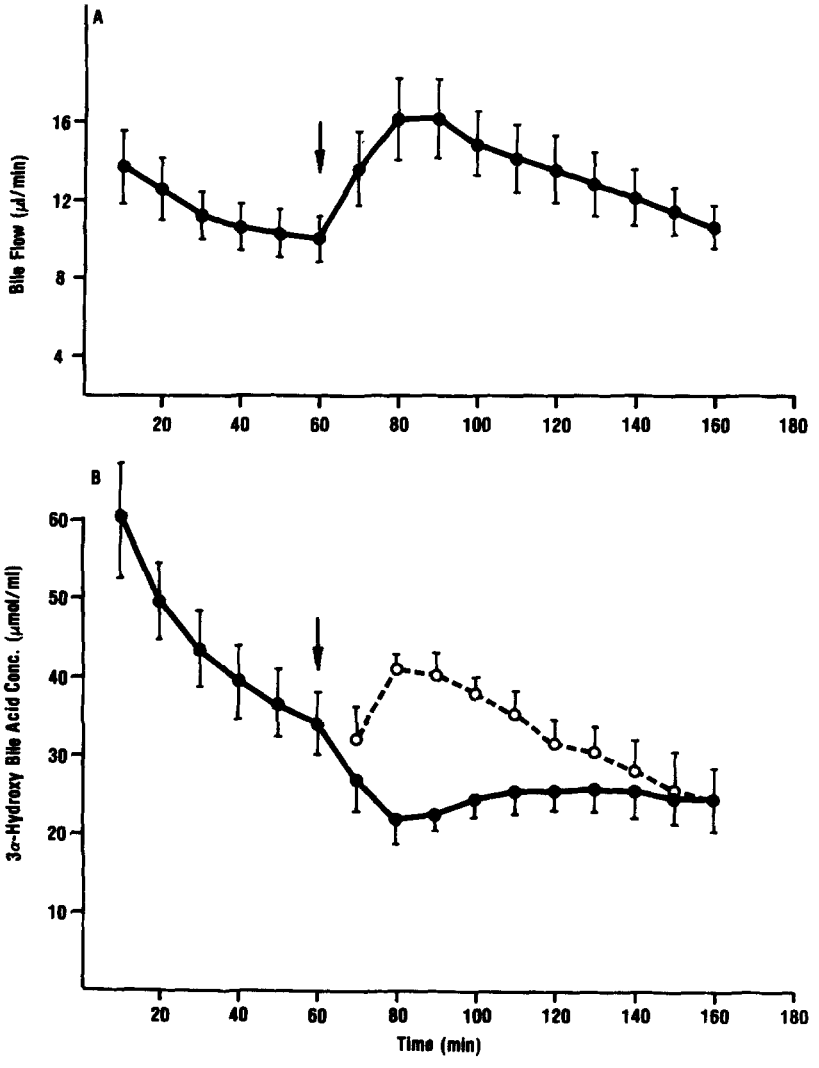

Fig. 4. A. Bile flow rate before and after intravenous administration of a load (5.7–6.3 mg) of isoetianic acid. B. 3α-hydroxysteroid concentration (as measured by 3α-hydroxysteroid dehydrogenase) in bile before and after β-glucuronidase hydrolysis. In both A and B, each point represents the mean of four studies. The SEM is in brackets and the arrows indicate the time at which the steroid was administered.

In contrast to the above observations, the biliary secretion of the label of [3α-^{3}H]isoetianic acid was significantly lower (only 15–18% of dose secreted in bile after 20 h; Fig. 2 and Table 1, nos. 1, 2). Notably, most of the label was associated with water and the content of tritium in bile water increased with time. No tritium was detected in water in the first 30 min of bile collection; at 2 h, one-third of biliary tritium was in water; and by the end of the study, more than 95% of the label was in tritiated water. Overall, only 5.6 and 8.1% of the dose was recovered from bile as organic tritium. All of the label in plasma collected at the end of the study was in the form of tritiated water. Urine collected over the first 3 h had 30 and 50% of urinary label in water versus 94 and 96% of the label in water for the 3–20 h urine samples. Only 0.5 and 1.1% of the dose was recovered in solids dissolved in urine. With 67 and 72% of the dose estimated to be distributed in total body water, total recoveries of administered tritium were 90.2 and 83.7%. The above results are in sharp contrast to those of earlier studies with [3β-^{3}H]etianic acid which showed efficient biliary secretion of label with only 9–15% of the administered dose recovered as tritiated water (5).

Administration of isoetianic acid in milligram amounts produced a choleresis (**Fig. 4A**) similar to that produced by a comparable dose of etianic acid (5), with bile flow increasing significantly above control levels. Bile samples assayed with 3α-hydroxysteroid dehydrogenase (which is specific for the 3α configuration and gives no reaction with 3β-hydroxysteroids), before and after treatment with β-glucuronidase, revealed a significant increase in the concentration of biliary 3α-hydroxy bile acids after β-glucuronidase hydrolysis. This increase was correlated significantly with both the measured increases in bile flow and with isotope secretion in bile, and provides indirect evidence for both epimerization of the 3-OH group and hydroxyl-linked glucuronidation of the product.

These results established that the nearly complete removal of 3α-^{3}H and inversion of the 3-hydroxyl group takes place during the hepatic uptake and biliary secretion of isoetianic acid. This conclusion is fully consistent with the analysis of the acid fraction recovered after β-glucuronidase hydrolysis from bile of rats given [2,2,4,4-^{3}H]isoetianic acid. As demonstrated by TLC analysis of the methylated acid fraction, 95% of the label migrated with etianic acid methyl ester and only about 1% with

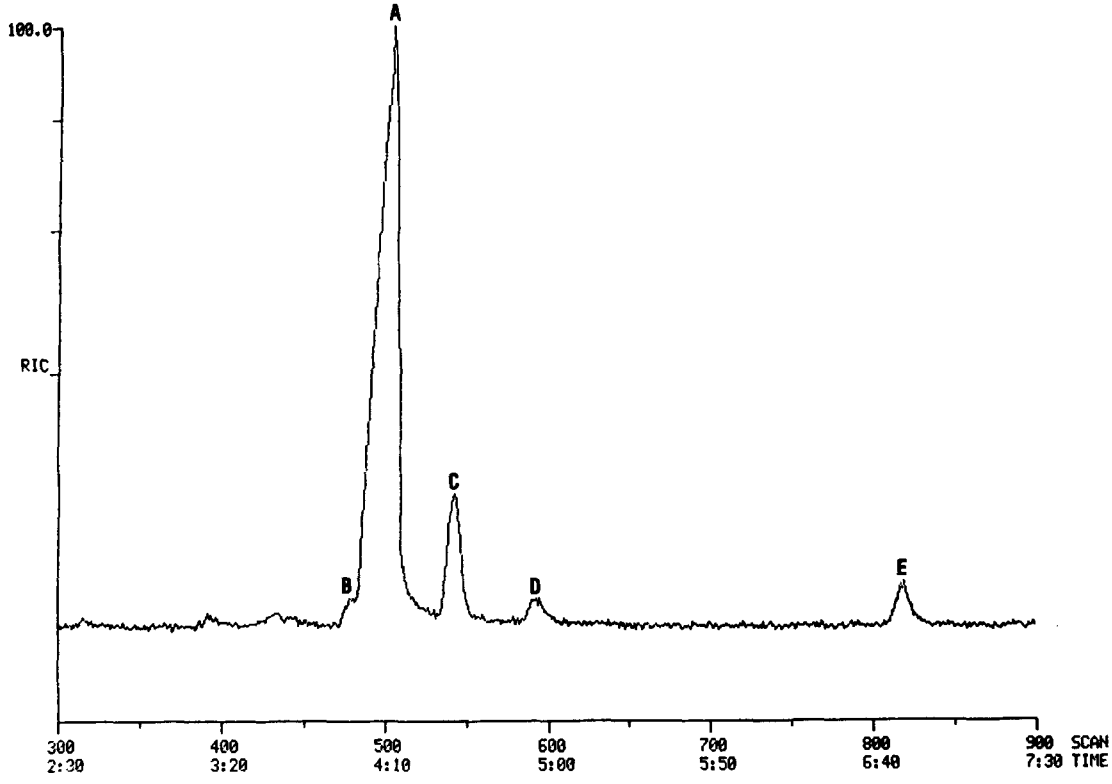

Fig. 5. GC-MS analysis of methyl ester, TMS ether derivatives of isoetianic acid metabolites isolated from bile. Identification of peaks by retention of time: A: etianic acid (3α,5β), methyl ester TMS ether, 91.1%; B: isoetianic acid (3β,5β), methyl ester TMS ether, 2.0%; C: etianic acid (3α,5β), ethyl ester TMS ether, 4.6%; D. dihydroxylated etianic acid (3α,5β) methyl ester TMS ether, 1.2%; E: cholesterol, TMS ether, 1.1%.

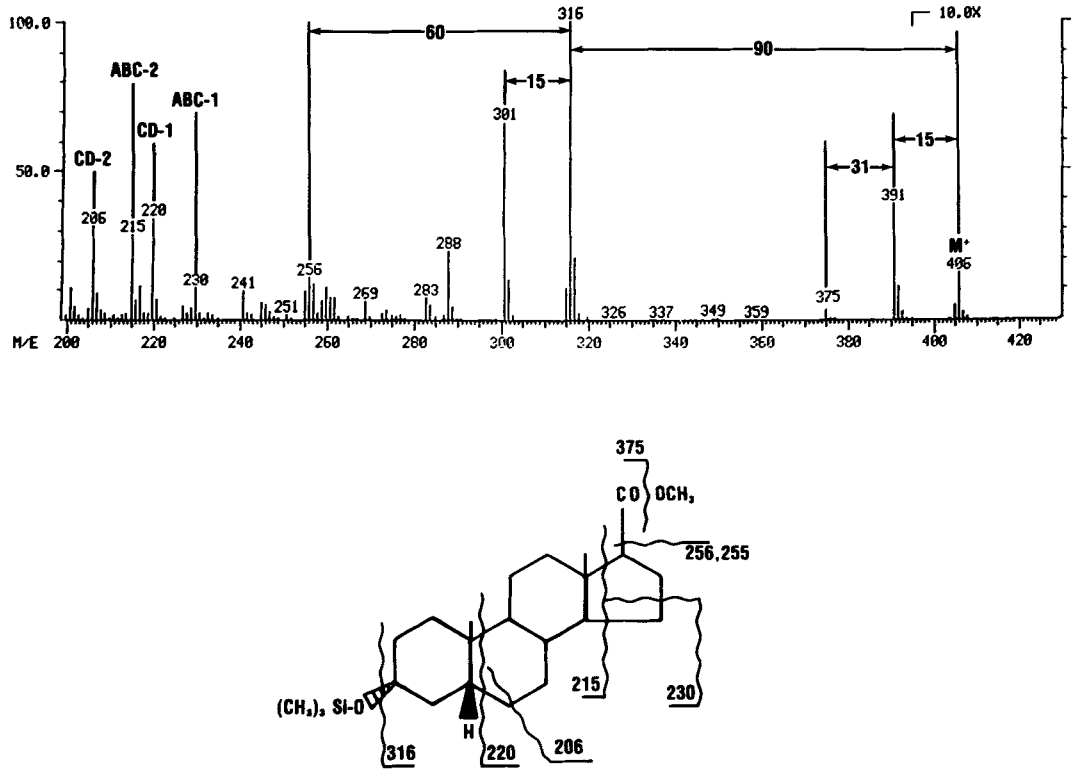

Fig. 6. 22 eV Electron impact mass spectrum of the methyl ester, TMS ether of etianic acid, the major metabolite of isoetianic acid (peak A, Fig. 5).

isoetianic acid methyl ester. The inversion of the C-3 configuration also was confirmed by GC–MS analysis of the TMS ethers of the purified monohydroxy bile acid methyl esters (**Fig. 5**). The two major components were the silyl ethers of the methyl ester (mass spectrum shown in **Fig. 6**) and the ethyl ester[2] of etianic acid (peaks A and C) while only about 2.0% of the mixture was identified as the derivative of isoetianic acid (peak B). All three compounds were identified by direct GC–MS comparison with authentic derivatives. A minor component (peak D) gave a spectrum compatible with the methyl ester, TMS ether of a dihydroxylated etianic acid. Several such products, formed in higher yields, were observed in similar experiments with etienic acid (11).

As shown by TLC, about 95% of the isotope present in bile was associated with polar derivatives corresponding to hydroxyl- and carboxyl-linked glucoronides which were clearly separated in the alkaline solvent system and have been fully identified previously (7). The rate of secretion of these two products was determined for rats given 7 and 30 mg doses of isoetianic acid (Table 1, nos. 9–14) and the kinetics of the secretion of labeled metabolites was followed quantitatively for the first hour (**Fig. 7**). At all times, the hydroxyl-linked glucuronide was the major metabolite, accounting for 90% of the labeled conjugates at 10 min and 65% at 60 min.

Under normal conditions, C_{24} bile acids are found in bile primarily as amino acid conjugates (14), however in vivo studies with C_{20} bile acids have shown no detectable amino acid conjugation (5). Our studies in rats, both in vivo and in vitro, have demonstrated the biosynthesis of 3-O, 6-O, and carboxyl-linked glucuronides of a number of C_{24}, as well as short-chain bile acids (10, 15, 16). Glucuronides of C_{24} bile acids are known to exist in humans (17–20) but appear in quantity only in chronic liver disease and then are found primarily in urine (17, 19, 20). Although the patterns of biliary secretion of C_{20} and C_{24}

[2]Formation of the ethyl ester may result from the solvolysis of carboxyl-linked glucuronides during extraction. Details of MS analysis: peak A, identical to the authentic methyl ester TMS ether (MeTMS) derivative of etianic acid; peak B, *m/z* M^+ and M–Me ions absent, 316 (M-TMSOH, 100), 301 (M-TMSOH-Me, 57), 215 (23), 206 (11), 108 (53), identical to the authentic METMS derivative of isoetianic acid; peak C, *m/z* 420, (M^+, 0.3), 405 (M–Me, 27), 375 (M–EtO, 7), 330 (M-TMSOH, 100), 315 (M-TMSOH-Me, 73), 302 (M-TMSOH-C_2H_4, 25), 283 (9), 274 (11), 269 (9), 257 (15), 256 (21), 255 (11), 241 (13), 234 (18), 230 (13), 220 (22), 215 (25), 108 (37), identical to the ethyl ester of etianic acid prepared by trans-esterification of the methyl ester with ethanol/*p*-toluenesulfonic acid; peak D, *m/z* 479 (M–Me, 72), 419 (M–HCOOMe–Me, 21), 410 (3), 404 (M-TMSOH, 3), 389 (19), 375 (M-TMSOH-EtOH, 3), 367 (4), 341 (7), 330 (5), 314 (M–2 × TMSOH, 17), 299 (M–2 × TMSOH–Me, 12), tentatively identified as the MeTMS derivative of a dihydroxylated etianic acid; peak E, identical with cholesterol TMS ether.

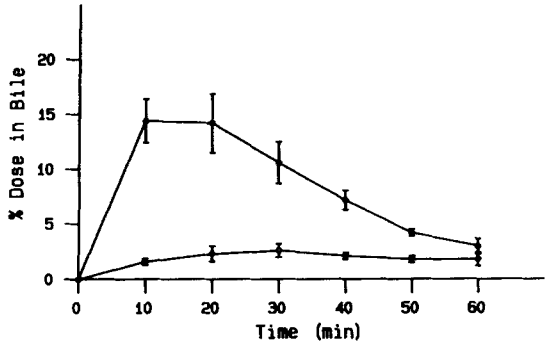

Fig. 7. Biliary secretion of carboxyl-linked (open symbols) and hydroxyl-linked (closed symbols) glucuronides of etianic acid. Quantitation was by analytical TLC (see text for details). Each point represents the mean of four experiments, the SEM is in brackets.

bile acids are superficially similar, the precise mechanisms for their hepatic metabolism and secretion are not known. It is possible that the C_{20} acids, lacking the distinctive 5-carbon side-chain of bile acids, may be processed by mechanisms related to those for bilirubin, bromosulphthalein or steroid hormones (21, 22).

In addition to the TLC comparison, both glucuronides were isolated and purified and, in the form of methyl ester acetate derivatives, their identification was fully confirmed by low eV electron impact MS and protein NMR. The analysis included comparison of the biliary metabolites with both types of protected (i.e., methylated, acetylated) glucuronides synthesized by the Koenigs-Knorr reaction and obtained biosynthetically from in vitro incubations with rat liver microsomes (7). These spectra, as discussed previously (7, 11), permitted clear-cut distinction of the substitution site and configuration at C-3.[3]

The prevalent, but not complete, loss of the 3-tritium of isoetianic acid and the inversion of the 3-hydroxyl group is consistent with a 3-oxo-5β-androstane-17β-carboxylic acid intermediate. Importantly, this ketone (Table 1, no. 19) is efficiently cleared from plasma and rapidly secreted in bile in a manner similar to isoetianic and etianic acids (11). The high retention of the [2,2,4,4-^{3}H]isoetianic acid label rules out 2- or 3-unsaturated intermediates. Shefer et al. (23) have demonstrated that both isochenodeoxycholic and isoursodeoxycholic acids are efficiently secreted in rat bile as chenodeoxycholic and ursodeoxycholic acids, and that the inversion of the 3-hydroxy group occurs in the liver. These results are in accord with the data presented here. However, in contrast to the metabolism of [3-^{3}H]isoetianic acid, administered [3-^{3}H]isoursodeoxycholic acid was recovered as a labeled metabolite, i.e., as [3-^{3}H]ursodeoxycholic acid, suggesting that the epimerization did not involve formation of the 3-keto intermediate. A possible alternative is that, in a cycle of oxidation and reduction that has been shown to occur with 3β-^{3}H-labeled lithocholic and chenodeoxycholic in the presence of purified 3α-hydroxysteroid dehydrogenase (24–26), the ^{3}H is transferred to a nicotinamide cofactor and then back to the original C_3 position. Björkhem et al. (27) have used 3β-^{3}H labeling to measure the rate of oxidoreduction of different α-oriented bile acid hydroxyl groups during their enterohepatic circulation in man. In this case also, the authors considered the possibility of the return of label to the oxidized bile acid upon reduction.

Whatever the mechanism, it can be assumed from the efficiency of the conversion of isoetianic to etianic acid that the 3α-hydroxy-5β-stereoisomer is the preferred configuration for biliary secretion of these steroids. It appears that the glucuronyltransferase involved in the conjugation process is also highly specific for the 3α configuration. This is supported by studies demonstrating that only carboxyl-linked glucuronides are formed when 3β-hydroxy short-chain (C_{20}–C_{23}) bile acids are incubated with rat liver microsomes (7). When 3α-hydroxy compounds are used as substrates for microsomal glucuronyl transferase, both hydroxyl- and carboxyl-linked glucuronides are produced (7).

Biliary secretion curves were the same whether microgram or milligram quantities of isoetianic acid were administered. Also, these curves were nearly identical when either isoetianic or etianic acids were given, and the choleresis produced by isoetianic acid duplicated that resulting from injection of etianic acid (5). All of these observations suggest that there is a preferred stereochemistry in the hepatic transport of short-chain bile acids and implicate pre-existent hepatic enzyme systems of considerable capacity. However, the precise biochemical pathways for the hepatic metabolism of these short-chain bile acids remain to be elucidated.

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[3]Mass spectra of these protected glucuronides are reported in ref. 28.

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