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STUDIES ON STEROIDS

CCXXXVIII.* DETERMINATION OF BILE ACIDS IN LIVER TISSUE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY WITH NEGATIVE ION CHEMICAL IONIZATION DETECTION

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SUMMARY

A method for the determination of bile acids in 2–10 mg of human liver tissue by gas chromatography (GC) in combination with negative ion chemical ionization (NICI) mass spectrometry is described. Unconjugated, glycine- and taurine-conjugated bile acids labelled with ^{18}O and ^{2}H were used as internal standards. The preparation of these compounds was attained by the exchange reaction of the carbonyl group with $H_2{}^{18}O$, followed by metal hydride reduction. Bile acids in solubilized liver tissue were extracted with a Sep-Pak C_{18} cartridge, separated into the unconjugated, glycine- and taurine-conjugated fractions by ion-exchange chromatography on piperidinohydroxypropyl-Sephadex LH-20 and then derivatized to the pentafluorobenzyl ester–dimethylethylsilyl ethers. Subsequent resolution of each fraction into lithocholate, deoxycholate, chenodeoxycholate, ursodeoxycholate and cholate was attained by GC on a cross-linked 5% phenylmethyl silicone fused-silica capillary column where bile acids were monitored with a characteristic carboxylate anion $[M - 181]^-$ in the NICI mode using isobutane as a reagent gas. The newly developed method was applied to the quantitation of bile acids in liver tissue with satisfactory sensitivity and reliability.

* For Part CCXXXVII, see H. Hosoda, R. Tsukamoto, K. Shoriken, W. Takasaki and T. Nambara, *Chem. Pharm. Bull.*, 36 (1988) 3525. In this paper the following trivial names and abbreviations are used: lithocholic acid (LCA) = 3α-hydroxy-5β-cholan-24-oic acid; chenodeoxycholic acid (CDCA) = 3α,7α-dihydroxy-5β-cholan-24-oic acid; deoxycholic acid (DCA) = 3α,12α-dihydroxy-5β-cholan-24-oic acid; ursodeoxycholic acid (UDCA) = 3α,7α-dihydroxy-5β-cholan-24-oic acid; cholic acid (CA) = 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid; G = glycine-conjugated; T = taurine-conjugated.

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INTRODUCTION

In recent years, considerable interest has been directed to the biodynamics of bile acids in man in connection with the diagnosis of hepatobiliary diseases[1]. A reliable method is, therefore, urgently needed for the trace analysis of bile acids in liver tissue. Among various methods, gas–liquid chromatography–mass spectrometry (GC–MS) is a powerful tool for the profile analysis of trace compounds and has been applied to the determination of bile acids in liver tissue using the electron impact ionization mode[2]. In the previous study, we disclosed that the combined use of derivatization to the pentafluorobenzyl (PFB) ester–dimethylethylsilyl (DMES) ether and capillary GC–MS with negative ion chemical ionization (NICI) detection is much more promising with respect to the sensitivity and versatility for the determination of bile acids[3]. The present paper deals with the separation and determination of unconjugated, glycine- (G) and taurine-conjugated (T) bile acids in liver tissue by GC–NICI–MS using stable isotope-labelled bile acids as internal standards (I.S.).

EXPERIMENTAL

Materials

Bile acids and cholylglycine hydrolase were supplied by Sigma (St. Louis, MO, U.S.A.) and DMES-imidazole was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Glycine- and taurine-conjugated bile acids were prepared in these laboratories[4]. The Sep-Pak C_{18} cartridge and Pre Pak-500/C_{18} were obtained from Waters Assoc. (Milford, MA, U.S.A.). All other chemicals employed were of analytical reagent grade. Solvents were purified by distillation prior to use. Piperidinohydroxypropyl-Sephadex LH-20 (PHP-LH-20) (acetate form, 0.6 mequiv./g)[5] and carboxymethyl-Sephadex LH-20 (CM-LH-20) (K^+ form, 1 mequiv./g)[6] were prepared in the manner previously reported. All glassware used was silanized with trimethylchlorosilane.

Gas chromatography–mass spectrometry

Capillary GC–MS was carried out using a VG Analytical MM12030 quadrupole mass spectrometer equipped with an Hewlett-Packard HP 5790A gas chromatograph. Isobutane was used as a reagent gas. A cross-linked 5% phenylmethyl silicone fused-silica capillary column (20 m × 0.3 mm I.D.) (J & W Scientific, Folsom, CA, U.S.A.) was inserted into the ion source through the direct inlet. The carrier gas was helium at a linear velocity of 60 cm/s. The test samples were injected through a Van den Berg solventless injector with an inlet pressure of 0.8 kg/cm^2. The injection port, column oven and ion source were kept at 280, 285 and 270°C, respectively. The ionization energy was 70 eV and the emission current was 400 μA.

Preparation of [^{18}O]-labelled bile acids

The methyl esters of LCA, CDCA, DCA and CA were subjected to oxidation with chromium trioxide in acetic acid, followed by alkaline hydrolysis, yielding mono-, di- and trioxo-5β-cholanoic acids. In a similar fashion, glycine- and taurine-conjugated oxo bile acids were also prepared from the corresponding ethyl glycinate- and taurine-conjugates. These were unequivocally characterized by ^{1}H NMR spectroscopy[7,8], and their purities were greater than 99.5% as judged by high-perform-

ance liquid chromatography (HPLC)[9]. The oxo bile acid (10–20 mg) in 70% ethanol (5 ml) was applied to a column (10 cm × 18 mm I.D.) of CM-LH-20 (K^+ form, 5 g) and eluted with 70% ethanol (50 ml). The eluate was dried and recrystallized from methanol–diethyl ether. The potassium salt of the oxo bile acid (20 mg) thus obtained was dissolved in $H_2{}^{18}O$ (isotopic purity 98 atom%, 300 μl) and heated at 90°C for 50 h. After cooling, the solution was stirred with NaB^2H_4 (isotopic purity 97 atom%, 4 mg) for 1 h with cooling in ice, and then poured into 5% hydrochloric acid. Unconjugated and glycine-conjugated bile acids were extracted with ethyl acetate. In the case of taurine-conjugates, the reaction mixture was poured into 5% hydrochloric acid, neutralized with 5% potassium hydroxide and then passed through a column (19 mm × 20 mm I.D.) of Pre Pak-500/C_{18} (2 g). After washing with water (20 ml), the desired bile acids were eluted with ethanol (20 ml). The ^{18}O,^2H-labelled bile acids thus obtained were purified by means of column chromatography on silica gel and HPLC on a reversed-phase column[9].

Sample preparation

Liver biopsies were performed at the Third Department of Internal Medicine, Tohoku University School of Medicine, for histological examination. A part of the liver tissue was used for this work.

The sample preparation was carried out according to the method previously reported[2]. Liver tissue weighing 2–10 mg taken by diagnostic needle biopsy was rinsed with ice-cold saline, placed on a filter-paper until weighing and then put into a 10-ml PTFE test-tube. To this tube was added 5% sodium hydroxide (1 ml) containing 1–10 ng each of unconjugated, glycine- and taurine-conjugated ^{18}O,^2H-labelled bile acids as I.S., and the whole was heated at 80°C for 30 min to solubilize the tissue. A 100–200 μl aliquot was taken to determine the protein content by the method of Lowry *et al.*[10]. The remaining solution was neutralized with 10% hydrochloric acid and then passed through a Sep-Pak C_{18} cartridge. After washing with water (4 ml), bile acids were eluted with 90% ethanol (5 ml). The eluate was applied to a column (18 mm × 6 mm I.D.) of PHP-LH-20 (100 mg) and elution was carried out at a flow-rate of 0.2 ml/min. After washing with 90% ethanol (4 ml), unconjugated, glycine- and taurine-conjugated bile acids were fractionally separated by stepwise elution with 0.1 *M* acetic acid in 90% ethanol (5 ml), 0.2 *M* formic acid in 90% ethanol (5 ml) and 0.3 *M* acetic acid–potassium acetate in 90% ethanol (pH 6.5) (5 ml)[5,11]. Hydrolytic cleavage of glycine- and taurine-conjugates in each fraction was then performed by incubation at 37°C for 2 h with cholylglycine hydrolase (5 U) in 0.1 *M* acetate buffer (pH 5.6, 1 ml) containing 0.1 *M* 2-mercaptoethanol and 0.2 *M* EDTA disodium salt. To the incubation mixture was added 0.1 *M* sodium hydroxide (0.1 ml), and the whole was applied to a Sep-Pak C_{18} cartridge in the manner described above for removal of proteins and inorganic salts. The unconjugated, glycine- and taurine-conjugated fractions were evaporated to dryness under reduced pressure and then were derivatized to PFB ester–DMES ethers.

Derivatization

To the deconjugated bile acid were added 5% (v/v) PFB bromide in acetonitrile (60 μl) and diisopropylethylamine (10 μl), and the whole was allowed to stand at 37°C for 45 min. The reaction mixture was diluted in ethanol–acetonitrile (1:1, 1 ml), and

then applied to a Sep-Pak C_{18} cartridge impregnated with ethanol. The PFB esters were eluted with ethanol–acetonitrile (1:1, 4 ml). To the dried eluate were added DMES-imidazole (50 μl) and 1% pyridine in hexane (50 μl), and the whole was heated at 60°C for 1 h[12,13]. Following the addition of 1% each of methanol and pyridine in hexane (200 μl) to decompose the excess of silylating reagent, the solution was evaporated under a stream of nitrogen. The residue was redissolved in 1% pyridine in hexane and injected onto the GC–MS system.

RESULTS AND DISCUSSION

Preparation of stable isotope-labelled bile acids

The reversed isotope dilution technique in combination with GC–MS provides a useful methodology for the trace analysis of important biological materials with high accuracy, specificity and sensitivity. For this purpose, target compounds labelled with ^2H have been commonly used as I.S. In the previous paper, we demonstrated that bile acid PFB ester–DMES ethers exhibited characteristic negative ions $[M - PFB]^-$, which contain oxygen atoms in both hydroxyl and carboxyl groups, as base peaks in NICI–MS[3]. This finding strongly suggested that an ^{18}O-labelled I.S. would be useful for the trace analysis of bile acids by GC with selected-ion monitoring (SIM). The incorporation of ^{18}O atoms into prostaglandins was attained through the carboxyl moeity by chemical or enzymatic hydrolysis of the ester bond in $H_2{}^{18}O$[14,15]. In order to obtain ^{18}O-labelled bile acids with high isotopic purity, this procedure requires four to five cycles of esterification of the carboxyl group followed by hydrolysis of the ester. Moreover, the formation of amide bonds with glycine and taurine through the carboxyl group results in loss of the ^{18}O label. Consequently, introduction of ^{18}O atoms into the hydroxyl groups on the steroid nucleus was attempted by the use of an exchange reaction of the carbonyl oxygen atom[16]. Initially, the reactivities of the carbonyl groups at C-3, C-7 and C-12 towards the exchange reaction were examined with 3- and 7-dehydro CDCA and 12-dehydro DCA. It is well known that such an exchange reaction proceeds more easily under basic conditions. In addition, the alkali metal salt of the carboxylic acid is fairly soluble in water. Hence, oxo bile acids were converted into the potassium salts by passage through a lipophilic cation-exchange gel, CM-LH-20 (K^+ form). The potassium salt was then dissolved in $H_2{}^{18}O$, whose isotopic purity was 98 atom%, and allowed to stand at 37 or 90°C. Aliquots of the resulting solution were taken at certain times and subjected to reduction with sodium borohydride. Following derivatization to the PFB ester–DMES ether, the content of ^{18}O in CDCA and DCA labelled with heavy isotope was determined by GC–SIM using a characteristic ion, $[M - 181]^-$. As illustrated in Fig. 1, the reaction rate was found to be dependent upon the position of the carbonyl group. The content of ^{18}O at C-3 increased along with the reaction time up to 2 h and reached the theoretical value at 37°C. On the other hand, the contents at C-7 and C-12 remained almost unchanged, even though the reaction time was prolonged for 6 h at 37°C. When the exchange reaction was performed at 90°C, even for carbonyl groups at C-7 and C-12, the ^{18}O content was elevated to a plateau at 45 h. The marked difference in the reactivity may be explained in terms of the steric hindrance of the carbonyl carbon atom. The position of the ^{18}O atom incorporated was further confirmed. Bile acids were transformed into the methyl ester–DMES ether derivatives and then subjected

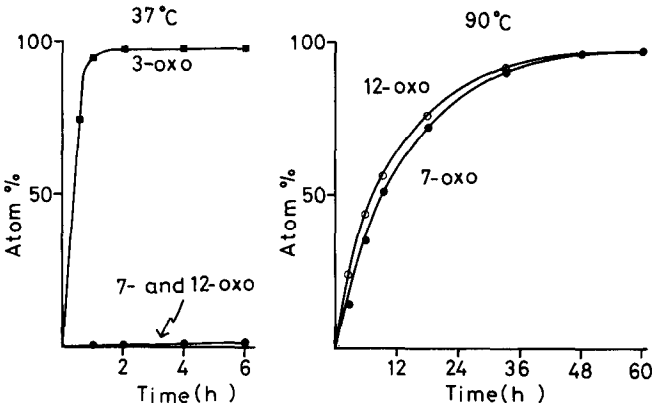

Fig. 1. Time courses for incorporation of ^{18}O atom into oxo bile acids.

to MS with the electron impact ionization mode. As compared with the non-labelled dihydroxy bile acids, the molecular ion was observed at two units to higher masses, while the fragment ion formed by elimination of two dimethylethylsilanol groups was at the same mass number. No exchange with the heavy isotope occurred at the carboxyl group under these conditions.

On the basis of these results, the preparation of unconjugated, glycine- and taurine-conjugated bile acids labelled with ^{18}O atom was then undertaken. Potassium 5β-cholanates having the carbonyl groups at C-3, C-7 and C-12, prepared by chromium trioxide oxidation from common bile acids and ion exchange on CM-LH-20 (potassium form), were dissolved in $H_2{}^{18}O$ (isotopic purity of 98%) and heated at 90°C for 50 h. Following metal hydride reduction and, if necessary, hydrolytic cleavage of glycine- and taurine-conjugates with cholylglycine hydrolase, the ^{18}O contents were determined as PFB esters–DMES ethers. It is evident from the data listed in Table I that ^{18}O was incorporated into oxo bile acids at each position in the theoretical amounts. Labelled UDCA, that is the positional isomer of CDCA, was also obtained by metal hydride reduction of 3,7-didehydro CDCA as a minor product. It is well known that the introduction of a number of ^{2}H atoms into a molecule causes a somewhat smaller retention value in GC. Such a kinetic isotope effect was negligible

TABLE I

ATOM% EXCESS OF ^{18}O-LABELLED BILE ACIDS*

Bile acid	*Number of ^{18}O*			
	0	*1*	*2*	*3*
LCA	2.4	97.6		
CDCA	<0.1	5.8	94.2	
DCA	<0.1	5.9	94.1	
CA	<0.1	0.5	8.3	91.2

* Corrected for background and natural abundance of stable isotopes.

for ^{18}O-labelled bile acids and, hence, no marked difference in the retention time was found even in labelled CA having three ^{18}O atoms.

Since the natural abundance of stable isotopes causes intense isotope peaks especially in the high mass region, a difference of more than 3 mass units between the labelled and unlabelled compounds is recommended. As for lithocholates, the two substrates differ from each other by only 2 mass units. Moreover, labelled lithocholates would be contaminated with more than 2% of unlabelled species owing to the isotopic purity of $H_2{}^{18}O$ used. Therefore, further labelling with ^2H was undertaken. The ^{18}O-labelled oxo bile acids were reduced with sodium borodeuteride (NaB^2H_4) (isotopic purity 97%) and the heavy isotope contents in the product were estimated in the manner described above. As shown in Table II, the ratios of the unlabelled fragment to the fully labelled fragment in [^{18}O,^2H]mono-, -di- and -trihydroxylated bile acids were found to be 1/500, 1/2000 and 1/10 000, respectively. This result implies that these labelled bile acids can also serve as carriers, preventing the loss of target compounds in the clean-up procedure. Any labelled isotopes were not eliminated during alkaline hydrolysis and solvolysis. A calibration graph was constructed by plotting the ratio of the peak height of each bile acid to that of the corresponding I.S. against the weight ratio of bile acid to the corresponding I.S. For unconjugated, glycine- and taurine-conjugated UDCA, corresponding [^{18}O,^2H]CDCAs were used as the I.S.s. The calibration graphs for unconjugated bile acids using 1 ng each of the corresponding I.S. are shown in Fig. 2. The linearity was good in the range of 0.01–20 for LCA, 0.005–100 for dihydroxylated bile acids and 0.01–500 for CA. Almost the same calibration graphs were obtained for glycine- and taurine-conjugates. A typical selected-ion recording of a synthetic mixture of unconjugated bile acids is illustrated in Fig. 3, where the monitoring ions were 461/464 for LCA, 563/569 for DCA, CDCA and UDCA and 665/674 for CA.

TABLE II

RATIOS OF UNLABELLED TO LABELLED FRAGMENT IN STABLE ISOTOPE-LABELLED BILE ACIDS

Compound	*Peak height ratio*		
	461/464	*563/569*	*665/674*
[3-^{18}O,3-^2H]LCA	0.0022		
[3-^{18}O,3-^2H]GLCA	0.0016		
[3-^{18}O,3-^2H]TLCA	0.0020		
[3,7-$^{18}O_2$,3,7-$^2H_2$]CDCA		0.0001	
[3,7-$^{18}O_2$,3,7-$^2H_2$]GCDCA		<0.0001	
[3,7-$^{18}O_2$,3,7-$^2H_2$]TCDCA		<0.0001	
[3,12-$^{18}O_2$,3,12-$^2H_2$]DCA		0.0005	
[3,12-$^{18}O_2$,3,12-$^2H_2$]GDCA		0.0006	
[3,12-$^{18}O_2$,3,12-$^2H_2$]TDCA		0.0001	
[3,7,12-$^{18}O_3$,3,7,12-$^2H_3$]CA			<0.0001
[3,7,12-$^{18}O_3$,3,7,12-$^2H_3$]GCA			<0.0001
[3,7,12-$^{18}O_3$,3,7,12-$^2H_3$]TCA			<0.0001

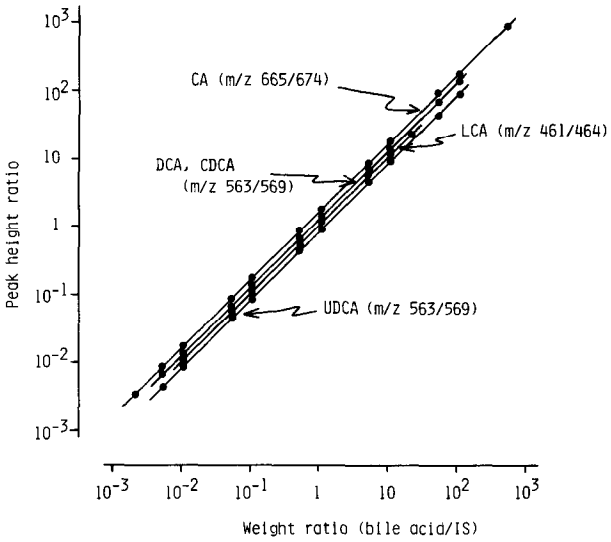

Fig. 2. Calibration graphs for bile acids.

Determination of bile acids in liver tissue

A standard procedure for the determination of bile acids in liver tissue is shown in Fig. 4. A liver tissue specimen was solubilized with an alkaline solution containing unconjugated, glycine- and taurine-conjugated [^{18}O,^{2}H]bile acids as I.S.s, according to the method of Yanagisawa *et al.*[2], and bile acids were extracted with a Sep-Pak C_{18} cartridge. The GC–MS technique has inevitable disadvantages such as the loss of information about the conjugated form. Therefore, bile acids were separated into the unconjugated, glycine- and taurine-conjugated fractions by ion-exchange chromatography on PHP-LH-20 prior to deconjugation[5,11]. After enzymatic hydrolysis of the amide bonds, bile acids in each fraction were derivatized to the PFB esters. In the previous study, removal of the excess of PFB bromide was carried out by means of

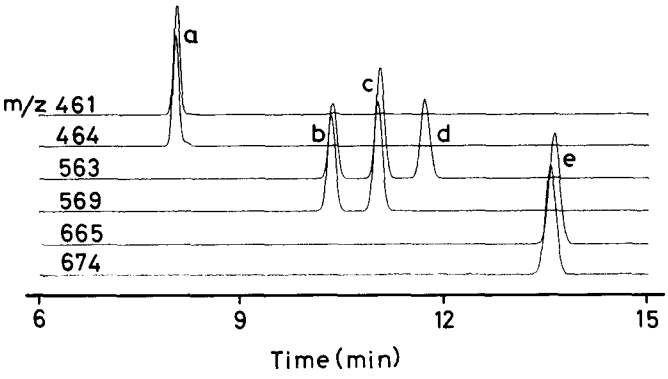

Fig. 3. GC with NICI selected-ion recording of bile acids as the PFB ester–DMES ether derivatives. a = LCA; b = DCA; c = CDCA; d = UDCA; e = CA.

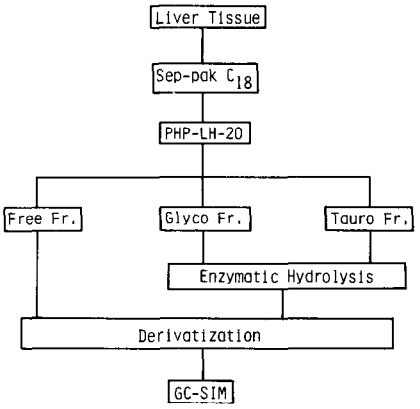

Fig. 4. Procedure for determination of bile acids in liver tissue.

column chromatography on silica gel[17]. However, this clean-up procedure proved to be unfavourable for trace analysis of bile acids because of the low recovery. Therefore, reversed-phase partition chromatography on a Sep-Pak C_{18} cartridge was employed for this purpose. The reaction mixture was applied to the cartridge and the cartridge was washed with methanol–acetonitrile. The bile acid PFB esters were recovered quantitatively, the excess of reagent remaining on the cartridge. Subsequently, the PFB esters were derivatized to the DMES ethers according to the method previously reported[2,12,13].

Applying the standard procedure to human liver, bile acids were determined with satisfactory reproducibility. Known amounts of bile acids were added to the solubilized test samples corresponding to approximately 2 mg of liver tissue, and their recoveries were estimated. In these experiments, the I.S.s were added prior to solubilization or derivatization. As listed in Table III, all bile acids exhibited satisfactory recoveries. The present method was applied to the simultaneous determination of bile acids in liver tissue obtained by diagnostic needle biopsy, and the results obtained are collected in Table IV. An amount of 1 μg wet weight of liver tissue corresponded to about 90 μg of protein. A typical selected-ion recording of the unconjugated bile acids in human liver tissue is illustrated in Fig. 5. The peaks of LCA, DCA, CDCA, UDCA and CA on the chromatogram represent approximately 3, 28, 34, 5 and 49 fmol as injected amounts, respectively.

In this study, we developed a new method for the determination of bile acids in liver tissue by GC–SIM with NICI detection. Although ^{2}H-labelled bile acids have been commonly used as I.S.s, the preparation of these compounds with high isotopic purity needs a time-consuming and tedious procedure. Hence, ^{2}H labelling has been employed only for unconjugated bile acids. For the determination of bile acids, the observed value should be corrected owing to the low recoveries of glycine- and taurine-conjugates[2]. The present method for introducing ^{18}O atoms into the hydroxyl groups made possible the preparation of unconjugated, glycine- and taurine-conjugated bile acids labelled with a stable isotope by a simple procedure. The group separation on PHP-LH-20 can serve to provide information about the conjugated form at C-24. Common bile acids in liver tissue were separated into LCA, DCA,

TABLE III

RECOVERY OF UNCONJUGATED BILE ACIDS ADDED TO SOLUBILIZED LIVER TISSUE

Bile acid	*Tissue (ng/mg*)*	*Added (ng/mg*)*	*Expected (ng/mg*)*	*Found (ng/mg*)*	*Recovery (% ± S.D.**)*
Internal standards added prior to solubilization					
LCA	0.019	0.051	0.070	0.069	98.6 ± 5.2
DCA	0.201	0.100	0.301	0.291	96.7 ± 4.9
CDCA	0.256	0.102	0.358	0.350	97.8 ± 3.7
UDCA	0.052	0.053	0.105	0.101	96.2 ± 4.8
CA	0.521	0.101	0.622	0.628	101.0 ± 4.2
GLCA	0.150	0.105	0.255	0.249	97.6 ± 5.1
GDCA	4.02	1.03	5.05	5.00	99.0 ± 3.9
GCDCA	4.82	1.00	5.82	5.70	79.9 ± 3.8
GUDCA	0.432	0.102	0.534	0.530	99.3 ± 5.8
GCA	6.50	1.06	7.56	7.58	100.3 ± 4.7
TLCA	0.130	0.101	0.231	0.227	98.3 ± 4.3
TDCA	0.789	0.508	1.297	1.30	100.2 ± 4.5
TCDCA	1.89	0.511	2.401	2.40	100.0 ± 5.2
TUDCA	0.184	0.102	0.286	0.280	97.9 ± 6.5
TCA	1.90	0.502	2.402	2.39	99.5 ± 5.7
Internal standards added prior to derivatization					
LCA	0.021	0.051	0.072	0.059	81.9 ± 12.3
DCA	0.144	0.100	0.244	0.210	86.1 ± 7.5
CDCA	0.184	0.102	0.286	0.240	83.9 ± 6.7
UDCA	0.036	0.053	0.089	0.071	79.8 ± 10.4
CA	0.267	0.101	0.368	0.302	82.1 ± 9.2
GLCA	0.038	0.105	0.143	0.113	79.0 ± 24.8
GDCA	2.40	1.03	3.43	2.92	85.1 ± 9.8
GCDCA	3.56	1.00	4.56	4.10	89.9 ± 8.4
GUDCA	0.362	0.102	0.464	0.371	80.0 ± 18.7
GCA	2.96	1.06	4.02	3.22	80.1 ± 9.6
TLCA	0.057	0.101	0.158	0.124	78.5 ± 12.2
TDCA	0.567	0.508	1.075	0.881	82.0 ± 12.6
TCDCA	1.02	0.510	1.530	1.30	85.0 ± 11.3
TUDCA	0.125	0.102	0.227	0.177	78.0 ± 24.6
TCA	1.00	0.502	1.502	1.127	75.0 ± 11.0

* Given in ng/mg liver tissue in wet weight.
** $n = 8$.

CDCA, UDCA and CA as PFB ester–DMES ethers on a capillary column coated with 5% phenylmethyl silicone. Monitoring with characteristic negative ions, $[M - PFB]^-$, unconjugated bile acids, which comprised less than 10% of the total amount, in 2–10 mg of the liver tissue specimen were determined with a quantitation limit of 10 pg/mg tissue.

In man, bile acids are also conjugated with glucuronic acid and sulphuric acid to form glucuronides and sulphates, respectively. The preparation of these bile acid conjugates labelled with ^{18}O and their use for GC–MS are being undertaken in these laboratories. It is hoped that the availability of an excellent method for the simultaneous determination of bile acids in liver tissue with satisfactory sensitivity and reliabil-

TABLE IV

AMOUNTS OF BILE ACIDS IN HUMAN LIVER TISSUE DETERMINED BY THE PRESENT METHOD

Results are given in ng/mg protein.

Bile acid	*Patient 1**	*Patient 2**	*Patient 3**	*Patient 4***
CA	2.50	6.52	4.86	5.23
CDCA	3.22	7.74	6.83	3.69
DCA	4.20	1.65	2.35	2.88
UDCA	0.45	0.92	1.03	0.71
LCA	1.21	1.62	2.08	0.43
GCA	29.35	35.76	85.12	59.15
GCDCA	46.72	141.24	198.20	71.22
GDCA	44.46	11.76	40.25	48.16
GUDCA	3.41	1.92	11.63	7.30
GLCA	2.02	4.81	4.79	0.76
TCA	11.23	24.63	29.19	20.13
TCDCA	11.41	50.05	40.84	20.55
TDCA	8.12	3.87	12.15	11.35
TUDCA	0.83	0.79	3.00	2.49
TLCA	2.06	2.67	6.25	1.15

* Chronic inactive hepatitis.
** Acute hepatitis.

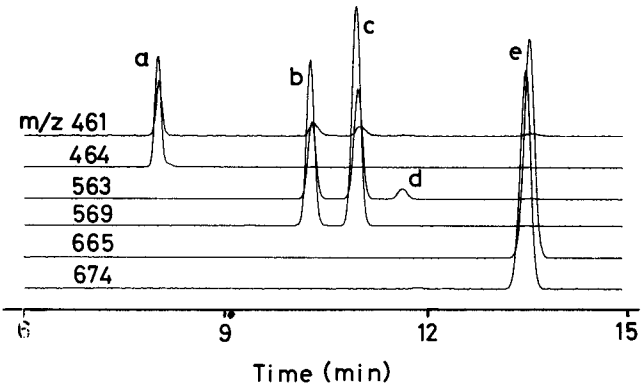

Fig. 5. GC with NICI selected-ion recording of unconjugated bile acids in solubilized human liver tissue as the PFB ester–DMES ether derivatives.

ity may provide much more precise knowledge on the metabolic profile of bile acids and serve as a diagnosis for hepatobiliary diseases.

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