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CAPILLARY GAS CHROMATOGRAPHY OF HYDROXYLATED BILE ACID STEREOISOMERS OF THE *allo* AND NORMAL SERIES

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SUMMARY

Relative retenton times and methylene unit (MU) values were determined for 56 bile acids of the *allo* (5α, A/B *-trans*) and normal (5β, A/B-*cis*) series, which differ from one another in the number, position and configuration of the hydroxyl groups at positions C-3, C-6, C-7 and/or C-12. The bile acids were derivatized to their methyl ester O-trimethylsilyl ethers (Me-TMS) and methyl ester O-dimethylethylsilyl ethers (Me-DMES), and chromatographed on fused-silica capillary columns, chemically bonded with non-selective OV-1 and selective PEG-20M liquid phases. Of the four possible combinations of derivatives and columns examined, Me-DMES on OV-1 and Me-TMS on PEG-20M were most suitable. The differences in the MU values between the 5α- and 5β-series, and between the Me-DMES and Me-TMS ether derivatives, are also summarized. These retention data would be helpful in the identification of closely related bile acid stereoisomers or for estimation of their structures.

INTRODUCTION

Capillary columns, because of their superior resolving properties, have largely supplanted conventional packed columns for gas chromatographic (GC) analysis of complex mixtures. The former columns (coated) have been used successfully in the analysis of bile acids[1–6] and their conjugates[7], despite a tendency of the stationary liquid phase to bleed at elevated temperatures. Since bile acids, even as their more volatile derivatives, require a temperature of over 200°C (usually 240–280°C) for good resolution, the availability of chemically bonded fused-silica capillary columns of higher thermal stability has prompted us to explore their use for separation of bile acids, and to compare the results with those obtained[6] on coated columns for the

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complete set of 26 theoretically possible 5β-cholanoic acids substituted by one to three hydroxyl groups at positions 3, 7 and 12.

Published GC studies of bile acids and their conjugates have mainly been limited to the analysis of the 5β-cholanoic acids, so that our access to the recently completed set of 26 5α-acids[8] makes feasible the comparison of the two sets of hydroxy bile acids (epimeric at C-5) using the more stable bonded columns.

For the present work we chose two fused-silica capillary columns of differing polarities, one chemically bonded with non-polar OV-1, the other with polar PEG-20M. The bile acids were analyzed as their methyl ester O-trimethylsilyl ether (Me-TMS) and methyl ester O-dimethylethylsilyl ether (Me-DMES)[9,10] derivatives because of their easy preparation and excellent GC properties.

EXPERIMENTAL

Sample and reagents

Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid, LCA) was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan), deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid, DCA) and cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid, CA) from Wako Pure Chemical Industries (Osaka, Japan). The remaining bile acid samples used were from collections in our laboratory, which included new *allo* bile acids recently reported by us[8].

The silylating reagents, TMS–HT (hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine) and N-dimethylethylsilylimidazole (DMESI), were obtained from Tokyo Kasei Kogyo Co.

Gas chromatography

A Shimadzu GC-7A gas chromatograph equipped with a flame ionization detector, capillary column split injector and data-processing system (Chromatopac C-R3A) was used isothermally. It was fitted with chemically bonded fused-silica capillary columns of two different polarities and operated under the following conditions: OV-1 (HiCap-CBP1) (18 m × 0.2 mm I.D., film thickness 0.25 μm) non-polar stationary phase, 270°C, helium linear velocity 32 cm/s, splitting ratio 1:80; PEG-20M (DB-WAX) (15 m × 0.25 mm I.D., film thickness 0.25 μm) polar stationary phase, 240°C, helium linear velocity 13 cm/s, splitting ratio 1:170. The OV-1 and PEG-20M columns were obtained from Shimadzu (Kyoto, Japan) and J & W Scientific, respectively.

Relative retention time (RRT) was expressed relative to the methyl ester silyl ether derivatives of DCA, and the methylene unit (MU) was determined using C_{22}–C_{38} *n*-alkanes.

Derivatization

Initially, free bile acid samples were converted into their C-24 methyl esters in the usual manner. The methyl esters were then derivatized to their complete Me-TMS and Me-DMES ethers for capillary GC determinations as follows: to 0.1–0.2 mg of each bile acid methyl ester, 100 μl of a silylating reagent (TMS-HT or DMESI) were added and allowed to stand for 30 min at room temperature. The bile acid methyl ester reacted readily. After the reaction, 1–3 μl of the supernatant were injected into the GC column simultaneously with an internal reference standard.

RESULTS AND DISCUSSION

Preliminary study

A mixture of seven bile acids, LCA, chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid, CDCA), ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid, UDCA), DCA, hyodeoxycholic acid (3α,6α-dihydroxy-5β-cholan-24-oic acid, HDCA), CA and hyocholic acid (3α,6α,7α-trihydroxy-5β-cholan-24-oic acid, HCA) and cholesterol, commonly found in bile extracts and frequently used for evaluating GC columns for their resolving efficiency, was used in a preliminary study of the four possible combinations of derivatives and columns chosen.

As shown in Fig. 1, the combinations Me-DMES on OV-1 and Me-TMS on PEG-20M gave clean baseline separations of all eight compounds. The order of elution (LCA < cholesterol < DCA < CDCA < HDCA < UDCA < CA < HCA), with the former combination was related to the number of hydroxyl groups in the compounds[3], while with the latter combination, the elution order was reversed. The remaining two combinations, Me-TMS on OV-1 and Me-DMES on PEG-20M (not illustrated), did not afford clean separations. The degree of separation and elution order depended on the number and nature of the silyloxy groups as well as on the type of column used. The results suggest that the combinations of Me-DMES on OV-1 and Me-TMS on PEG-20M would be suitable for analyzing bile acid mixtures extracted from biological fluids.

Main study

Table I and II show retention data for the four combinations —the two types of methyl ester silyl ether derivatives on the two columns selected for the entire two sets of 5α- and 5β-compounds plus the two hyo acids, HDCA and HCA— expressed

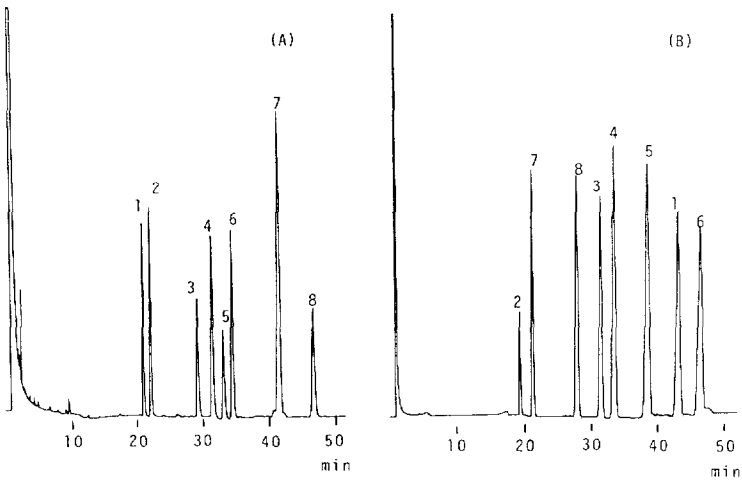

Fig. 1. Capillary GC of a mixture of seven common bile acids and cholesterol as their (A) Me-DMES ether derivatives on OV-1 and (B) Me-TMS ether derivatives on PEG-20M. For conditions see text. Peak identification: 1 = LCA; 2 = cholesterol; 3 = DCA; 4 = CDCA; 5 = HDCA; 6 = UDCA; 7 = CA; 8 = HCA.

TABLE I

RRT AND MU VALUES OF THE METHYL ESTER SILYL ETHER DERIVATIVES OF 5α- AND 5β-BILE ACIDS ON OV-1

RRT was expressed relative to the methyl ester silyl ether derivatives of DCA [3α,12α-$(OH)_2$-5β], 17.06 min for Me-TMS and 26.72 min for Me-DMES. The designations 5α and 5β refer to 5α- and 5β-cholanoates, respectively.

Hydroxyl substituent	*TMS*				*DMES*			
	RRT		*MU*		*RRT*		*MU*	
	5α	*5β*	*5α*	*5β*	*5α*	*5β*	*5α*	*5β*
None	0.53	0.48	29.41	29.06	0.34	0.30	29.41	29.06
3α	0.86	0.86	31.41	31.41	0.71	0.71	32.66	32.66
3β	1.07	0.85	32.32	31.33	0.89	0.71	33.56	32.66
7α	0.60	0.63	29.92	30.12	0.48	0.51	31.06	31.26
7β	0.82	0.74	31.18	30.79	0.65	0.60	32.29	31.88
12α	0.64	0.61	30.22	30.00	0.50	0.46	31.18	30.89
12β	0.66	0.59	30.30	29.81	0.52	0.47	31.32	30.98
3α,7α	1.02	1.05	32.10	32.25	1.06	1.08	34.29	34.35
3α,7β	1.14	1.17	32.59	32.68	1.18	1.19	34.73	34.77
3β,7α	1.04	0.96	32.23	31.90	1.10	0.99	34.46	33.98
3β,7β	1.46	1.17	33.60	32.68	1.54	1.23	35.81	34.87
3α,12α	0.92	1.00	31.73	32.00	0.95	1.00	33.86	34.04
3α,12β	0.94	0.95	31.81	31.86	0.95	0.95	33.86	33.86
3β,12α	1.16	0.98	32.67	31.98	1.21	0.99	34.84	33.98
3β,12β	1.27	1.00	32.98	32.00	1.35	1.05	35.26	34.22
7α,12α	0.65	0.90	30.28	31.62	0.67	0.70	32.45	32.59
7α,12β	0.67	0.71	30.43	30.61	0.67	0.70	32.45	32.59
7β,12α	0.89	0.86	31.57	31.41	0.87	0.83	33.49	33.28
7β,12β	0.97	0.88	31.95	31.51	1.00	0.90	34.04	33.59
3α,6α		1.09		32.38		1.15		34.58
3α,7α,12α	1.03	1.06	32.11	32.28	1.41	1.44	35.44	35.52
3α,7α,12β	1.03	1.02	32.11	32.10	1.31	1.31	35.12	35.12
3α,7β,12α	1.15	1.53	32.62	33.75	1.43	1.56	35.49	35.88
3α,7β,12β	1.25	1.20	32.89	32.74	1.57	1.49	35.87	35.67
3β,7α,12α	1.07	0.98	32.32	31.98	1.43	1.31	35.49	35.12
3β,7α,12β	1.18	1.00	32.69	32.00	1.53	1.31	35.75	35.12
3β,7β,12α	1.50	1.18	33.40	32.69	1.84	1.44	36.53	35.52
3β,7β,12β	1.75	1.29	34.29	33.05	2.29	1.73	37.42	36.28
3α,6α,7α		1.28		33.02		1.62		36.02

as the usual RRT and MU values*. As expected, the heavier Me-DMES derivatives are eluted more slowly than the corresponding Me-TMS compounds from each column. With few exceptions, the Me-DMES derivatives emerged from the OV-1 column according to the number of hydroxyl groups, as noted in the preliminary study. With the other three combinations, no general retention order was observed.

The twelve monohydroxylated esters of the C-5 epimeric series were well resolved as their silyl ethers on both columns (Fig. 2). In each series the presence of an

* The MU values reported previously[6] for the 5β-compounds should be revised[11].

TABLE II

RRT AND MU VALUES OF THE METHYL ESTER SILYL ETHER DERIVATIVES OF 5α- AND 5β-BILE ACIDS ON PEG-20M

RRT was expressed relative to the methyl ester silyl ether derivatives of DCA [3α,12α-$(OH)_2$-5β], 24.02 min for Me-TMS and 42.28 min for Me-DMES.

Hydroxyl substituent	*TMS*				*DMES*			
	RRT		*MU*		*RRT*		*MU*	
	5α	*5β*	*5α*	*5β*	*5α*	*5β*	*5α*	*5β*
None	0.96	0.86	35.80	35.37	0.59	0.52	35.80	35.37
3α	1.19	1.40	36.67	37.27	0.98	1.17	37.58	38.29
3β	1.74	1.13	38.10	36.47	1.46	0.96	39.04	37.54
7α	0.67	0.76	34.48	34.96	0.54	0.62	35.40	35.88
7β	1.18	1.06	36.64	36.20	0.94	0.85	37.52	37.14
12α	0.76	0.73	34.96	34.79	0.59	0.55	35.80	35.45
12β	0.74	0.63	34.86	34.26	0.59	0.51	35.80	35.35
3α,7α	0.88	1.07	35.52	36.24	1.00	1.12	37.71	38.12
3α,7β	1.29	1.50	36.94	37.52	1.36	1.57	38.79	39.32
3β,7α	1.04	0.84	36.14	35.36	1.15	0.89	38.17	37.29
3β,7β	1.97	1.27	38.55	36.87	2.14	1.37	40.49	38.84
3α,12α	0.81	1.00	35.19	35.98	0.86	1.00	37.18	37.71
3α,12β	0.88	0.94	35.52	35.76	0.92	0.99	37.04	37.67
3β,12α	1.29	0.87	36.96	35.45	1.36	0.89	38.44	37.29
3β,12β	1.32	0.81	37.05	35.19	1.45	0.89	39.00	37.29
7α,12α	0.43	1.66	32.81	37.89	0.50	1.32	34.75	38.68
7α,12β	0.48	0.51	33.24	33.45	0.50	0.52	34.75	35.37
7β,12α	0.47	0.81	35.45	35.19	0.86	0.79	37.18	36.81
7β,12β	0.88	0.74	35.52	34.86	0.92	0.79	37.40	36.81
3α,6α		1.21		36.74		1.32		38.68
3α,7α,12α	0.54	0.69	33.65	34.55	0.88	0.98	37.25	37.58
3α,7α,12β	0.62	0.71	34.16	34.67	0.86	0.89	37.18	37.29
3α,7β,12α	0.82	*	35.24	*	1.08	*	38.01	*
3α,7β,12β	0.91	1.00	35.63	35.98	1.22	1.32	38.44	38.68
3β,7α,12α	0.65	0.56	34.38	33.78	0.99	0.78	37.67	36.75
3β,7α,12β	0.76	0.58	34.96	33.89	1.05	0.94	37.99	37.47
3β,7β,12α	1.35	0.91	37.13	35.63	1.76	1.13	39.72	38.15
3β,7β,12β	1.44	0.91	37.39	35.63	2.04	1.23	40.27	38.47
3α,6α,7α		0.91		35.63		1.13		38.15

* See text.

hydroxy group at C-3, regardless of its configuration, caused a larger retarding effect than one at C-7 or C-12[12,13]. Furthermore, for the monohydroxy compounds, with all four combinations and with both 5α- and 5β-derivatives, the axially substituted epimer is eluted before the equatorial; the only exception is the 12-hydroxy-5β pair of epimers on three of the combinations.

A comparison between the six C-5 epimeric pairs at positions C-3, C-7 and C-12 shows the earlier elution of the 5β-derivatives of each pair, with the exception of the 7α-compounds. [Since conformationally the substituents at ring A are reversed

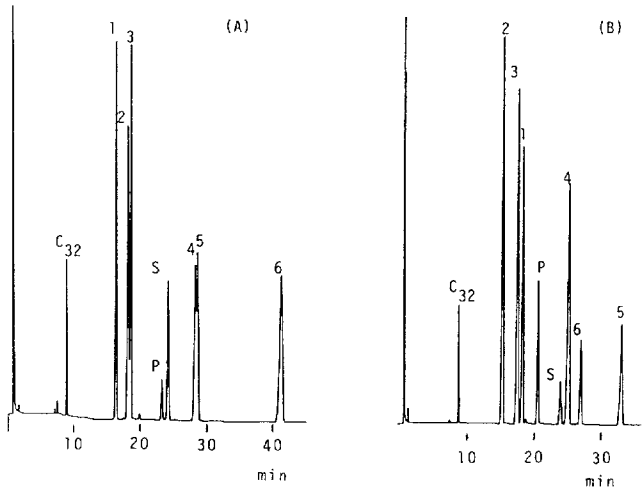

Fig. 2. Capillary GC of a mixture of the monohydroxylated stereoisomers of (A) 5α- and (B) 5β-bile acids as their Me-TMS ether derivatives on PEG-20M. Peak identification: 1 = 7α; 2 = 12β; 3 = 12α; 4 = 7β; 5 = 3α; 6 = 3β; S = 3α,12α(5β); P = methyl 5α- or 5β-cholanoate.

between the 5α- and 5β-compounds, the pairs compared at C-3 are 3α-OH(5α) *vs.* 3β-OH(5β) and 3β-OH(5α) *vs.* 3α-OH(5β)].

On the PEG-20M column, both 5α and 5β Me-TMS derivatives of the 7α-, 12α- and 12β-compounds were eluted before those of the unsubstituted parents, which is a reversal of the retarding effect of an hydroxy group otherwise found generally with all four combinations. A rationalization based on the degree of shielding of these groups by the side chain or on the A ring hindering interaction with the stationary phase is not completely convincing. The argument is weakened by the fact that both the A/B *cis* and A/B *trans* derivatives at these positions behave similarly.

As observed in previous studies by GC[12,13] and high-performance liquid chromatography (HPLC)[14], the retention times of polyhydroxylated compounds were not simply the sums of the contributions from the individual hydroxyls. This was true for all four combinations. Apparently the introduction of a second (or third) hydroxyl group affects the original interaction with the liquid phase in currently unpredictable ways.

Unlike the similar mobility of analogous di- and trihydroxylated compounds epimeric at C-5 found in a previous HPLC comparison[14], the two C-5 series behaved quite differently in GC. In the 5α-series, the individual member within each of the three diol groups had essentially the same elution order. The isomers with both silyloxyls axial (α,α) had the highest mobility, while the ones with equatorial (β,β) substituents had the lowest. However, with the 5β-series, the retention order within each group varied according to both the configuration of the substituent and the column used (Fig. 3). Nevertheless, as a group the 7,12-diols were generally eluted earlier than either the 3,7- or 3,12-diols, and the few exceptions are probably ascribable to the erratic effect of a 7- or 12-hydroxyl group in moderating the large retardation of the C-3 substituent.

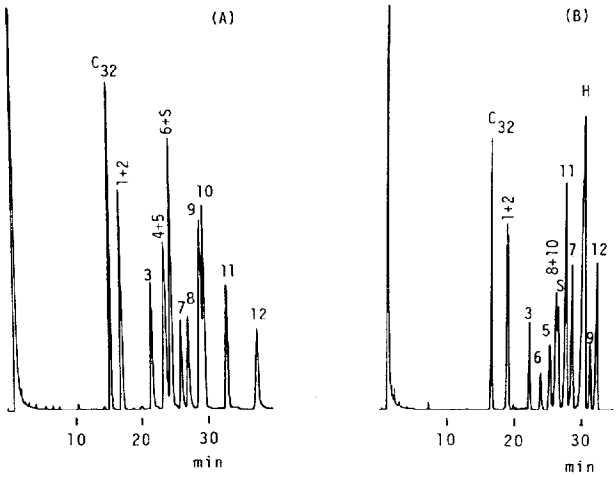

Fig. 3. Capillary GC of a mixture of the dihydroxylated stereoisomers of (A) 5α- and (B) 5β-bile acids as their Me-DMES ether derivatives on OV-1. Peak identification: 1 = 7α,12α; 2 = 7α,12β; 3 = 7β,12α; 4 = 3α,12α(5α); 5 = 3α,12β; 6 = 7β,12β; 7 = 3α,7α; 8 = 3β,7α; 9 = 3α,7β; 10 = 3β,12α; 11 = 3β,12β; 12 = 3β,7β; S = 3α,12α(5β); H = 3α,6α(5β).

The trihydroxy compounds likewise resemble the dihydroxy ones in that the retention order of analogous derivatives of the two series was dissimilar. The eight 5α-stereoisomers were completely resolved as their Me-TMS ethers on the PEG-20M column, emerging in the order $\alpha\alpha\alpha < \alpha\alpha\beta < \beta\alpha\alpha < \beta\alpha\beta < \alpha\beta\alpha < \alpha\beta\beta < \beta\beta\alpha < \beta\beta\beta$ (Fig. 4). Their Me-DMES derivatives on the PEG-20M column and both types of ether derivatives on the OV-1 column followed essentially the same elution order,

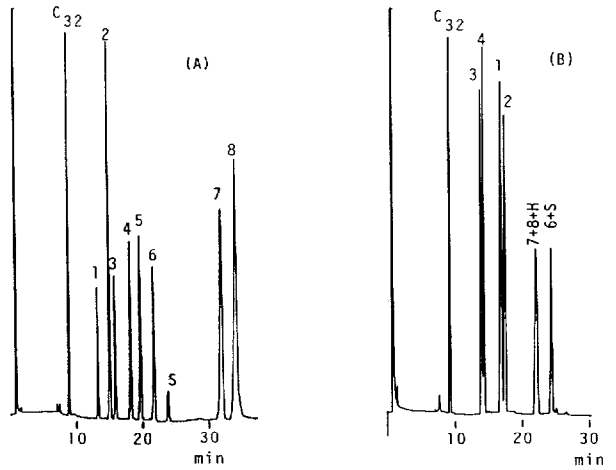

Fig. 4. Capilary GC of a mixture of the trihydroxylated stereoisomers of (A) 5α- and (B) 5β-bile acids as their Me-TMS ether derivatives on PEG-20M. Peak identification: 1 = 3α,7α,12α; 2 = 3α,7α,12β; 3 = 3β,7α,12α; 4 = 3β,7α,12β; 5 = 3α,7β,12α; 6 = 3α,7β,12β; 7 = 3β,7β,12α; 8 = 3β,7β,12β; S = 3α,12α(5β); H = 3α,6α,7α(5β).

although the resolutions were less clean. However, the relative mobility of the 5β-derivatives was dependent on both the type of ether derviative and the column used.

Summaries of the retention data are shown in Table III: $\Delta MU_{\alpha-\beta}$ gives the differences in MU values between analogous 5α- and 5β-derivatives, and ΔMU_{D-T}[3,9] gives the difference between the Me-TMS and Me-DMES values for the same compound on each of the two columns. A negative $\Delta MU_{\alpha-\beta}$ value denotes that the retention time of the 5β-derivative is longer than that of the 5α.

A comparison of the $\Delta MU_{\alpha-\beta}$ values on each column between the Me-TMS and Me-DMES derivatives shows that in general they have similar values, suggesting

TABLE III

$\Delta MU_{\alpha-\beta}$ AND ΔMU_{D-T} VALUES ON OV-1 AND PEG-20M

Hydroxyl substituent	$\Delta MU_{\alpha-\beta}$*				ΔMU_{D-T}**			
	OV-1		*PEG-20M*		*OV-1*		*PEG-20M*	
	TMS	*DMES*	*TMS*	*DMES*	5α	5β	5α	5β
None	0.35	0.35	0.43	0.43	0.00	0.00	0.00	0.00
3α	0.00	0.00	−0.60	−0.71	1.25	1.25	0.91	1.02
3β	0.99	0.90	1.63	1.50	1.24	1.33	0.94	1.07
7α	−0.20	−0.20	−0.48	−0.48	1.14	1.14	0.92	0.92
7β	0.39	0.41	0.44	0.38	1.11	1.09	0.88	0.94
12α	0.22	0.29	0.17	0.35	0.96	0.89	0.84	0.66
12β	0.49	0.34	0.60	0.45	1.02	1.17	0.94	1.09
3α,7α	−0.15	−0.06	−0.72	−0.41	2.19	2.10	2.19	1.88
3α,7β	−0.09	−0.04	−0.58	−0.53	2.14	2.09	1.85	1.80
3β,7α	0.33	0.48	0.78	0.88	2.23	2.08	2.03	1.93
3β,7β	0.92	0.94	1.68	1.65	2.21	2.19	1.94	1.97
3α,12α	−0.27	−0.18	−0.79	−0.53	2.13	2.04	1.99	1.73
3α,12β	−0.05	0.00	−0.24	−0.63	2.05	2.00	1.52	1.91
3β,12α	0.69	0.86	1.51	1.15	2.17	2.00	1.48	1.84
3β,12β	0.98	1.04	1.86	1.71	2.28	2.22	1.95	2.10
7α,12α	−1.34	−0.14	−5.08	−3.93	2.17	0.97	1.94	0.79
7α,12β	−0.18	−0.14	−0.21	−0.62	2.02	1.98	1.51	1.92
7β,12α	0.16	0.21	0.26	0.37	1.92	1.87	1.73	1.62
7β,12β	0.44	0.45	0.66	0.59	2.09	2.08	1.88	1.95
3α,6α						2.20		1.94
3α,7α,12α	−0.17	−0.08	−0.90	−0.33	3.33	3.24	3.60	3.03
3α,7α,12β	0.01	0.00	−0.51	−0.11	3.01	3.02	3.02	2.62
3α,7β,12α	−1.13	−0.39			2.87	2.13	2.77	
3α,7β,12β	0.15	0.20	−0.35	−0.24	2.98	2.93	2.81	2.70
3β,7α,12α	0.34	0.37	0.60	0.92	3.17	3.14	3.29	2.97
3β,7α,12β	0.69	0.63	1.07	0.52	3.06	3.12	3.03	3.58
3β,7β,12α	0.71	1.01	1.50	1.57	3.13	2.83	2.59	2.52
3β,7β,12β	1.24	1.14	1.76	1.80	3.13	3.23	2.88	2.84
3α,6α,7α						3.00		2.52

* Difference in the MU values between the 5α and 5β series; a negative value denotes that the retention time of the 5β-derivative is longer than that of the 5α.

** Difference in the MU values between the Me-DMES and Me-TMS ethers.

that the structure of the silyloxy group has little effect on the degree of separation. However, two notable exceptions to this generalization were observed with the 7α,12α-dihydroxy and the 3α,7β,12α-trihydroxy methyl esters, where the TMS ethers had much larger negative $\Delta MU_{\alpha-\beta}$ values than the corresponding DMES derivatives.

Under the conditions used in this study, a $\Delta MU_{\alpha-\beta}$ value of at least 0.16 would be required for baseline separation of a C-5 epimeric pair on the OV-1 column, whereas only 0.08 would be needed on PEG-20M. Accordingly, because only 21 (Me-TMS) and 19 (Me-DMES) pairs out of the 27 C-5 epimeric pairs examined would be adequately separated on OV-1, while all the pairs, regardless of the nature of the silyloxy groups, would be successfully separated with PEG-20M, the latter column is the better choice for resolving most C-5 epimers (this conclusion was reached by examination of the $\Delta MU_{\alpha-\beta}$ values). However, one drawback in the use of the PEG-20M column is its long retardation of the DMES derivatives, particularly the polyhydroxylated compounds. For example, the retention time of the Me-DMES ether of the 3α,7β,12α-acid (5β) was more than 1.5 h (hence not listed in Table II). The high polarity of this compound compared with other trihydroxy analogues was confirmed by thin-layer chromatography.

The addition of hydroxyl groups to the parent methyl 5α- and 5β-cholanoates produced nearly consistent increases in the ΔMU_{D-T} values[3]. Average ΔMU_{D-T} values observed for the mono-, di- and trihydroxylated compounds were as follows: 1.13, 2.10 and 3.07 on OV-1; 0.91, 1.86 and 2.92 on PEG-20M. The values on the PEG-20M column are generally somewhat smaller than those on the OV-1 column. Since the increment of approximately 1.0 unit separating the three classes of compounds (mono-, di- and trihydroxy) is essentially independent of the other structural characteristics, the determination of the ΔMU_{D-T} values for unknown bile acids affords a useful method of estimating the number of hydroxyl groups in the molecule. However, it should be noted that the 7α,12α-dihydroxy-5β-compound exhibits an unexpectedly small value on both liquid phases, and constitutes the one exception to the general finding.

In conclusion, fused-silica capillary columns, chemically bonded with either non-polar OV-1 or polar PEG-20M liquid phases, provide excellent separations of mono-, di- or trihydroxylated stereoisomers of both "allo" and "normal" bile acids as their Me-TMS and Me-DMES ether derivatives, even at elevated temperatures. Of the four combinations examined, Me-DMES on OV-1 and Me-TMS on PEG-20M are suitable for analyzing these compounds. The retention data would be helpful for identifying an unknown hydroxylated bile acid of the types examined in this work, or for estimating its structure.

REFERENCES

1 T. Laatikainen and A. Hesso, *Clin. Chim. Acta*, 64 (1975) 63.
2 G. Karlaganis and G. Paumgartner, *Clin. Chim. Acta*, 92 (1979) 19.
3 A. Fukunaga, Y. Hatta, M. Ishibashi and H. Miyazaki, *J. Chromatogr.*, 190 (1980) 339.
4 N. Tanida, Y. Hikasa and T. Shimoyama, *J. Chromatogr.*, 240 (1982) 75.
5 S. Barnes, R. Waldrop and D. G. Pritchard, *J. Chromatogr.*, 231 (1982) 155.
6 T. Iida, F. C. Chang, T. Matsumoto and T. Tamura, *J. Lipid. Res.*, 24 (1983) 211.
7 J. M. Street, D. J. H. Trafford and H. L. J. Makin, *J. Lipid Res.*, 27 (1986) 208.
8 T. Iida, T. Shinohara, T. Momose, T. Tamura, T. Matsumoto, T. Nambara and F. C. Chang, *Synthesis*, 12 (1986) in press.

9 H. Miyazaki, M. Ishibashi, M. Itoh and T. Nambara, *Biomed. Mass Spectrom.*, 4 (1977) 23.
10 H. Miyazaki, M. Ishibashi and K. Yamashita, *Biomed. Mass Spectrom.*, 5 (1978) 469.
11 C. J. W. Brooks, G. M. Barrett and W. J. Cole, *J. Chromatogr.*, 289 (1984) 231.
12 W. H. Elliott, R. L. B. Walsh, M. M. Mui, M. A. Thorne and C. M. Siegfried, *J. Chromatogr.*, 44 (1969) 452.
13 P. Eneroth and J. Sjövall, in P. P. Nair and D. Krichevsky (Editors), *The Bile Acids*, Vol. 1, Plenum, New York, 1971, p. 150.
14 T. Iida, T. Momose, T. Shinohara, J. Goto, T. Nambara and F. C. Chang, *J. Chromatogr.*, 366 (1986) 396.