Journal of Chromatography, 537 (1991) 345–356
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 758

Capillary gas chromatographic behavior of stereoisomeric bile acids with a *vicinal* glycol structure by their "mixed" alkylboronate derivatives

TAKASHI IIDA* and ICHIRO KOMATSUBARA
College of Engineering, Nihon University, Koriyama, Fukushima-ken, 963 (Japan)
FREDERIC C. CHANG
Department of Chemistry, Harvey Mudd College, Claremont, CA 91711 (U.S.A.)
and
JUNICHI GOTO and TOSHIO NAMBARA
Pharmaceutical Institute, Tohoku University, Aobayama, Sendai, 980 (Japan)
(First received May 2nd, 1990; revised manuscript received August 14th, 1990)

ABSTRACT

The capillary gas chromatographic (GC) behavior of 25 bile acids with a *vicinal* 6,7- or 3,4-glycol structure, differing from one another in the stereochemical configuration [diaxial *trans*, diequatorial *trans* and axial–equatorial (or *vice versa*) *cis*], was studied. Methylene unit (MU) values were determined for each of the bile acids as their nine classes of hydroxyl derivatives: complete methyl ester (Me)–trimethylsilyl (TMS) and Me–dimethylethylsilyl (DMES) ether and Me–acetate (Ac) derivatives, and their so-called "mixed" cyclic alkylboronate derivatives, Me–methylboronate (MB)–TMS, Me–MB–DMES, Me–MB–Ac, Me–*n*-butylboronate (*n*BB)–TMS, Me–*n*BB–DMES and Me–*n*BB–Ac. Changes in the MU values of each bile acid due to the different derivatizations were used for the determination of the number of hydroxyl groups and the stereochemical relationship of 1,2-diol groups in the molecules. The *cis*-glycol type of compound, regardless of its α,α- or β,β-configuration, formed the corresponding mixed cyclic alkylboronate derivatives. On the other hand, both the diaxial and diequatorial *trans*-glycols yielded a GC peak corresponding to the persilylated or peracetylated derivatives, although the latter compounds were occasionally accompanied by a second peak corresponding to the mixed alkylboronate–silyl ether derivative. The retention data would be useful for analysing bile acid mixtures extracted from biological samples.

INTRODUCTION

Numerous studies have been reported on the gas chromatographic (GC) separation, identification and quantification of various bile acids related to 5α- and 5β-cholanoic acids [1–4]. However, they were almost exclusively concerned with bile acids with isolated hydroxyl groups. Similar studies on 4- or 6-hydroxylation products of common bile acids, *i.e.*, lithocholic (3α-OH), deoxycholic [3α,12α-$(OH)_2$], chenodeoxycholic [3α,7α-$(OH)_2$], ursodeoxycholic [3α,7β-$(OH)_2$] and cholic [3α,7α,12α-$(OH)_3$] acids, which possess a *vicinal* 1,2-diol function in the molecules

0021-9673/91/$03.50

[5–7], have been limited to a few exceptions, probably owing to their restricted availability or non-existence.

A number of unusual bile acids with a 1,2-glycol structure at the 6,7- or 3,4-positions have recently been found in significant amounts in biological samples from patients with liver diseases and in newborn infants and fetuses [5–7]. These compounds are of great current interest in biological and metabolic studies. The qualitative and quantitative analyses of such bile acids have usually been performed by GC and GC–mass spectrometry (MS), after suitable GC derivatization.

Among various derivatization procedures, cyclic alkyl- or arylboronate derivatives (*e.g.*, methyl- [8–12] and *n*-butylboronates [9,13,14]) or cyclic dialkylsilylene derivatives (*e.g.*, di-*tert.*-butyl- [15,16] and diethylsiliconides [17]) as protecting groups of diols have been shown to be suitable for the GC and GC–MS of 1,2-, 1,3- or 1,4-dihydroxylated compounds such as steriods and prostaglandins. In particular, the so-called "mixed" cyclic alkylboronate–silyl ether or alkylboronate–ester derivatives, introduced by Brooks and co-workers [8,9], have been widely used for the characterization of various bifunctional steroids having both a 1,2- or 1,3-diol system and isolated hydroxyl groups in the molecules [8–13], although the technique requires further derivatization for the isolated hydroxyl groups that are unable to form the cyclic boronate. Cyclic boronate formation with an unknown compound yields specific structural information, and the derivatives generally are better resolved by chromatographic separation of a mixture. In addition, the cyclization product obtained provided a mass spectrum containing characteristic ions with prominent intensity.

As a result of work on a programme of synthesizing potential bile acid metabolites, a series of di-, tri- and tetrahydroxylated bile acids with a *vicinal* 3,4- or 6,7-glycol structure, which differ from one another in the stereochemical configuration of the 1,2-diol system [*i.e.*, diaxial *trans*-, diequatorial *trans*- and axial–equatorial (or *vice versa*) *cis*-glycols], are now available. In this paper, we describe the capillary GC behavior of 25 sterically different bile acids as their mixed methyl- and *n*-butylboronate derivatives and their complete methyl ester–trimethylsilyl (Me–TMS) and methyl ester–dimethylethylsilyl (Me–DMES) ether and methyl ester–acetate (Me–Ac) derivatives. Although the GC behavior of some 3,6,7-trihydroxy bile acids with a *cis*-glycol structure as their mixed methylboronate–TMS ether derivatives is known [9], analogous *trans*-glycols have not been reported hitherto.

EXPERIMENTAL

Samples and reagents

All the stereoisomeric 5α- and 5β-cholanoic acids having a *vicinal* 3,4- or 6,7-glycol structure were from collections in our laboratory, which include new and natural bile acids recently synthesized by us [18–20].

The silylating reagents, TMS–HT (hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine) and N,N-dimethylethylsilylimidazole (DMESI), were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Methylboronic acid was purchased from Aldrich (Milwaukee, WI, U.S.A.). *n*-Butylboronic acid and 4-dimethylaminopyridine were available from Tokyo Kasei Kogyo. All solvents were of analytical-reagent grade and used without further purification.

GC instrument and column

A Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector and data-processing system (Chromatopac C-R6A) was used isothermally. It was fitted with an aluminum-clad flexible fused-silica capillary column (25 m × 0.25 mm I.D.) with a thin film (0.1 μm) of bonded and cross-linked methylpolysiloxane (equivalent to OV-101) and operated under the following conditions: carrier gas (helium) flow-rate, 1.5 ml/min; splitting ratio, 1:50; and column temperature, 270°C. The column, HiCap CBPM1, was purchased from Shimadzu (Kyoto, Japan).

Derivatization procedures

Bile acid samples were first converted into the C-24 methyl esters by the usual diazomethane method [1]. Each of the bile acid methyl esters was then subjected to the nine classes of hydroxyl derivatizations prior to GC, and their structures are depicted in Fig. 1, as exemplified by a 5β-3,6,7-triol. The mixed alkylboronate deriv-

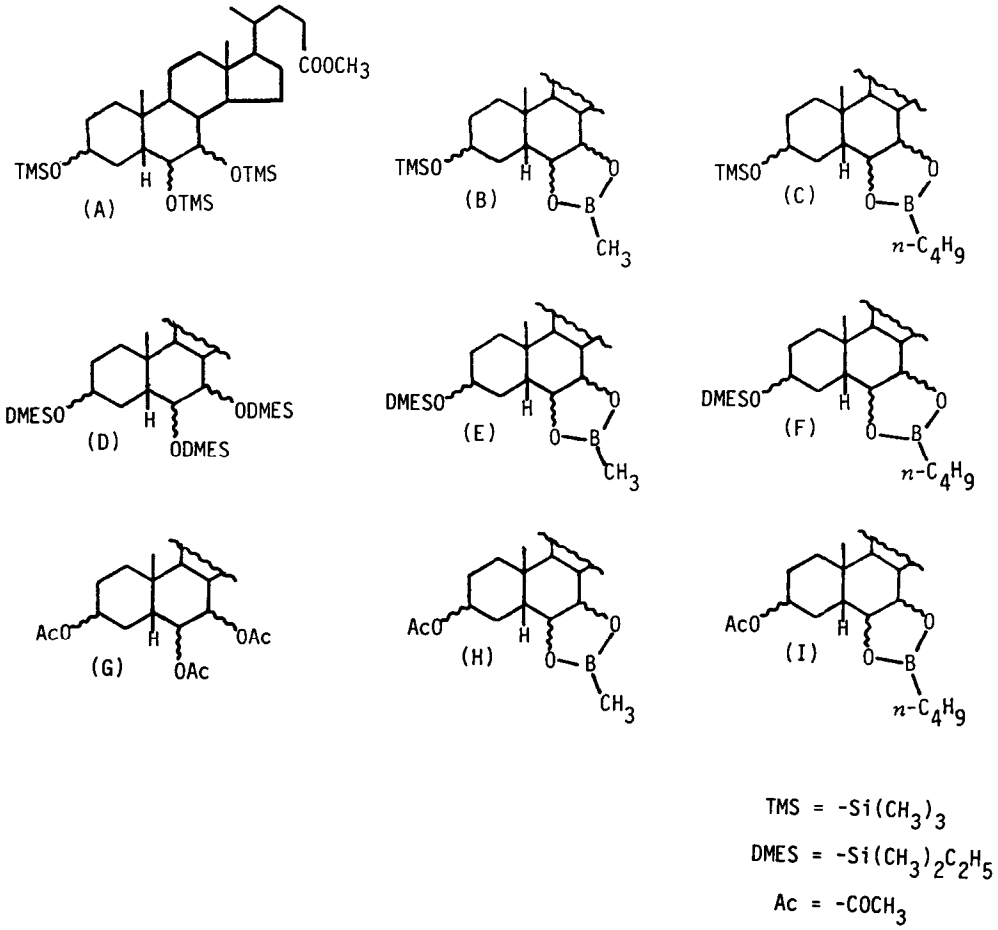

Fig. 1. Derivatizations examined.

TABLE I

MU VALUES OF THE Me–TMS (A), Me–MB–TMS (B), Me–*n*BB–TMS (C), Me–DMES (D), Me–MB–DMES (E), Me–*n*BB–DMES (F), Me–Ac (G), Me–MB–Ac (H) AND Me–*n*BB–Ac (I) DERIVATIVES OF BILE ACIDS

Position and cofiguration of hydroxyls	Me–TMS (A)	Me–MB–TMS (B)	Me–*n*BB–TMS (C)	Me–DMES (D)	Me–MB–DMES (E)	Me–*n*BB–DMES (F)	Me–Ac (G)	Me–MB–Ac (H)	Me–*n*BB–Ac (I)
3α	31.26			32.67			32.47		
3α,6α	32.30			34.62			34.76		
3α,7α	32.12			34.44			33.99		
3α,7β	32.53			34.78			34.92		
3α,12α	31.88			34.13			33.35		
3α,7α,12α	32.24			35.64			34.35		
3α,7β,12α	32.87			35.77			35.65		
3α,6α,7α	33.15	33.15	35.15	36.12	34.30	36.45	35.54	34.05	36.15
3α,6α,7β	34.33	33.61	35.75	37.13			36.43		
3α,6β,7α	32.26			35.30			35.71		
3α,6β,7β	33.30	32.98	35.41	36.31	34.18	36.64	36.78	34.05	36.57
3β,6α,7α	33.05	32.76	34.83	36.12	34.18	36.22	35.38	33.89	35.82
3β,6α,7β	34.62	33.35	35.64	37.28			36.09		
3β,6β,7α	31.88			35.09			35.38		
3β,6β,7β	33.84	33.33	35.67	36.62	34.59	36.94	36.43	34.13	36.74
3α,6α,7β (5α)	34.88	33.21	35.65	37.93			36.78		
3α,6β,7β (5α)	33.46	32.98	35.34	36.44	34.18	36.56	36.99	34.30	36.66
3α,6α,7α,12α	33.33	33.39	35.29	37.28	35.33	37.37	35.72	34.48	36.62
3α,6α,7β,12α	34.26	33.89	36.21	37.84			37.00		
3α,6β,7α,12α	32.42			36.48			36.01		
3α,6β,7β,12α	32.93	33.30	35.68	36.72	35.33	37.78	37.26	34.78	37.37
3α,6α,7β,12α (5α)	34.69	33.53		38.60			37.36		
3α,4β	33.58	31.79	34.65	35.55			34.39		
3β,4α	31.76			33.89			33.92		
3β,4β	33.02	31.84	34.84	35.19	31.88	34.82	34.16	31.84	34.88
3α,4β,7α	33.96		35.41	36.78		36.30	35.46		
3β,4β,7α	34.23	32.00	34.84	36.91	33.02	35.74	35.54	33.15	35.96
3α,4β,12α	34.11			37.06			34.94		
3β,4α,12α	32.84			35.87			34.81		
3β,4β,12α	33.18	32.48	35.43	36.15	33.45	36.33	34.94	32.89	35.89
3α,4β,7α,12α	34.68		35.06	38.39		37.56	35.58		
3β,4β,7α,12α	34.42	32.39	35.05	37.98	34.44	37.12	35.95	33.72	36.46

atives (B, C, E, F, H and I) were prepared in two steps by a combination of boronic cyclization followed by silylation or acetylation [8,9].

Complete methyl ester–trimethylsilyl (Me–TMS; A) and methyl ester–dimethylethylsilyl (Me–DMES; D) ether derivatives

To each bile acid methyl ester (50–100 μg), silylating reagent (TMS–HT or DMESI, 50 μl) was added and the mixture was allowed to stand for 30 min at room temperature [4].

Complete methyl ester–acetate (Me–Ac; G) derivatives

To each bile acid methyl ester (50–100 μg), acetic anhydride (20 μl), dry pyridine (30 μl) and 4-dimethylaminopyridine (10 μg) were added and the mixture was heated at 60°C for 30 min [9]; for 3,4-glycols, acetylation was carried out at 120°C for 2 h.

Methyl ester–cyclic alkylboronate derivatives

To each bile acid methyl ester (50–100 μg), methylboronic (or *n*-butylboronic) acid (50 μg) and dry pyridine (50 μl) were added and the mixture was heated at 60°C for 30 min.

Methyl ester–methylboronate–trimethylsilyl (Me–MB–TMS; B), methyl ester–n-butylboronate–trimethylsilyl (Me–nBB–TMS; C), methyl ester–methylboronate–dimethylethylsilyl (Me–MB–DMES; E), and methyl ester–n-butylboronate–dimethylethylsilyl (Me–nBB–DMES; F) ether derivatives

Mixed cyclic boronate–silyl ether derivatives were prepared by a slight modification of the procedure of Brooks and co-workers [8,9]. After methylation and alkylboronation, the resulting derivative, prepared as above was treated with silylating reagent (TMS–HT or DMESI, 50 μl) and the mixture was allowed to stand for 30 min at room temperature.

Methyl ester–methylboronate–acetate (Me–MB–Ac; H) and methyl ester–n-butylboronate–acetate (Me–nBB–Ac; I) derivatives

After methylation and alkylboronation, the resulting derivative, prepared as above, was treated with acetic anhydride (20 μl), dry pyridine (30 μl) and 4-dimethylaminopyridine (10 μg) and the mixture was heated at 60°C for 30 min (or at 120°C for 2 h for 3,4-glycols).

After the reactions, an aliquot of the sample solutions was injected directly into the GC system without clean-up [21] simultaneously with an internal reference standard.

RESULTS AND DISCUSSION

Twenty-five bile acids possessing a *vicinal* 6,7- or 3,4-glycol structure differing in stereochemical configuration were used to study the GC behavior accompanying various hydroxyl derivatizations. Table I shows the 25 compounds examined plus seven common bile acids and the retention data observed for the nine classes of derivatives: Me–TMS (A), Me–MB–TMS (B), Me–*n*BB–TMS (C), Me–DMES (D),

ME–MB–DMES (E), Me–*n*BB–DMES (F), Me–Ac (G), Me–MB–Ac (H) and Me–*n*BB–Ac (I). The reaction products usually afforded a sharp and symmetrical peak that facilitates the separation of isomers when analyzed using a non-selective fused-silica capillary column (HiCap CBPM1). Retention data were expressed as methylene unit (MU) values. In order to minimize the discrepancies in the MU values [7], the same column was used throughout the determination of the retention data for all the compounds.

GC behaviors of complete silyl ether and acetate derivatives

As bile acids have conventionally been analyzed as their complete Me–TMS, Me–DMES or Me–Ac derivatives [1,2], we initially examined the GC behavior of the 25 acids. The ten stereoisomers of 3,6,7-triols including two 5α- (*allo*) series of compounds were well resolved as their Me–TMS ether derivatives on this column, emerging in the order 3β,6β,7α < 3α,6β,7α < 3β,6α,7α < 3α,6α,7α < 3α,6β,7β < 3α,6β,7β (5α) < 3β,6β,7β < 3α,6α,7β < 3β,6α,7β < 3α,6α,7β (5α). Their Me–DMES ether derivatives followed essentially the same elution order, as shown in Fig. 2a. The 3,6,7,12-tetrahydroxy stereoisomers likewise resemble the 3,6,7-trihydroxy compounds in that the relative mobilities of analogous derivatives follow a similar order,

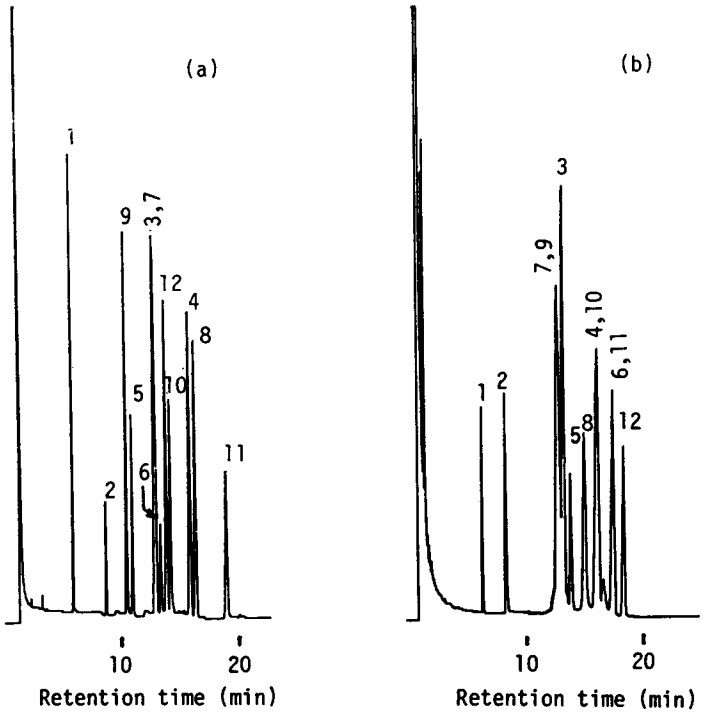

Fig. 2. Capillary GC of a mixture of the 3,6,7-trihydroxy stereoisomers as their (a) Me–DMES and (b) Me–Ac derivatives. Peaks: 1 = C_{32}; 2 = deoxycholic acid; 3 = 3α,6α,7α; 4 = 3α,6α,7β; 5 = 3α,6β,7α; 6 = 3α,6β,7β; 7 = 3β,6α,7α; 8 = 3β,6α,7β; 9 = 3β,6β,7α; 10 = 3β,6β,7β; 11 = 3α,6α,7β (5α); 12 = 3α,6β,7β (5α).

i.e., 3α,6β,7α,12α < 3α,6β,7β,12α < 3α,6α,7α,12α < 3α,6α,7β,12α < 3α,6α,7β,12α (5α).

It is evident from the above observations that, for each silyl ether derivative, 6β,7α-diaxial *trans*-glycols are eluted much faster than the corresponding 6α, 7β-diequatorial *trans*-analogs; compounds with a 6α,7α- or 6β,7β-axial–equatorial (or *vice versa*) *cis*-glycol structure appear between the two *trans*-epimers. A similar elution order was also observed with the silyl ether derivatives of the stereoisomeric 3,4-glycols (diaxial 3β,4α < axial–equatorial 3β,4β < diequatorial 3α, 4β), except for the 3,4,7-trihydroxy isomers, in which the 3α,4β,7α-triol shows a smaller retention time than the 3β,4β,7α-counterpart.

As expected [4,22,23], the trend of the differences in the retention times was in good agreement with earlier observations. Thus, the Me–DMES ethers have longer retention times than the corresponding Me–TMS ethers owing to the heavier ethyl group, and the MU values obtained for the Me–DMES ethers were increased nearly consistently by *ca.* 1 unit per one hydroxyl group in comparison with those of the corresponding Me–TMS ethers. Average differences in the MU values between the two silyl ether derivatives were 2.20 ($n=7$) for di-, 2.95 ($n=17$) for tri- and 3.79 ($n=7$) for tetrahydroxylated bile acids.

The elution order of the Me–Ac derivatives (G) of the bile acids differed from that found for the corresponding silyl ethers mentioned above [24,25], and the following general elution order of 6,7-glycols was observed: equatorial–axial *cis*- < diaxial *trans*- < diequatorial *trans*- < axial–equatorial *cis*-glycols. However, the peaks of derivatives of several isomeric pairs of the 3,6,7-triols were found to overlap (Fig. 2b). In addition, under mild acetylation conditions at 60°C for 30 min, the Me–Ac derivative of the 3,4-glycol type of compounds often gave two well resolved peaks, probably owing to steric hindrance. These compounds were therefore acetylated by more drastic conditions at 120°C for 2 h and, as expected, afforded essentially a single peak. These observations suggest that this derivative is not suitable for the profile analysis of the type of compound [26].

GC behaviors of mixed alkylboronate–silyl ether and alkylboronate–acetate derivatives

The mixed alkylboronate derivatives were prepared in two steps by a slight modification of the method published by Brooks and co-workers [8,9]. The method involves alkylboronic cyclization combined with silylation or acetylation. Fig. 3 shows representative chromatograms of mixtures of the nine classes of derivatives [six mixed cyclic alkylboronates (B,C,E,F,H,I) and three unmixed (A,D,G)] of each of 3 β,4β,12α- and 3α,6β,7α-trihydroxy-5β-cholanoic acid. Fig. 3a, for the derivatives of the axial–equatorial *cis*-glycol 3β,4β,12α-triol acid, shows a distinct separation of the components with single well shaped peaks, whereas Fig. 3b, for the corresponding mixture of derivatives of the diaxial *trans*-glycol 3α,6β,7α-stereoisomer shows only three poorly resolved peaks.

Essentially identical retention GC behaviours were found for all of the other *cis*- and *trans*-glycol types of compounds. The results indicate that under the derivatization conditions used, the *cis*-glycols (6α,7α-, 6β,7β- and 3β,4β-), regardless of α,α- or β,β-configuration, form "mixed" alkylboronate derivatives [9], whereas the *trans*-glycols (6α,7β-, 6β,7α-, 3α,4β- and 3β,4α-), not being able to form cyclic esters, are derivatized to one of the persilyl or peracetate forms.

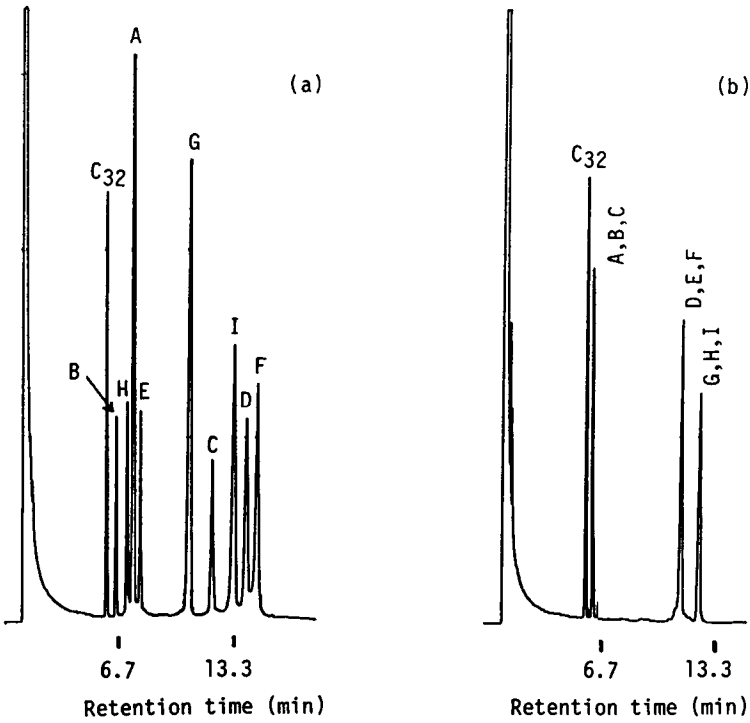

Fig. 3. Capillary GC of a mixture of the hydroxyl derivatization products of (a) 3β,4β,12α- and (b) 3α,6β,7α-trihydroxy-5β-cholanoic acids. Peaks: A = Me–TMS; B = Me–MB–TMS; C = Me–*n*BB–TMS; D = Me–DMES; E = Me–MB–DMES; F = Me–*n*BB–DMES; G = Me–Ac; H = Me–MB–Ac; I = Me–*n*BB–Ac.

However, with the diequatorial *trans*-glycols (6α,7β- or 3α,4β-), although in most instances they resemble their diaxial *trans*-analogs in not forming mixed boronate derivatives, in some instances they do give two well separated peaks, particularly when the two-step derivatization process is used to form the Me–MB–TMS and Me–*n*BB–TMS derivatives (see Table I). In these instances one peak corresponds to the mixed alkylboronate–silyl ether and the other the persilyl ether, indicating that partial formation of the cyclic derivative had taken place. Although the former is usually much smaller than the latter (Fig. 4), the ratio of the two peaks is hardly influenced by the boronation conditions examined [reaction time (30, 60 and 120 min) and reaction temperature (60, 80 and 100°C)], as exemplified by 5β-3α,6α,7β-triol. Peak identification must therefore be carried out cautiously when interpreting the GC results for naturally occurring diequatorially hydroxylated bile acids as the mixed alkylboronate–silyl ether derivatives.

From the above findings, the changes in the MU values due to cyclic alkylboronate formation were calculated. The results are given in Table II, where the $\Delta[Um]_{B-S}$ and $\Delta[Um]_{B-A}$ are the MU increments [22] based on the Me–TMS (B–A and C–A) and Me–DMES (E–D and F–D) ethers and Me–Ac (H–G and I–G), respectively, for the same compound which forms the alkylboronate ester; a negative

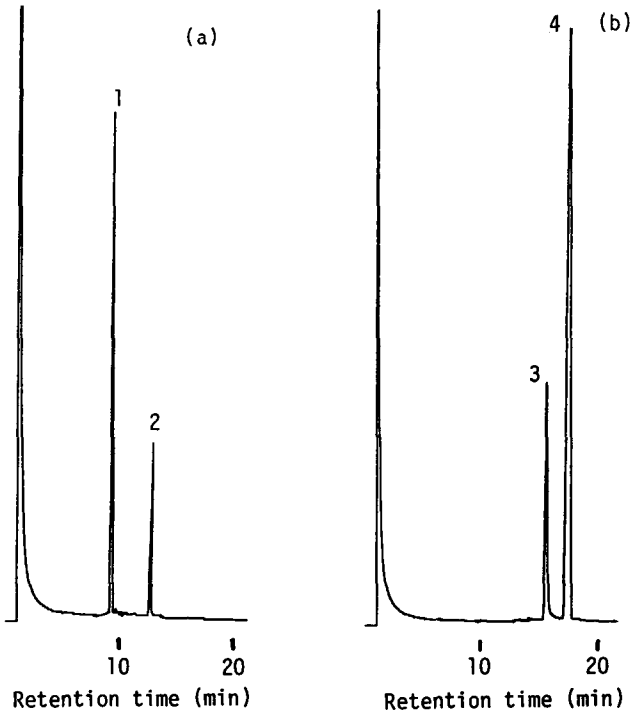

Fig. 4. Capillary GC of the products of diequatorial *trans*-glycols, (a) 3α,6α,7β- and (b) 3α,4β,7α-trihydroxy-5β-cholanoic acids, derivatized to their Me–*n*BB–TMS and Me–*n*BB–DMES ethers, respectively. Peaks: 1 = Me–TMS; 2 = Me–*n*BB–TMS; 3 = Me–*n*BB–DMES; 4 = Me–DMES.

value denotes that the retention time of the mixed alkylboronate derivatives is shorter than that of the corresponding persilylated or peracetylated compounds. The $\Delta[Um]_{B-S}$ and $\Delta[Um]_{B-A}$ values observed were in the ranges –2.23 to 0.37 (B–A) for Me–MB–TMS, 0.38 to 2.75 (C–A) for Me–*n*BB–TMS, –3.89 to –1.39 (E–D) for Me–MB–DMES, –1.17 to 1.06 (F–D) for Me–*n*BB–DMES, –2.73 to –1.24 (H–G) for Me–MB–Ac and –0.33 to 0.95 (I–G) for Me–*n*BB–Ac. The Me–*n*BB–TMS ethers always give large positive $\Delta[Um]_{B-S}$ values, whereas the Me–MB–DMES ether and Me–MB–Ac derivatives gave large negative values. Further, with regard to these derivatives, 6β,7β-glycols usually show a larger value than the corresponding 6α,7α-isomers, with a few exceptions, thus permitting a facile determination of the stereochemical relationship. The other derivatives showed positive or negative values depending on the structure of substrates.

In order to clarify further the general features of cyclic alkylboronations, the MU and $\Delta[Um]_{D-T}$ values were expressed graphically, where $\Delta[Um]_{D-T}$ is the difference in the MU values between the analogous DMES and TMS ether derivatives (*e.g.*, Me–*n*BB–DMES *vs.* Me–*n*BB–TMS) for the same compound on this column [22]. Fig. 5 shows the interrelationship between the $\Delta[Um]_{D-T}$ values and the number of hydroxyl groups in a molecule. As mentioned above, a plot of the $\Delta[Um]_{D-T}$ value *vs.* number of hydroxyl groups for the Me–DMES and Me–TMS ethers [22] showed

TABLE II

$\Delta[Um]_{B-S}$ AND $\Delta[Um]_{B-A}$ VALUES OBSERVED FOR AXIAL–EQUATORIAL (OR *VICE VERSA*) *CIS*- AND DIEQUATORIAL *TRANS*-GLYCOLS

Position and configuration of hydroxyls	$\Delta[Um]_{B-S}$ [a]				$\Delta[Um]_{B-A}$ [b]	
	B–A (Me–MB–TMS –Me–TMS)	C–A (Me–*n*BB–TMS –Me–TMS)	E–D (Me–MB–DMES –Me–DMES)	F–D (Me–*n*BB–DMES –Me–DMES)	H–G (Me–MB–Ac –Me–Ac)	I–G (Me–*n*BB–Ac –Me–Ac)
3α,6α,7α	0	2.00	–1.82	0.33	–1.49	0.61
3α,6α,7β	–0.72	1.42				
3α,6β,7β	–0.32	2.11	–2.13	0.33	–2.73	–0.21
3β,6α,7β	–1.27	1.02				
3β,6α,7α	–0.29	1.78	–1.94	0.10	–1.49	0.44
3β,6β,7β	–0.51	1.83	–2.03	0.32	–2.30	0.31
3α,6α,7β (5α)	–1.67	0.77				
3α,6β,7β (5α)	–0.48	1.88	–2.26	0.12	–2.69	–0.33
3α,6α,7α,12α	0.06	1.96	–1.95	0.09	–1.24	0.90
3α,6α,7β,12α	–0.37	1.95				
3α,6β,7β,12α	0.37	2.75	–1.39	1.06	–2.48	0.11
3α,6α,7β,12α (5α)	–1.16					
3α,4β	–1.79	1.07				
3β,4β	–1.18	1.82	–3.31	–0.37	–2.32	0.72
3α,4β,7α		1.45		–0.48		
3β,4β,7α	–2.23	0.61	–3.89	–1.17	–2.39	0.42
3β,4β,12α	–0.70	2.25	–2.70	0.18	–2.05	0.95
3α,4β,7α,12α		0.38		–0.83		
3β,4β,7α,12α	–2.03	0.63	–3.54	–0.86	–2.23	0.51

[a] Differences in the MU values [22] between the persilyl and the corresponding "mixed" alkylboronate–silyl ether derivatives.
[b] Differences in the MU values [22] between the peracetate and the corresponding "mixed" alkylboronate–acetate derivatives.

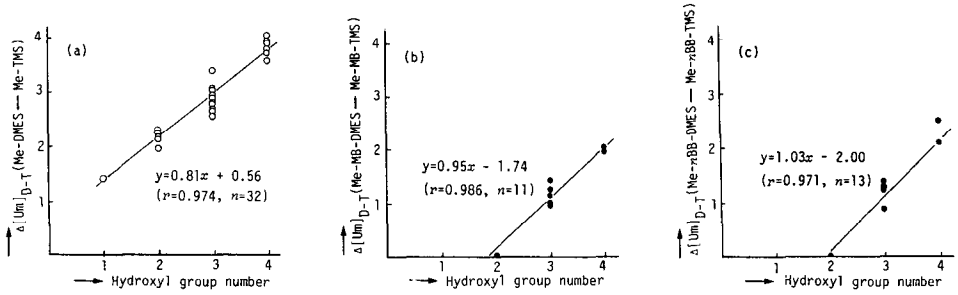

Fig. 5. Relationship of hydroxyl group number to $\Delta[Um]_{D-T}$ value of (a) Me–DMES *vs.* Me–TMS; (b) Me–MB–DMES *vs.* Me–MB–TMS; and (c) Me–MB–DMES *vs.* Me–*n*BB–TMS. (○) Persilyl and (●) alkylboronate–silyl ether peaks.

good linearity (Fig. 5a), defined as $y = 0.81x + 0.56$ ($r = 0.974$, $n = 32$), indicating that the addition of hydroxyl groups produces nearly consistent increases (*ca.* 1 unit per hydroxyl group) in the $\Delta[Um]_{D-T}$ values. On the other hand, plots of the $\Delta[Um]_{D-T}$ value *vs.* number of hydroxyl groups for Me–MB–DMES and Me–MB–TMS ethers afforded the regression line expressed as $y = 0.95x - 1.74$ ($r = 0.986$, $n = 11$) (Fig. 5b) with a much smaller intercept (*ca.* –2 units in the $\Delta[Um]_{D-T}$ value) and with a similar slope. The line consisted of values of the derivatives of axial–equatorial (or *vice versa*) *cis*-glycols and those of one of the two peaks arising from some diequatorial *trans*-glycols mentioned above. The $\Delta[Um]_{D-T}$ value of –2 units reflects the greater reduction in retention time achieved by replacing two *vicinal* DMES groups (rather than two TMS groups) with a cyclic boronate. The value of 2 thus arises from the mass difference of 28 (2DMES–2TMS), and would be similar for the other types of cyclic boronate derivatives (Me–*n*BB–DMES and Me–*n*BB–TMS) (Fig. 5c).

There were also close correlations between the MU values of the mixed *n*-butylboronates with those of the corresponding methylboronate derivatives (Fig. 6), and a statistically significant correlation was found for each of the three graphs. For example, the regression line with a large intercept and with a smaller slope of less than

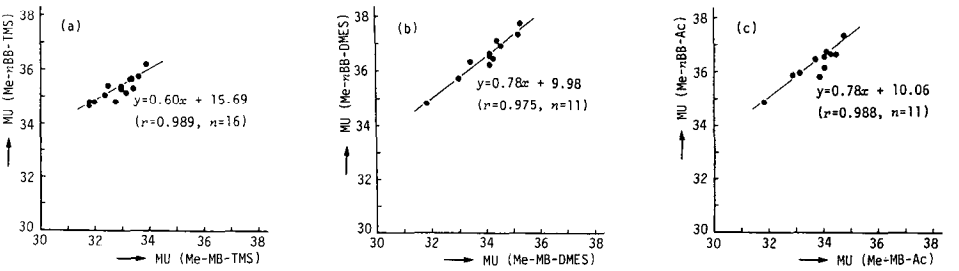

Fig. 6. Relationship between the MU values of (a) Me–*n*BB–TMS and Me–MB–TMS, (b) Me–*n*BB–DMES and Me–MB–DMES and (c) Me–*n*BB–Ac and Me–MB–Ac.

1.0, expressed as $y = 0.60x + 15.69$ ($r = 0.989$, $n = 16$) (Fig. 6a), was obtained from the data for the Me–*n*BB–TMS *vs.* Me–MB–TMS ethers. Analogously, regression lines with a similar intercept and slope to the line in Fig. 6a were also observed for the MU values for the other two combinations [Me–*n*BB–DMES *vs.* Me–MB–DMES (Fig. 6b) and Me–*n*BB–Ac *vs.* Me–MB–Ac (Fig. 6c)] by changing the boronate esters from methyl to the heavier *n*-butyl group.

The above high correlations suggest that if either the $\Delta[Um]_{D-T}$ value or the MU value for the mixed methyl (or the corresponding *n*-butyl) boronation product of an unknown bile acid is known, the number of hydroxyl groups and the presence or absence and the stereochemical relationship (*cis* and *trans*) of a *vicinal* glycol structure can be characterized mathematically by applying the regression equations.

In conclusion, the capillary GC behavior of bile acids with a *vicinal* glycol structure, as their mixed cyclic alkylboronate derivatives, on a non-polar capillary column provided important information concerning the presence or absence and/or the stereochemical configuration of the 1,2-diol groups. Of the several derivatives examined, Me–MB–DMES or Me–*n*BB–TMS seems to be most suitable. The retention data would be helpful for identifying an unknown bile acid of the types examined in this study, or for establishing its structure.

REFERENCES

1 P. Eneroth and J. Sjövall, in P. P. Nair and K. Kritchevsky (Editors), *The Bile Acids*, Vol.1, Plenum Press, New York, 1971, p. 121.
2 J. M. Street and K. D. R. Setchell, *Biomed. Chromatogr.*, 2 (1988) 229.
3 W. H. Elliott, R. L. B. Walsh, C. M., M. M. Mui, M. A. Thorne and C. M. Siegfried, *J. Chromatogr.*, 44 (1969) 452.
4 T. Iida, T. Momose, T. Tamura, T. Matsumoto, J. Goto, T. Nambara and F. C. Chang, *J. Chromatogr.*, 389 (1987) 155.
5 W. H. Elliott, in H. Danielsson and J. Sjövall (Editors), *Sterols and Bile Acids*, Elsevier, Amsterdam, 1985, p. 303.
6 K. D. R. Setchell, P. P. Nair and D. Kritchevsky (Editors), *The Bile Acids*, Vol.4, Plenum Press, New York, 1988.
7 R. Dumaswala, K. D. R. Setchell, L. Zimmer-Nechemias, T. Iida, J. Goto and T. Nambara, *J. Lipid Res.*, 30 (1989) 847.
8 C. J. W. Brooks, W. J. Cole, H. B. McIntyre and A. G. Smith, *Lipids* 15 (1980) 745.
9 C. J. W. Brooks, G. M. Barrett and W. J. Cole, *J. Chromatogr.*, 289 (1984) 231.
10 S. Takatsuto, B. Ying, M. Morisaki and N. Ikekawa, *J. Chromatogr.*, 239 (1982) 233.
11 N. Ikekawa, S. Takatsuto, T. Kitsuwa, H. Saito, T. Morishita and H. Abe, *J. Chromatogr.*, 290 (1984) 289.
12 S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, 34 (1986) 3435.
13 K. Ichimura, H. Yamanaka, K. Chiba, T. Shinozuka, Y. Shiki, K. Saito, S. Kusano, S. Ameniya, K. Oyama, Y. Nozaki and K. Kato, *J. Chromatogr.*, 375 (1986) 5.
14 B. Johansson and I. Fromark, *J. Chromatogr.*, 341 (1985) 462.
15 C. J. W. Brooks, W. J. Cole and G. M. Barrett, *J. Chromatogr.*, 315 (1984) 119.
16 C. J. W. Brooks and W. J. Cole, *Analyst (London)*, 110 (1985) 587.
17 H. Miyazaki, M. Ishibashi, M. Itoh and K. Yamashita, *Biomed. Mass Spectrom.*, 11 (1984) 377.
18 T. Iida, T. Momose, T. Tamura, T. Matsumoto, F. C. Chang, J. Goto and T. Nambara, *J. Lipid Res.*, 30 (1989) 1267.
19 T. Iida, T. Momose, F. C. Chang, J. Goto and T. Nambara, *Chem. Pharm. Bull.*, 37 (1989) 3323.
20 T. Iida, I. Komatsubara, S. Yoda, J. Goto, T. Nambara and F. C. Chang, *Steroids*, in press.
21 T. Iida, T. Itoh, K. Hagiwara, F. C. Chang, J. Goto and T. Nambara, *Lipids*, 24 (1989) 1053.
22 H. Miyazaki, M. Ishibashi, M. Itoh and T. Nambara, *Biomed. Mass Spectrom.*, 4 (1977) 23.
23 A. Fukunaga, Y. Hatta, M. Ishibashi and H. Miyazaki, *J. Chromatogr.*, 190 (1980) 339.
24 P. A. Szczepanik, D. L. Hachey and P. D. Klein, *J. Lipid Res.*, 17 (1976) 314.
25 P. A. Szczepanik, D. L. Hachey and P. D. Klein, *J. Lipid Res.*, 19 (1978) 280.
26 G. Janssen, S. Toppet, F. Compernolle and G. Parmentier, *Steroids*, 53 (1989) 677.