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DERIVATIVES OF MENTHOLS AND MENTHOGLYCOLS FOR USE IN GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

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SUMMARY

The use of TMSi derivatives has permitted us to obtain a better separation of the four menthol stereoisomers than the one obtained generally with the alcohols on a polar column. This separation is carried out with an SE-30 non-polar column with 5 % of liquid phase.

For menthoglycol and neomenthoglycol, two levels of silylation have been defined. With the use of bis-(trimethylsilyl)-acetamide or bis-(trimethylsilyl)-trifluoroacetamide, each isomer gives two mono-TMSi derivatives, the 3 and 8-mono-TMSi ether. On the other hand, under catalysis conditions the result is only one derivative of each isomer, the 3,8-di-TMSi ether.

The formation of menthoglycol and neomenthoglycol cyclic boronates is fast and gives stable derivatives with excellent chromatographic properties.

Heptafluorobutyrate derivatives of menthol stereoisomers, menthoglycol and neomenthoglycol can be detected at the subnanogram level by an electron capture GLC detector.

Structural determination of these derivatives has been carried out by mass spectrometry. All these derivatives are suitable for the analysis by combined gas chromatography–mass spectrometry. This method has been applied to the characterization of the four menthol isomers in mint oil.

INTRODUCTION

During the last five years, important progress has been made in the analysis of natural substances, especially in the steroid field, by means of the appropriate volatile derivatives in gas–liquid chromatography (GLC).

Until now the experiments on the analysis of terpene alcohols by gas chromatography was usually accomplished by the use of very polar stationary phases with a high degree of impregnation[1,2]; these conditions are necessary for the good separation of terpene alcohols and especially of their stereoisomers. This procedure is inconvenient for analysis by gas chromatography coupled with mass spectrometry: considerable background noise on the mass spectra occurs due to bleeding of the liquid phase. On the other hand, the polyhydroxylated monoterpenes are adsorbed, often irreversibly,

on the chromatographic columns and the tertiary hydroxyl groups can be thermally dehydrated in the injector block of the chromatograph.

We should like to describe in this article the use of some derivatives of alcohols in the terpene series which avoid these disadvantages during gas phase analysis.

TMSi derivatives of some monoterpene alcohols including menthol have been prepared by LIU *et al.*[3], and by ADCOCK AND BETTS[4] for the purpose of making their separation easier by thin-layer chromatography. But the study of menthol stereoisomer TMSi derivatives and their separation by GLC were not considered.

The aim of this study is to show that it is possible to obtain excellent separations of terpene alcohols and their stereoisomers by gas chromatography with very thermally stable columns with a small loading of liquid phase, when these alcohols are used as appropriate derivatives. The TMSi derivatives were obtained from bis-(trimethylsilyl)-acetamide[5] (BSA) and bis-(trimethylsilyl)-trifluoroacetamide[6] (BSTFA) reagents.

In order to protect the *tert.*-8-hydroxyl groups in the terpene series, two methods were explored: trimethylsilylation under catalysis conditions and the formation of cyclic boronate diols.

Finally, the heptafluorobutyrate esters (HFB derivatives) allow an ultrasensitive assay of the terpene alcohols.

The structures of the different derivatives prepared in this study were determined by mass spectrometry.

As model compounds for this study, the four stereoisomers of *p*-menthanol-3 (menthol, neomenthol, isomenthol and neoisomenthol) whose difficult separation is very important from a practical point of view and the stereoisomers of *p*-menthanediol-3,8 (menthoglycol and neomenthoglycol) whose tertiary hydroxyl group is sensitive to dehydration were chosen.

EXPERIMENTAL

GLC instruments, columns and separation conditions

A Packard model 7400 gas chromatograph, with a hydrogen-flame ionisation detection system or with an electron capture detection system (^{3}H), was employed. The glass columns (9 ft. × 2 mm I.D.) were packed with 5 % SE-30 (or 1 % OV-1) (methylsiloxane polymers) or 5 % OV-17 (phenylmethylsiloxane polymer) on 120–140 mesh size-graded, acid-washed and silanised Gas-Chrom P[7]. The Gas-Chrom P and the liquid phases were obtained from Supelco, Inc.

Separations, and determinations of methylene unit (MU) values were carried out by temperature programming at a rate of 1°/min, from 70°. The methylene unit values were obtained with even-numbered straight-chain hydrocarbons, C_{10}, C_{12}, C_{14} and C_{16} (Fluka) according to HORNING *et al.*[5]: they are similar to the retention indices, according to KOVÁTS[8], a coefficient 100 being applied to the first values to obtain the second values. The KOVÁTS indices were initially determined under isothermal conditions.

The nitrogen pressures at 100° were 32 p.s.i. (SE-30) and 30 p.s.i. (OV-17). The rates of flow of the carrier gas, hydrogen and air were respectively 25 ml/min; 30 ml/min and 300 ml/min. The injector block temperature was 240° and the detector chamber temperature was 260°.

Mass spectrometry

Structural studies of derivatives were carried out by gas chromatography–mass spectrometry with a Perkin Elmer, Model 270 instrument. The column was a 9 ft. × 2 mm glass coil with 1 % SE-30 liquid phase on 100–120 mesh Gas-Chrom P: column packing was prepared in the usual way[7]. The accelerating voltage was 70 eV. The ion source temperature was 200°.

Preparation of TMSi derivatives

Silylation reactions were carried out by dissolving 1 mg of monoterpene alcohol in the silylating reagent (BSA or BSTFA). A solvent, pyridine (PYR), was employed in a few instances. The final concentration was 1 μg/μl.

TMSi derivatives were prepared in the following way:

(*A*) *BSA*. Reaction mixtures were allowed to stand at room temperature.

(*B*) *BSTFA–PYR* (10:2). The menthoglycol and neomenthoglycol were not easily soluble in the silylating reagents at room temperature: in this instance, it was necessary to use pyridine as solvent.

The mixture BSTFA–PYR was used at room temperature or at 60°. Under these conditions, the same reaction products as those under the conditions A were generally obtained.

However, the reagents peaks were eluted more rapidly, which is an advantage in the course of analysis of compounds of low molecular mass.

(*C*) *BSTFA–PYR–TMCS*. Pyridine was used as a solvent. The ratios of BSTFA, PYR, TMCS reagents were 10:2:2 or 10:2:1. Reaction mixtures were heated in an oven at 80°.

The catalysed reaction conditions are known to achieve silylation of hindered hydroxyl groups, like the 11β- or *tert.*-17α-hydroxyl groups in steroid series[9]. So, silylation of the *tert.*-8-hydroxyl group in the monoterpene series is expected under these conditions.

Preparation of n-butylboronates[10]

A 1.7 mg sample of menthoglycol or neomenthoglycol and 1 mg of butylboronic acid (molar proportion, 1:1) were dissolved in pyridine. The progress of the reaction was checked by GLC; complete reaction was observed in about 3 min, at room temperature.

Preparation of heptafluorobutyrates

For the preparation of (sub)microgram amounts, the vapor phase method[11,12] was used.

A 1 μg sample of monoterpene alcohol, dissolved in hexane, was applied to a small roll or gauze. The gauze is then suspended in a 10 ml closed vessel above 0.75 ml of a benzene–heptafluorobutyric anhydride (HFBA) mixture (2:1).

The esterification reaction was conducted at room temperature. For menthol stereoisomers, the reaction period was 30 min. The reaction was not complete for menthoglycol and neomenthoglycol, even after 5 h: a mono-ester and di-ester mixture was obtained.

The derivative was eluted from the gauze with benzene (100 μl).

The solution method, with HFBA or heptafluorobutyrylimidazole (HFBI) as reagents, was used for the preparation of milligram amounts.

(*a*) *With HFBA*[13]. An 0.5 mg sample of alcohol was esterified with HFBA in benzene (1:1, 0.25 ml) at 70°. The residue, after evaporation under nitrogen, was dissolved in hexane; final concentrations were 1 mg/ml for flame ionisation detection (FID) or for GLC–MS analysis and 1 μg/ml for electron capture detection (ECD).

(*b*) *With HFBI*[14]. A 1 mg sample of alcohol was allowed to react with HFBI in acetonitrile (1:1; 0.2 ml) at 60°. The derivative was extracted with hexane (final concentration: 1 mg/ml). Samples were diluted before use with GLC with ECD to a concentration of between 0.05–1 μg/ml; 1 μl was introduced into the gas chromatograph.

Sources of menthol stereoisomers, menthoglycol and neomenthoglycol.

These have been described in a preceding publication[2]. BSA and BSTFA reagents were obtained from Pierce Chemical Co. and the "*pro analysi*" grade solvents were provided by Merck.

TABLE I

METHYLENE UNIT (MU) VALUES DETERMINED WITH 5% SE-30 AND 5% OV-17 COLUMNS FOR DERIVATIVES OF MENTHOL STEREOISOMERS, MENTHOGLYCOL AND NEOMENTHOLGLYCOL

Compound	*MU values*[a]	
	SE-30	*OV-17*
Menthol		
p-Menthan-3-ol	13.41	12.61
3-Trimethylsilyloxy	12.52	12.60[b]
Neomenthol		
p-Menthan-3-ol	13.32	12.48
3-Trimethylsilyloxy	12.25	12.19
Isomenthol		
p-Menthan-3-ol	[c]	[c]
3-Trimethylsilyloxy	12.41	12.46
Neoisomenthol		
p-Menthan-3-ol	13.51	12.72
3-Trimethylsilyloxy	12.65	12.67
Menthoglycol		
p-Menthan-3,8-diol (*trans*)	13.14[d]	—
3-Trimethylsilyloxy	14.10	14.88
8-Trimethylsilyloxy	14.55	[e]
3,8-Di-(trimethylsilyloxy)	15.35	15.09
n-Cyclobutylboronate	14.95	15.93
3-Mono-(heptafluorobutyrate)	11.96	12.02
3,8-Di-(heptafluorobutyrate)	13.88	13.29
Neomenthoglycol		
p-Menthan-3,8-diol (*cis*)	12.92[d]	—
3-Trimethylsilyloxy	13.79	14.49
8-Trimethylsilyloxy	14.34	[f]
3,8-Di-(trimethylsilyloxy)	15.12	14.81
n-Cyclobutylboronate	14.65	15.65
3-Mono-(heptafluorobutyrate)	11.86	11.92
3,8-Di-(heptafluorobutyrate)	13.45	12.79

[a] Determined with 5% SE-30 and 5% OV-17 columns by temperature programming at a rate of 1°/min, from 70°.
[b] TMSi derivatives of menthol and neoisomenthol are not separated on this liquid phase.
[c] Isomenthol is eluted with menthol.
[d] The free alcohol peaks show a marked tailing with SE-30 and with OV-17.
[e] The peak is eluted as a shoulder posterior to the di-TMSi derivative peak.
[f] The peak is eluted with the mono-TMSi derivative of the *trans* isomer.

RESULTS AND DISCUSSION

The methylene unit values of the derivatives are given in Table I.

TMSi derivatives

Different conditions of silylation, without catalyst (A, B) or with catalyst (C) were applied to each compound according to the hindrance of the alcohol function to be silylated.

The TMSi ethers of the menthol stereoisomers were obtained by silylation methods (A) or (B). Their formation was complete after a reaction period of 15 min. The TMSi derivatives of menthol were eluted from the chromatographic columns, at about 100°.

The stationary phase SE-30 is particularly suitable for the separation of TMSi derivatives of the four menthol stereoisomers as is shown by Fig. 1.

The isomer couples which differ in their hydroxyl configuration (peaks A, C and peaks B, D) are the ones which are the best separated. The difficult separations between menthol and isomenthol on one hand and menthol and neoisomenthol on the other hand, are satisfyingly achieved with a Purnell coefficient higher than 1.

This resolution allows us to separate small amounts of neomenthol, isomenthol

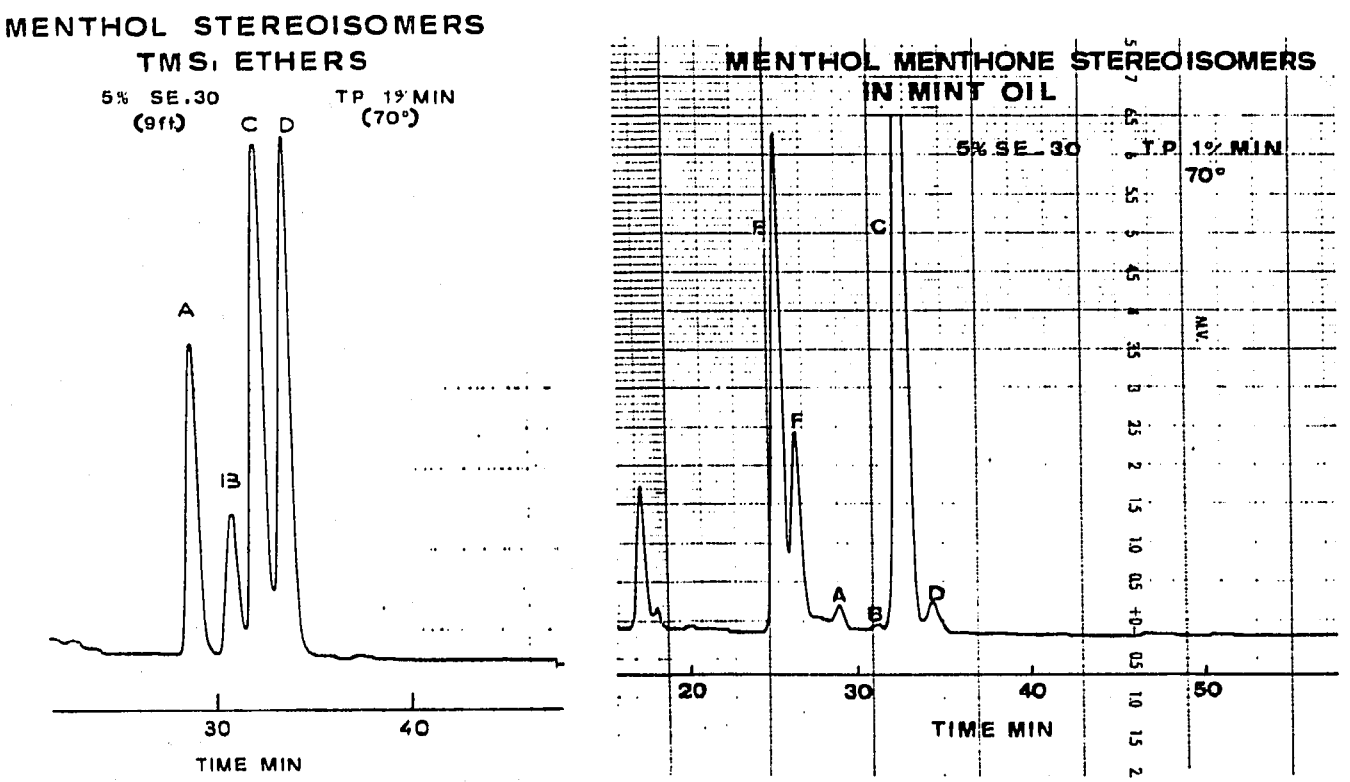

Fig. 1. GLC separation of TMSi ethers resulting from the reaction of menthol stereoisomers with BSA, at room temperature. The ethers are those of neomenthol, (A); isomenthol, (B); menthol, (C); and neoisomenthol, (D).

Fig. 2. Part of a chromatogram of a silylated mint oil, showing peaks corresponding to A = neomenthol TMSi; B = isomenthol TMSi; C = menthol TMSi; D = neoisomenthol TMSi; E = menthone; F = isomenthone.

and neoisomenthol from the main peak of menthol in mint oil, as is shown in Fig. 2.

Neomenthoglycol (I) and menthoglycol (II) silylation leads to two levels of silylation according to the experimental conditions.

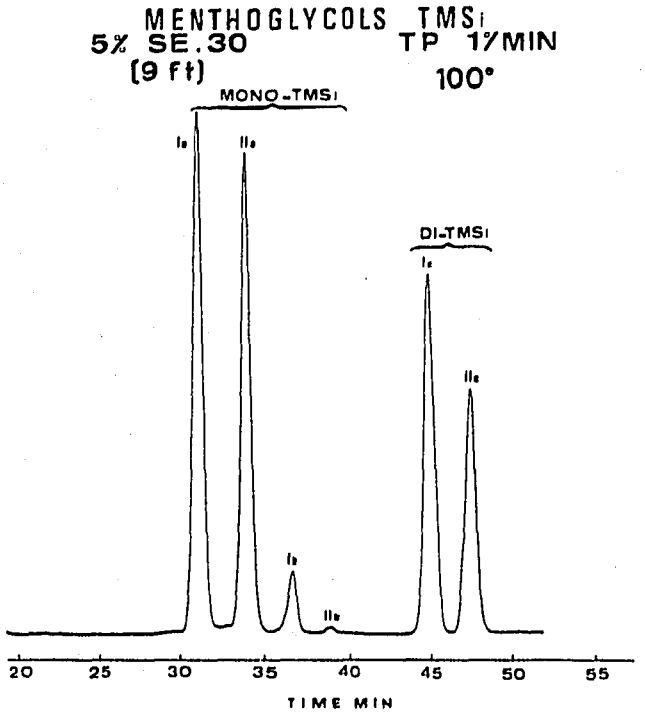

Fig. 3. GLC separation of derivatives obtained in the course of the reaction of menthoglycol (II) and neomenthoglycol (I) with BSA–PYR–TMCS, 10:2:1, at 80°. These derivatives are the 3-mono-ethers (Ia, IIa), the 8-mono-ethers (Ib, IIb) and the 3,8-di-ethers (Ic, IIc). Only the mono-TMSi ethers a and b are obtained under noncatalysed reaction conditions (A) or (B). The di-TMSi ethers c are obtained quantitatively under catalysed reaction conditions (C), after a reaction period of 1 h 30 min for (I) and 8 h for (II).

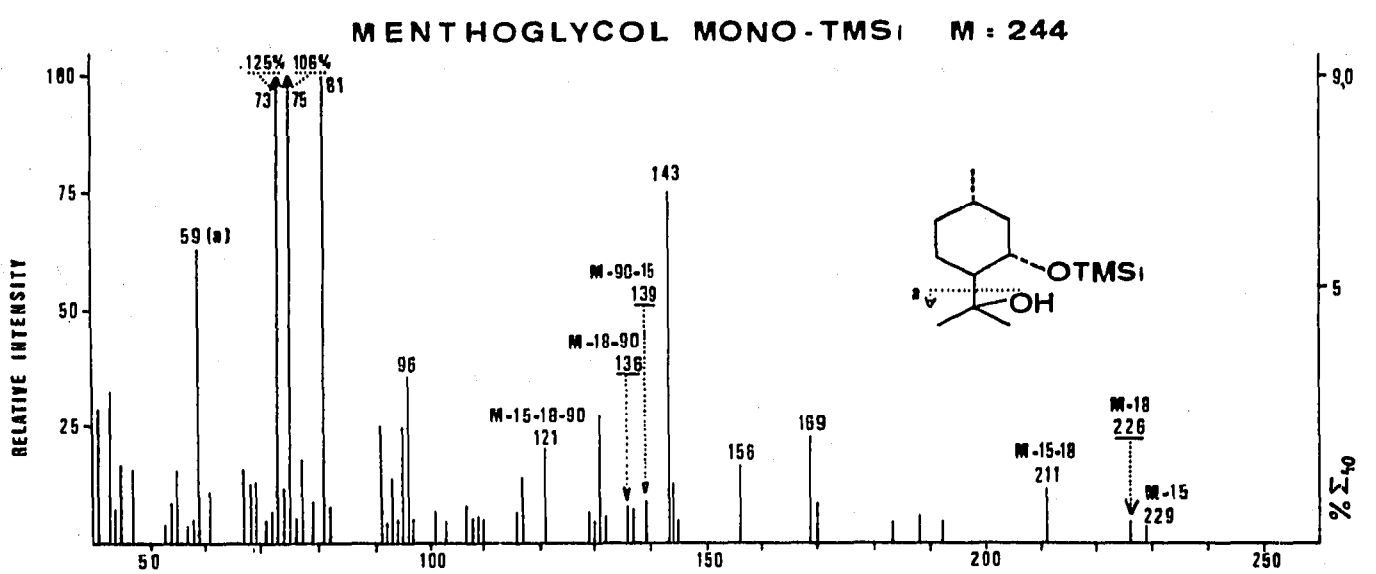

Fig. 4. Mass spectrum for the mono-TMSi ether of menthoglycol (3 mono-ether). Peaks providing structural information include those at the following a.m.u. values: 229 (M-15), 226 (M-18), 211 (M-15-18), 139 (M-90-15), 136 (M-90-18), 121 (M-15-18-90), 96, 81, 59. Peaks at 73 and 75 a.m.u. are usually found in spectra for TMSi ethers.

With the noncatalysed reactions (A) or (B), every stereosiomer gives two derivatives a and b. The reaction is complete after 30 min at room temperature.

With the catalysed reaction (C) at 80°, only one derivative c for every stereoisomer is obtained quantitatively.

Fig. 3 shows the separation of these different derivatives. The chromatogram was obtained during the course of the catalysed reaction: derivatives a and b are converted to c as the reaction progresses.

Mass spectra of the compounds Ia and IIa (Fig. 4) are characteristic of mono-TMSi derivatives. The ion fragments at the following a.m.u. values, 229 (M-15), 226 (M-18), 139 (M-15-90), 136 (M-18-90) and 121 (M-15-18-90) indicate the presence of trimethylsilyloxy and hydroxyl groups. The peak at 59 a.m.u. which has also been described in the α-terpineol spectrum[15,16] results from fragmentation of a-type and is characteristic of compounds with a hydroxyisopropyl group. These results indicate that the structure of derivatives Ia and IIa is that of a 3-mono-TMSi ether.

The structure of the derivatives Ib and IIb was found by mass spectrometry to be that of an 8-mono-TMSi ether. Fig. 5 shows the mass spectrum of IIb: the fragment at M-15-18 (211 a.m.u.) arises from the expulsion of a methyl group and water, the loss of water proving that a free hydroxyl group is present. The peaks at M-15-90 (139 a.m.u.) and M-15-18-90 (121 a.m.u.) show that the derivatives Ib and IIb have one TMSi group. The base peak at 131 a.m.u. characterizes the cleavage of a trimethylsilyloxyisopropyl group and locates the OTMSi group in the molecule at position 8.

The structure of derivatives obtained under silylation conditions (C) has also been studied by mass spectrometry. Mass spectra of these derivatives indicate that two TMSi groups are present (fragments at M-15 (301 a.m.u.), M.15-90 (211 a.m.u.) and at M-15-2 × 90 (121 a.m.u.)). The base peak at 131 a.m.u. characterizes the loss of the trimethylsilyloxyisopropyl group. Derivatives Ic and IIc are 3,8-di-TMSi ethers.

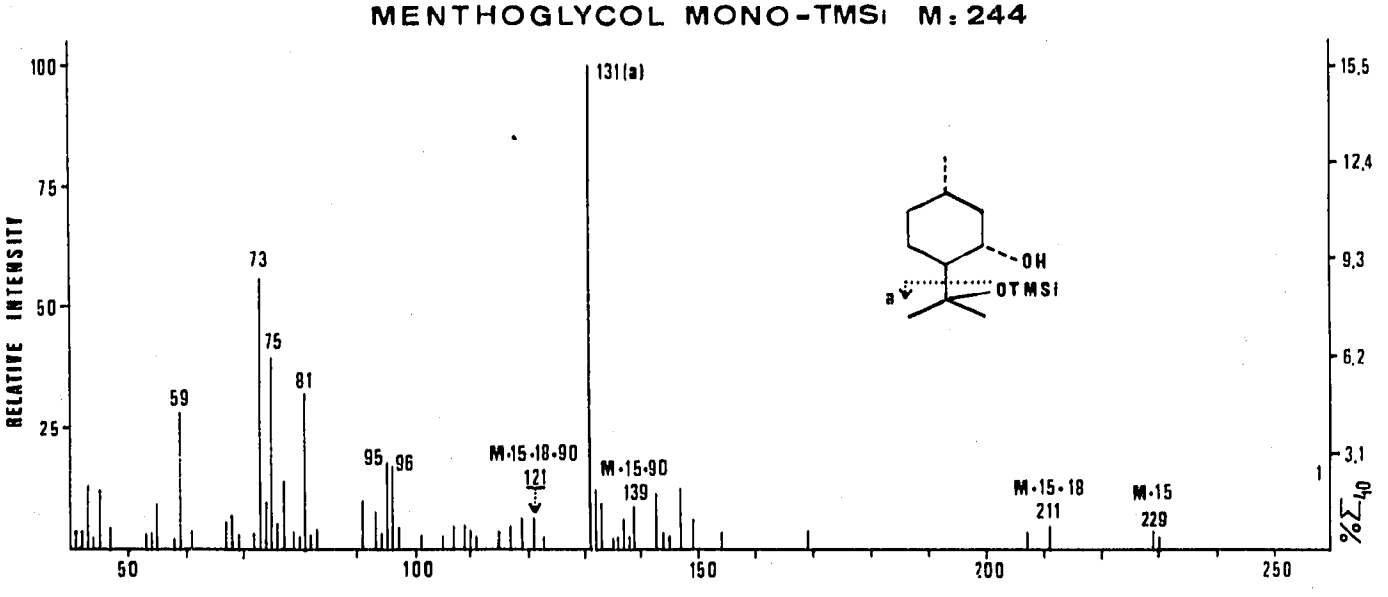

Fig. 5. Mass spectrum for the mono-TMSi ether of menthoglycol (8-mono-ether). Peaks providing structural information include those at the following a.m.u. values: 229 (M-15), 211 (M-15-18), 139 (M-15-90), 131 (a), 121 (M-15-18-90), 96, 81. The base peak (*m/e* 131) corresponds to cleavage adjacent to the trimethylsilyloxy group.

Mass spectra of these derivatives, as well as those of HFB derivatives and *n*-butylboronates, contain fragments at 81 a.m.u. and 96 a.m.u. corresponding respectively, according to THOMAS AND WILLHALM[17], to cyclohexene and methylcyclohexene ions.

The conversion of mono-TMSi ethers of menthoglycol and neomenthoglycol into di-TMSi ethers occurs even without catalyst, when the reaction mixture is heated at 80°, but the conversion yield is low. This conversion is very fast (30 min for the neomenthoglycol) at 80° with a catalyst (BSTFA, TMCS and PYR in the ratio 10:2:2).

The formation rate of di-TMSi derivative of the *cis* isomer was compared to that of the *trans* isomer, when BSTFA, PYR and TMCS were used in the ratio 10:2:1 and is shown in Fig. 6.

The conversion rate is much greater for the *cis* isomer than for the *trans* isomer. In comparison with the interpretation given by ZIMMERMAN AND ENGLISH[18] in their free menthoglycols studies, we may suppose that the formation of an intramolecular hydrogen bonding between the tertiary hydroxyl and 3-ÖTMSi groups leads to a pseudobicyclic system. This system is of a *cis*-decalin type for neomenthoglycol and of a *trans*-decalin type for menthoglycol. The system of the *cis*-decalin type being less stable than that of the *trans*-decalin type, the pseudocyclic ring opening would be easier for neomenthoglycol and the silylation of the alcohol function therefore is faster.

The di-TMSi derivatives which have the advantage of giving only one peak per isomer, also possess a response higher than that of the mono-TMSi derivatives: k_1 =

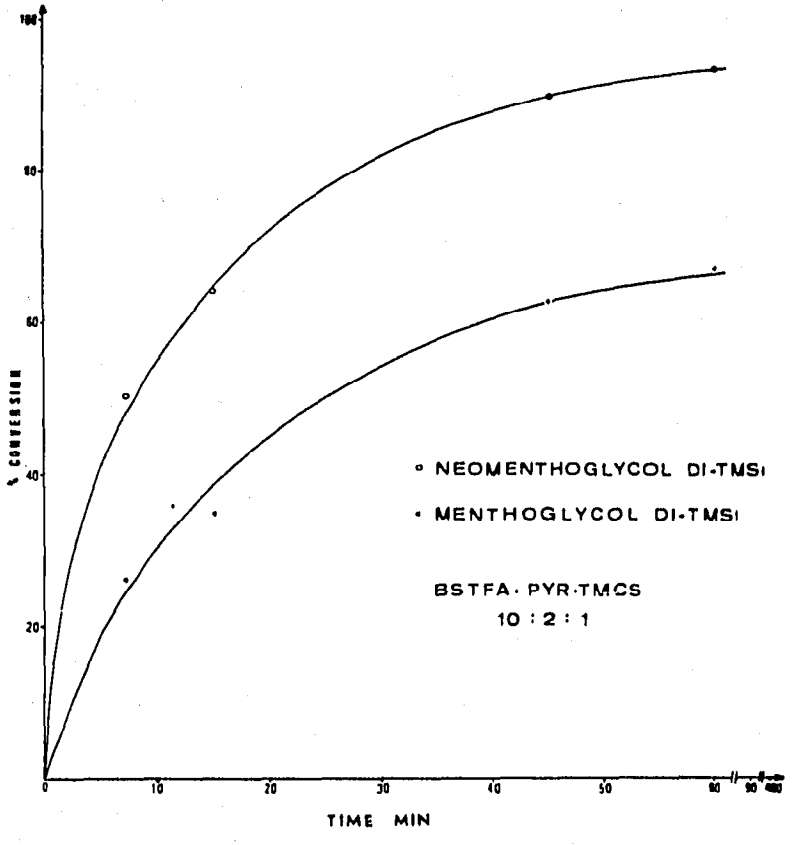

Fig. 6. Kinetics of conversion of the 3-mono-TMSi ether to the 3,8-di-TMSi ether for menthoglycol and neomenthoglycol, with BSTFA–PYR–TMCS, 10:2:1, at 80°. *n*-Hexadecane was used as internal standard.

0.7 for the di-TMSi derivatives and $k_1 = 0.9$ for the mono-TMSi derivatives; the *n*-hexadecane was taken as a reference ($k_1 = 1$).

Cyclic n-butyl boronate esters

The cyclic *n*-butyl boronates of menthoglycol and neomenthoglycol (Fig. 8) have very good gas chromatographic properties: their formation is almost instantaneously

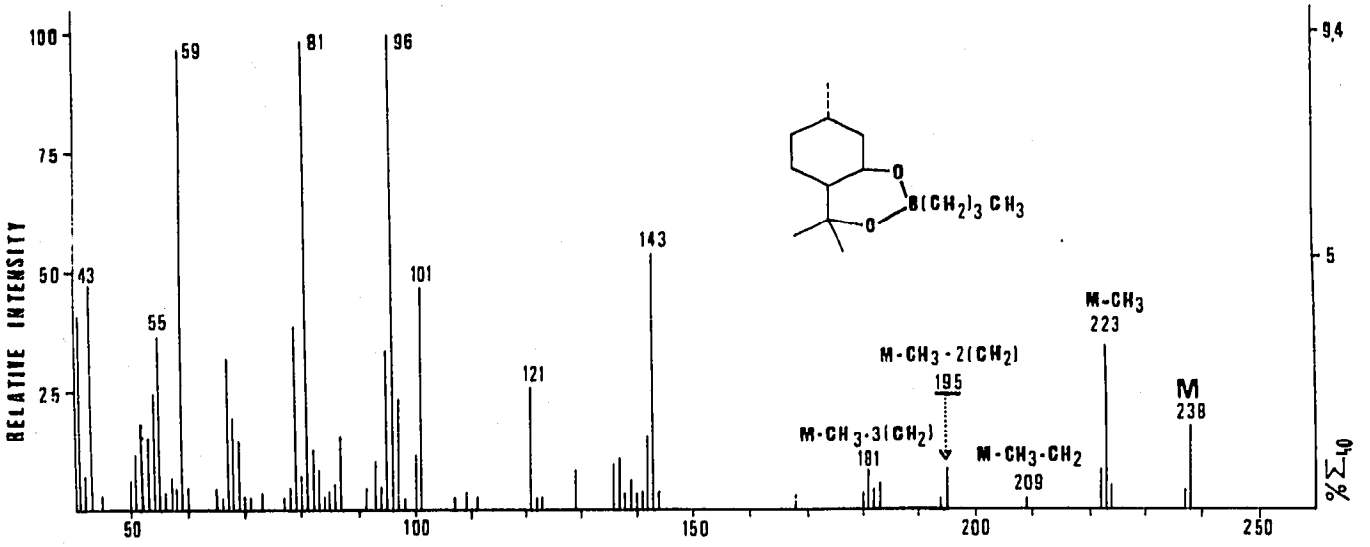

Fig. 7. Mass spectrum for the neomenthoglycol *n*-butylboronate, $M = 238$. Peaks providing structural information include those at the following a.m.u. values: 238 (M), 223 (M-CH_3), 209 (M-CH_3-CH_3), 195 (M-CH_3-2 CH_2), 181 (M-CH_3-3 CH_2), 101, 96, 81, 59.

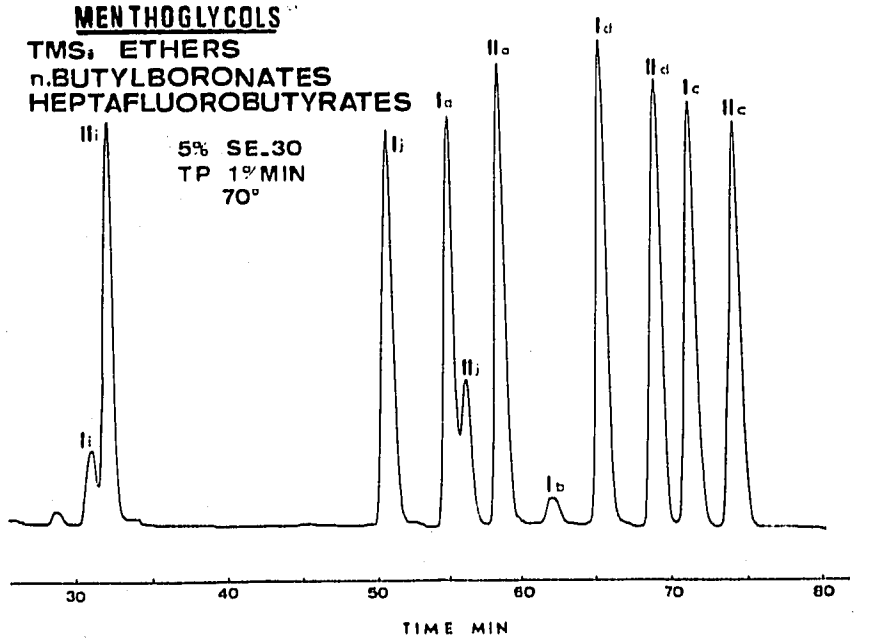

Fig. 8. Separation with 5% SE-30 phase of menthoglycol (II) and neomenthoglycol (I) derivatives: 3-mono-TMSi-neomenthoglycol (Ia) and 3-mono-TMSi-menthoglycol (IIa); 8-mono-TMSi-neomenthoglycol (Ib); 3,8-di-TMSi-neomenthoglycol (Ic) and 3,8-di-TMSi-menthoglycol (IIc); 3-mono-HFB-neomenthoglycol (Ii) and 3-mono-HFB-menthoglycol (IIi); 3,8-di-HFB-neomenthoglycol (Ij) and 3,8-di-HFB-menthoglycol (IIj); neomenthoglycol 3,8-*n*-butylboronate (Id) and menthoglycol 3,8-*n*-butylboronate.

complete (after 3 min) as shown by GLC and each isomer gives only one derivative.

The structure of these derivatives was verified by mass spectrometry. The spectra of the two isomers, one of which is illustrated in Fig. 7 (molecular ion at 238 a.m.u. and base peak at 96 a.m.u.) are qualitatively similar: only the ion fragments at 55 a.m.u. and 121 a.m.u. are very different quantitatively.

Heptafluorobutyrate esters

The acylation of menthol stereoisomers with HFBI, at 60°, leads to HFB derivatives which were separated on 1 % OV-1 liquid phase under isothermal conditions. Their separation is illustrated in Fig. 9 and should be compared with the separation of the TMSi derivatives shown in Fig. 1.

The HFB derivatives of menthol stereoisomers may be detected at the nanogram level by electron capture techniques.

The acylation of menthoglycol and neomenthoglycol with HFBA gives monoesters Ii, IIi and di-esters Ij, IIj. The chromatogram shown in Fig. 8 shows the separation of all the menthoglycol and neomenthoglycol derivatives prepared in this study: the HFB esters are the most volatile of all these derivatives. The rate of conversion to di-HFB derivatives for the *cis* isomer is greater than that for the *trans* isomer: in Fig. 8, after the same reaction period, we observe a proportion of the *cis* di-HFB

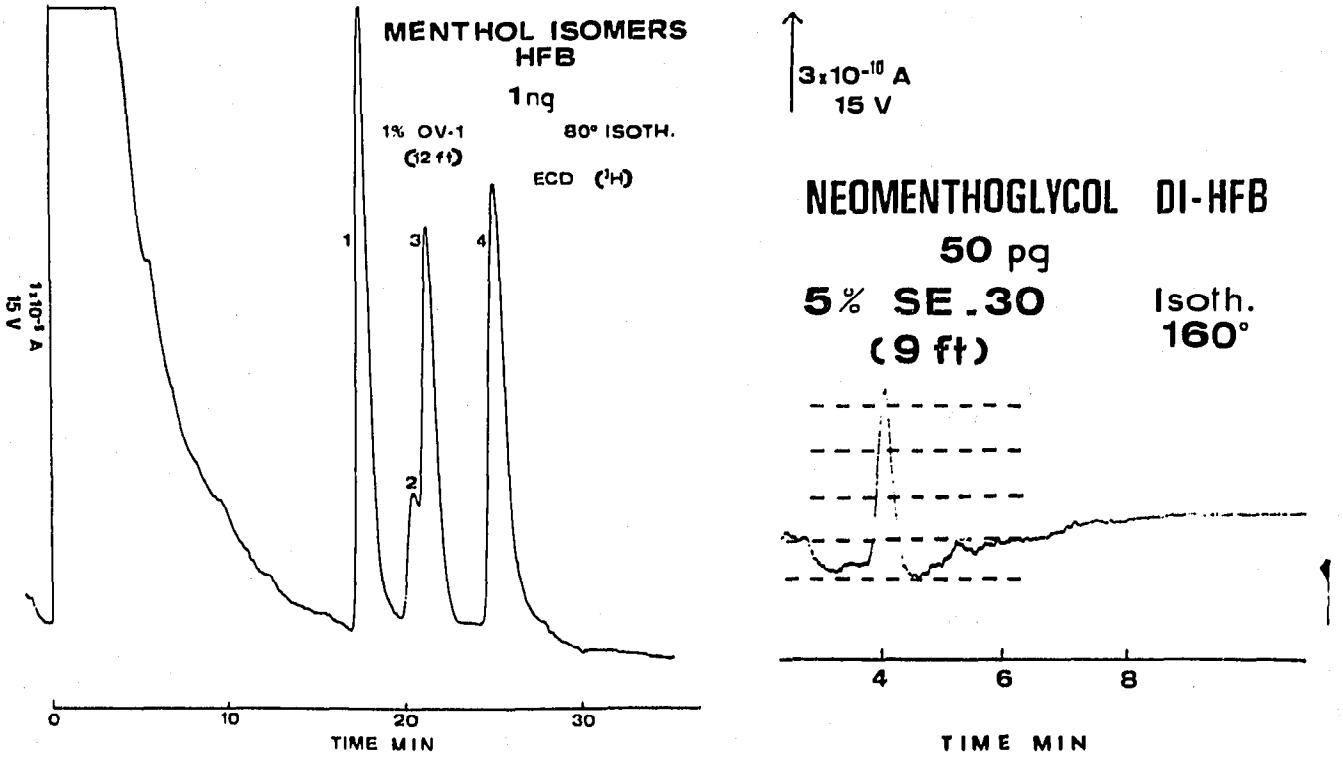

Fig. 9. Detection at nanogram level of neomenthol HFB (1), isomenthol HFB (2), menthol HFB (3) and neoisomenthol HFB (4). The separation of these HFB derivatives should be compared with the separation shown in Fig. 1. Reaction conditions: HFB derivatives were prepared with HFBI, at 60°. The reaction period was 30 min.

Fig. 10. Detection of 50 picograms of di-HFB derivative of neomenthoglycol. This derivative was prepared with HFBA, at 60°, by the solution method; the vapor phase method was also used for preparing subnanogram amounts of this derivative.

derivative (I) greater than that of the *trans* di-HFB derivative (II). This result is similar to the one which was obtained with the menthoglycol or neomenthoglycol di-silylation.

The structure of menthoglycol HFB derivatives was verified by mass spectrometry. The ion fragments providing structural information include those which are given in Table II. The di-HFB esters of menthoglycols may be detected at the subnanogram level. As it is shown in Fig. 10, the ratio signal/noise for 50 pg of compound is equal to 4.

TABLE II

PEAKS PROVIDING STRUCTURAL INFORMATION IN MASS SPECTRA OF MENTHOGLYCOL OR NEOMENTHOGLYCOL HFB DERIVATIVES

Peaks	*Ions a.m.u. values*		
	M+	*Base peak*	*Other peaks*
IIi (3-mono-HFB)	absent	41 (a-18)	169 (CF_2 CF_2 CF_3), 136 (M − 214 − 18 = M − HOOC CF_2 CF_2 CF_3 − H_2O), 119 (CF_2 CF_3), 81, 69 (CF_3)
IIj (3,8-di-HFB)	564 weak	81 cyclohexene ion	351 (M - 213 = M − OOC CF_2 CF_2 CF_3), 350 (M − 214), 255 (a), 169, 136 (M − 2 × 214), 119, 81, 69.

CONCLUSIONS

The use of the TMSi derivatives allows us to obtain a better separation of the four menthol stereoisomers than the one obtained generally with the alcohols on a polar column. This separation is carried out with an SE-30 non-polar column with 5 % loading of liquid phase, the temperature column being about 100°; and it permits the characterization of the menthol isomers in mint oil.

For menthoglycols, two levels of silylation have been defined. With the use of BSA or BSTFA, mono-TMSi derivatives are obtained; practically, these conditions are unsatisfactory, each isomer giving two derivatives, 3 and 8 mono-TMSi ethers. On the other hand, the use of catalysed conditions, at 80°, gives only one peak for each isomer: the 3,8-di-TMSi ether.

Menthoglycol and neomenthoglycol cyclic boronate formation is easy and gives stable derivatives with excellent chromatographic properties.

HFB derivatives of menthol stereoisomers, menthoglycol and neomenthoglycol may be detected at the subnanogram level with an electron capture GLC detector.

All these derivatives are suitable for the analysis by combined gas chromatography–mass spectrometry.

These methods may be applied to qualitative, quantitative and structural

analysis of terpene alcohols in perfumes and essential oils. They allow pharmacodynamic and biosynthesis studies of small amounts of terpene alcohols.

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