Notes

CHROM. 5140

Analysis of some hydroxy fatty compounds as their trimethylsilyl ethers by gas–liquid chromatography

In the gas–liquid chromatography (GLC) analysis of hydroxy fatty esters either as such or as their acetates, their high polarity and low volatility gave rise to long retention times and thus made quantitation somewhat unsatisfactory. The introduction of trimethylsilyl (TMS) ethers has allowed the analysis of a large number of these acids. Thus WOOD *et al.*[1] reported good resolutions of mono- and diglycerides as their TMS ethers. The retention times of various ricinoleic acid derivatives showed that the TMS derivative eluted approximately four times faster than the acetylated derivative and five times faster than the methyl ester[2]. A comparison of the GLC resolution of diastereoisomeric polyhydroxystearates as TMS and trifluoroacetyl derivatives revealed that the trifluoroacetyl derivatives were more useful for the analysis of hydroxy derivatives especially for resolving *erythro* and *threo* isomers[3]. There are a few more reports on the analysis of fatty alcohols as their TMS ethers[4,5]. In continuation of our work on the GLC of TMS ethers of hydroxy compounds[6], we now report the analysis of some long-chain hydroxy fatty acids.

Experimental

The hydroxy derivatives were prepared by established procedures of hydroxylation, lithium aluminium hydride and catalytic reductions, etc.[7]. The procedure of WOOD *et al.*[1] was employed for silylation. Thus the hydroxy ester (20 mg) taken in pyridine (0.5 ml) was treated with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml). After a reaction time of 30 sec during which the contents were intimately mixed, the reaction mixture was kept for 5 min. After addition of 5 ml of water, the silylated mixture was extracted with light petrol (b.p. 40–60°). The petroleum ether extracts were washed with water and dried, and solvent was removed on a steam bath. GLC of this material was carried out in an F & M model 1609 GLC unit provided with a flame ionisation detector. A 3% SE-30, silicone gum rubber, packed with Chromosorb W, 45–60 mesh, in a 2 ft. × 3/16 in. stainless-steel column was used. Other conditions of analysis were: hydrogen flow rate, 40 ml/min; nitrogen flow rate, 100 ml/min; air, 300 ml/min; injection port temp., 310°; block temp., 300°; attenuation, 1600; and chart speed, 4 min/in. Programming was carried out for the various TMS derivatives of different chain lengths from temperatures ranging from 130 to 220° at 5°/min.

Results and discussion

Table I gives the elution temperatures and retention times of various hydroxy fatty compounds analysed as their TMS ethers. Undecanol, undecanediol and undecanetriol had progressively higher retention temperatures and were well separated from each other. Among the C_{18} monohydroxystearates, the 2-hydroxy ester emerged first from the column followed by 9(10)-hydroxy and 12-hydroxy esters. But 18-hydroxystearate which carries a primary hydroxyl group is distinctly separable from

TABLE I

RETENTION TIMES OF VARIOUS TMS ETHERS OF HYDROXY FATTY COMPOUNDS

TMS ether	*Elution temp. (°C)*	*Absolute retention time (min)*
Temp. programming from 160 to 180° at 5°/min		
Methyl 2-hydroxystearate	183	4.8
Methyl 9(10)-hydroxystearate	185	5.0
Methyl 12-hydroxystearate	186	5.2
Methyl 18-hydroxystearate	192	6.4
Temp. programming from 180 to 220° at 5°/min		
Methyl *threo*-9,10-dihydroxystearate	195	3.0
Methyl *erythro*-9,10-dihydroxystearate	196	3.2
Methyl 9,10,12,13-tetrahydroxystearate	206	5.2
Methyl 13(14)-hydroxydocosanoate	209	5.8
Methyl 2-hydroxydocosanoate	209	5.8
Methyl *erythro*-2,3-dihydroxydocosanoate	212	6.6
Methyl *erythro*-13,14-dihydroxydocosanoate	214	6.8
Methyl *threo*-13,14-dihydroxydocosanoate	214	6.8
1,13,14-Docosanetriol	220	8.0
Temp. programming from 130 to 180° at 5°/min		
Undecanol	142	2.4
1(10),11-Undecanediol	158	5.6
1,10,11-Undecanetriol	168	7.6

compounds carrying a secondary hydroxyl group and has a longer retention time. The TMS derivatives of positional isomers obtained by catalytic hydrogenation of *cis*-9,10-epoxystearic acid, *viz.*, 9- and 10-hydroxystearates, were not resolvable from each other as has been earlier observed for acetoxy analogues by TULLOCH[8].

Among the di- and polyhydroxy derivatives, the *threo* and *erythro* isomers are not separable. Tetrahydroxy derivatives had a longer retention time (5.2 min) than the dihydroxystearates (3.2 min). Similarly the hydroxy compounds of C_{22} series had higher elution temperatures than those of the C_{18} and C_{11} derivatives. The *threo*- and *erythro*-13,14-dihydroxydocosanoates were not separable and had higher retention times than the corresponding 2,3-dihydroxy isomers (2,3-dihydroxy, 6.6 min; 13,14-dihydroxy, 6.8 min). 1,13,14-Docosanetriol had the highest retention time (8.0 min) of all the compounds of C_{22} series analysed by GLC. Analysis on a polyester column was attempted to separate the stereoisomers but could not be used due to the bleeding of the stationary phase from the column at temperatures above 210°.

The silylation technique for the analysis of hydroxy isomers was used in the identification of the formoxylation product of *trans*-2-docosenoic acid. As the 2- and 3-hydroxy groups are in closer proximity to carboxyl than in other centrally located hydroxy fatty acids, the former pair were separated. Thus GLC analysis of the formoxylated product after methylation and silylation showed it to consist of 78% of 3-hydroxy- and 22% of 2-hydroxydocosanoic acids, respectively[7].

Conclusions

Hydroxy compounds with C_{11}, C_{18} and C_{22} chain lengths show significant differences in their retention times and are easily separable.

Mono-, di-, tri- and tetrahydroxy esters can be separated, their retention times increasing in the order stated. However *threo* and *erythro* isomers are not separable.

Methyl 18-hydroxystearate has a longer retention time than either the 12-hydroxy or 9(10)-hydroxy isomers and is separable from them.

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Received October 22nd, 1970

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