CHROM. 6010

GAS CHROMATOGRAPHY OF SULPHONYL TRIMETHYL SILYL ESTER DERIVATIVES OF PHENOLIC ACIDS

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SUMMARY

The sulphonyl trimethyl silyl ester derivatives of a group of related 4-hydroxyphenyl and 3,4-dihydroxyphenyl compounds were prepared. The gas chromatography of these was studied using stationary phases of increasing polarity and their behaviour was compared, with that of the trimethyl silyl ether/esters of the same compounds using their respective methylene unit values. It was shown that as the polarity of the phase increases the differentiation of the two types of derivative becomes greater. It should be possible to achieve a more selective separation of particular phenolic acids by careful choice of phase polarity.

INTRODUCTION

Interest in the metabolism of phenylalanine and tyrosine together with the excretion of associated metabolites in the urine[1-4] has brought about a more critical examination of the techniques available for the determination of these phenolic acids. To this end, gas chromatography appears to be uniquely suited and much work has been done in the development of general and more specific methods. This has revealed a conflict in approach and technique. The more specific methods devised for the important phenolic acids[5,6] require more complicated preparative techniques and formation of more sophisticated derivatives. These are not well suited to routine use in the analysis of large numbers of specimens. The more general techniques involve simple extraction and derivative formation. Of these the most convenient for routine use has been that involving the preparation and chromatography of the trimethyl silyl (TMS) ether/esters[7,8]. These suffer from the disadvantage of being much less specific. Using the OV-1 stationary phase (as with the SE-52 phase used by KAROUM *et al.*[7]) the peak due to vanilmandelic acid was sometimes obscured by that due to hippuric, homogentisic, 4-hydroxyphenyllactic and 3,4-dihydroxyphenylacetic acids. Although use of temperature programming increases the resolution it is still not completely satisfactory. Use of the methyl ester TMS ether derivatives was suggested but this necessitates the use of diazomethane which is both explosive and carcinogenic[9]. Thus it was thought to be unsuitable for routine use. Application

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TABLE I

MU VALUES OF TMS ETHER/ESTER AND SULPHONYL TMS ESTER DERIVATIVES OF PHENOLIC ACIDS

Gas chromatographic conditions as in the text.

Compound	*5% OV-1 phase*		*5% OV-17 phase*		*5% OV-210 phase*	
	TMS ether/ester derivative	*Sulphonyl TMS ester derivative*	*TMS ether/ester derivative*	*Sulphonyl TMS ester derivative*	*TMS ether/ester derivative*	*Sulphonyl TMS ester derivative*
Homovanillic acid	17.50	17.95	19.50	21.00	20.75	24.45
3,4-Dihydroxyphenylacetic acid	18.25	19.00	19.50	22.30	20.90	27.78
subsid. peak		18.75		20.95		24.60
4-Hydroxyphenyllactic acid	19.15	19.50	19.95	21.20	21.65	24.05
Vanilmandelic acid	18.70	19.15	19.95	21.75	21.45	26.25
3,4-Dihydroxymandelic acid	19.20	20.15	20.00	23.65	21.20	28.90
subsid. peak		19.75		21.40		25.80
subsid. peak						24.10
Ferulic acid	20.25	20.80	22.85	24.05	25.00	28.15
Caffeic acid	20.75	22.05	22.50	25.15	24.80	34.30
subsid. peak		21.45		24.10		29.85
subsid. peak						28.05

of the more polar OV-17 phase was reported to allow specific estimation of vanilmandelic and homovanillic acids[10]. While discrete peaks were obtained it was shown that these were contaminated by other substances which could be separated by thin-layer chromatography and electrophoresis (Fig. 1). It therefore became apparent that advantage could be gained from the use of a simple yet more specific derivative which would allow the application of more selective gas chromatography. For this purpose sulphonation of the phenolic acids prior to TMS ester formation was attempted.

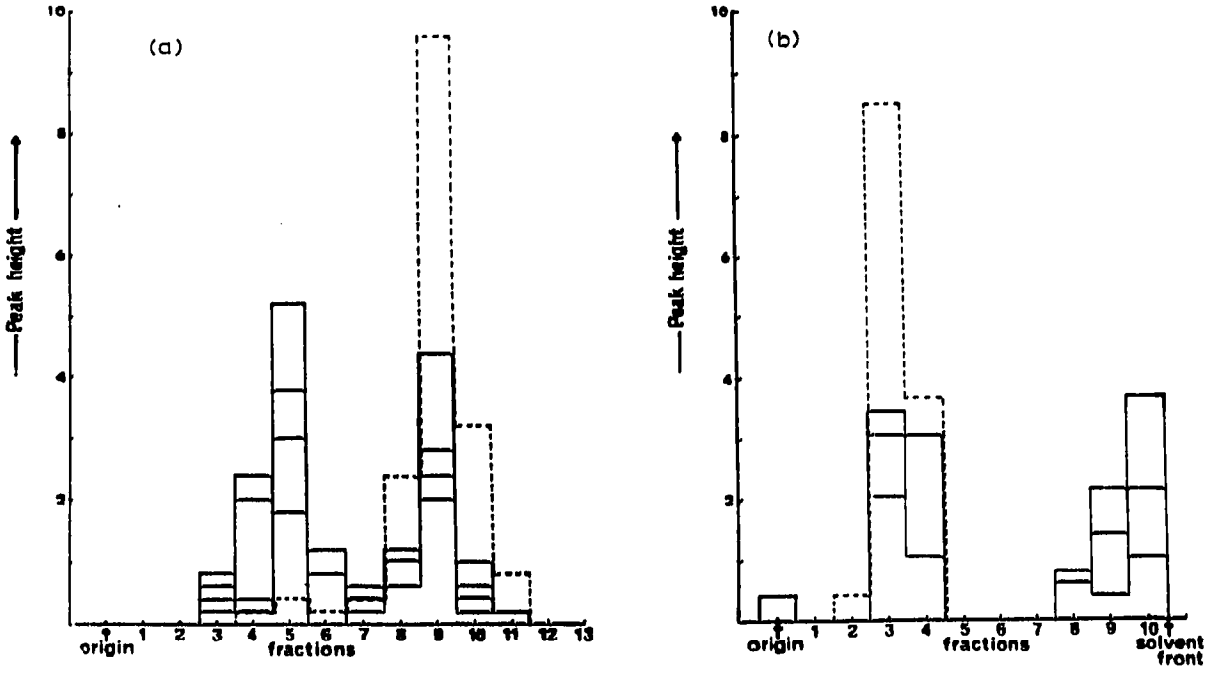

Fig. 1. The separation of vanilmandelic acid from its contaminant by (a) thin-layer electrophoresis at pH 3.9 (pyridine–glacial acetic acid–water as buffer) for 2 h using 600 V and (b) thin-layer chromatography using isopropanol–ammonia–water (8:1:1) as solvent. Urine was extracted[10], applied to the thin-layer plate and run as described. The plate was divided up and eluted in sections using 0.1 *N* HCl, this being extracted directly with diethyl ether. After evaporation of the organic phase, the residue was treated with BSTFA and gas chromatographed on OV-17. The peak height refers to peaks found on chromatography of the urine extract (————) which manifest the same retention time as that of a vanilmandelic acid standard treated similarly (– – – –).

METHOD

A few milligrams of each reference compound, as its free acid, was dissolved in 2 ml of ethyl acetate and to it was added 0.3 ml of chlorosulphonic acid. The reaction was incubated at 60° for 5 min. The solution was cooled and 2 ml of water were added slowly to it. The organic phase was increased by addition of a further 3 ml of ethyl acetate and extraction was performed. This was repeated using a further 2 × 3 ml of solvent. The ethyl acetate was pooled and re-extracted with 2 × 2 ml of 1 *M* sodium carbonate. The aqueous phases were pooled and made acid by addition of 5 *M* HCl. Extraction was then performed with 3 × 4 ml diethyl ether. This was dried with anhydrous sodium sulphate and evaporated to dryness. To the residue was added 0.1 ml of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) followed by incubation at 60° for 10 min. Of this solution, 5 μl was injected directly into the gas chromatograph.

The derivatives were chromatographed using OV-1, OV-17 and OV-210 phases prepared as 5 % w/w on 100–120 mesh Chromosorb G (acid washed, dimethylchlorosilane treated) with nitrogen carrier gas flow rate 40 ml/min. The OV-1 separation was achieved at 215°, the OV-17 at 225° and the OV-210 at 230°. The instrument used was a Pye Series 104 gas chromatograph fitted with 5-ft. glass columns and a flame ionisation detector. The column oven was operated at 350° and the flash heater at maximum. The ionisation amplifier attenuation was 2×10^3.

The prepared colums were calibrated to allow the determination of methylene unit (MU) values by the use of a number of straight chain hydrocarbons (C_{18}–C_{30} depending on the column) chromatographed under the same conditions as the derivatives.

RESULTS AND DISCUSSION

The sulphonyl derivatives of the phenolic acids were prepared by reaction with chlorosulphonic acid. This was performed at 60°. The optimum time of incubation was determined for vanilmandelic acid and 3,4-dihydroxyphenylacetic acid as representatives of the group. It was found that after 5 min the reactions had reached 98 % and 92 % of completion (Fig. 2).

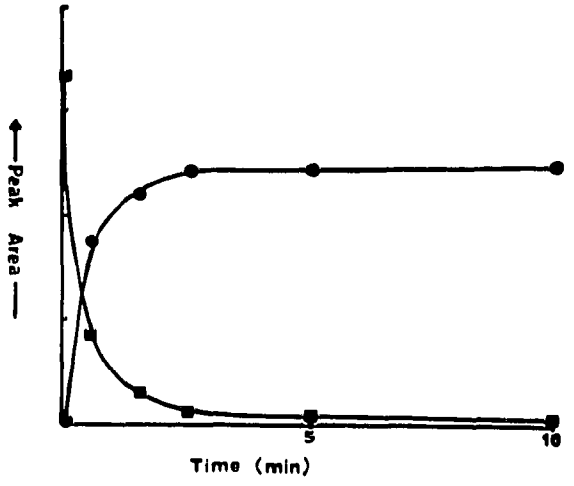

Fig. 2. Graph showing the rate of sulphonation of vanilmandelic acid using chlorosulphonic acid under the conditions stated in the text. After reaction the solutions were extracted, treated with BSTFA and chromatographed on 5% OV-17 as described. ■, TMS ether/ester of vanilmandelic acid; ●, sulphonyl TMS ester of vanilmandelic acid.

Each of the derivatives was chromatographed on its own. Then in each case original phenolic acid was added and after a further incubation the samples were rerun. Specimens of these chromatograms are shown in Fig. 3.

The MU values for the sulphonyl TMS ester derivatives and their corresponding TMS ether/esters were determined[11] using methyl silicone OV-1, phenyl methyl silicone OV-17 and trifluoropropyl methyl silicone OV-210 stationary phases. The results are shown in Table I. With all the phases the 4-hydroxy-3-methoxy parent compounds formed derivatives which yielded one single peak even when the incubation time was reduced to give incomplete reaction. With the 3,4-dihydroxy parent

compounds the shorter incubation gave rise to subsidiary peaks. With the OV-1 and OV-17 phases one subsidiary peak was found in each case, while with the OV-210 phase 3,4-dihydroxymandelic acid and caffeic acid gave two subsidiary peaks. The 3,4-dihydroxyphenylacetic acid gave only one peak.

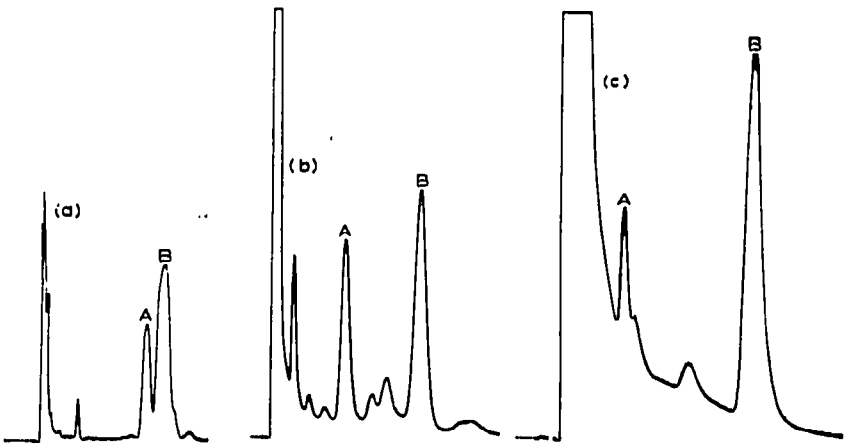

Fig. 3. Chromatograms showing the separation of the TMS ether/ester (A) and sulphonyl TMS ester (B) of vanilmandelic acid on (a) 5% OV-1, (b) 5% OV-17 and (c) 5% OV-210.

The results suggest that a single sulphonyl group is introduced in the case of the 4-hydroxy-3-methoxy compounds and that two such groups are introduced in the case of the 3,4-dihydroxy compounds. If with this second group of compounds incubation is incomplete the subsidiary peak appears representing the introduction of a single sulphonyl group. This is supported by calculation of the average relative

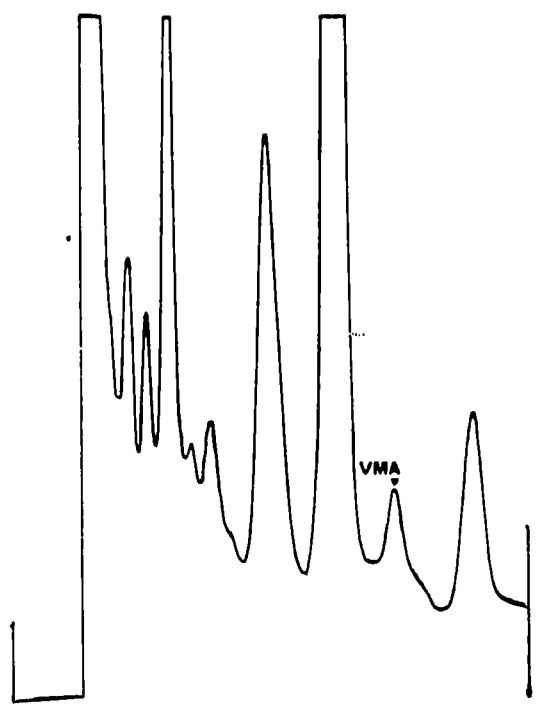

Fig. 4. Chromatogram of ethyl acetate extract of acidified normal urine, sulphonyl TMS ester derivative prepared as described, on 5% OV-17 at 220° showing the discrete peak due to vanilmandelic acid.

increase in MU values on formation of the sulphonyl TMS ester over the TMS ether/ester of the same compound. This is 0.49 and 1.48, equivalent to the introduction of one sulphonyl group with OV-1 and OV-17 phases respectively and 1.00 and 3.03 for the similar introduction of two sulphonyl groups. In the case of the OV-210 phase, separation of two subsidiary peaks is accounted for as introduction of the single sulphonyl group into either the 3 or 4 position. A similar relative increase in MU values is seen here as with the other phases, 3.95 and 8.02 for general introduction of one or two sulphonyl groups. The average difference between the 3 and 4 mono-substituted compounds on OV-210 is equivalent to 1.75 MU values.

From these results it can be seen that as the polarity of the stationary phase increases, so does the retention of the sulphonyl TMS ester as compared with the TMS ether/ester derivative. This is especially marked in the separation of the 4-hydroxy-3-methoxy compounds from their respective 3,4-dihydroxy compounds. Their ether/ester derivatives are not well separated, the separation being represented by an average difference in MU values of 0.31. Their sulphonyl ester derivatives are much better separated, this being represented by an average difference in MU values of 2.19. The use of the sulphonyl TMS ester derivatives therefore allows the better separation of these closely related compounds. It should be possible to choose a phase with much greater selectivity for particular phenolic acids. This must be studied further. The separation of the components of an ethyl acetate extract of an acidified normal urine of which sulphonyl TMS ester derivatives have been prepared is shown in Fig. 4. The position of the sulphonyl TMS ester of vanilmandelic acid has been identified and shows discrete separation from the other components. Thus it is hoped that this approach will provide a more specific way of estimating phenolic acids by gas chromatography.

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