THE GAS–LIQUID CHROMATOGRAPHIC BEHAVIOR OF SOME CLOSELY RELATED DISUBSTITUTED PHENYLACETIC ACIDS

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In connection with the development of a gas–liquid chromatographic (GLC) method for the determination of 3,4-dimethoxyphenylacetic acid in human urine[1]* the GLC behavior of this and a number of closely related acids (as their methyl esters) and certain of their derivatives has been investigated with two different stationary phases: F-60-Z, a slightly polar two-component combination phase[9], and the polar polyester NGA[10]**. Table I gives the retention data for 3,4-dimethoxyphenylacetic acid, the isomeric compounds 3-methoxy-4-hydroxy- and 3-hydroxy-4-methoxyphenylacetic acid, and for their acetyl, trifluoroacetyl and trimethylsilyl ether derivatives (as the methyl esters). With F-60-Z an excellent differentiation between the isomeric methoxy phenols is obtained (separation factor 3-OH:4-OH of 1.22). The higher molecular weight but less polar dimethoxy compound is eluted at an intermediate rate, a reflection of the low degree of polarity of this phase. With the more polar NGA 3,4-dimethoxyphenylacetic acid is eluted faster than either of the isomeric methoxyphenoxy compounds, as would be expected (see Fig. 1). The isomer separation factor is 1.26, with the 3-hydroxy-4-methoxy isomer again eluted more slowly. When a methoxy group is introduced into the 3-position of 4-methoxyphenylacetic acid the retention time (NGA) is increased approximately three-fold (see Table II). Under the same conditions the retention time of 4-hydroxyphenylacetic acid is more than twice that of 3,4-dimethoxyphenylacetic acid. The introduction of a methoxy group into the 3-position (to give 3-methoxy-4-hydroxyphenylacetic acid), however, instead of leading to a considerable increase in retention time*** actually results in a significant drop in retention time, even though molecular weight has been increased by thirty units and the compound still possesses a phenol group. This apparently anomalous result may be explained on the basis of hydrogen bonding between the phenol group at the 4-position and the oxygen of the methoxy group. The phenolic hydroxyl group is now less available for interaction with the stationary phase, and this decrease in

* This aromatic acid would be expected to result from the action of monamine oxidase upon 3,4-dimethoxyphenylethylamine[2,3], a compound suggested to be related to abnormal mental behavior and shown by a number of workers to be present in the urine of schizophrenic individuals[3–8].

** For a comprehensive review of the GLC of aromatic acids see reference[11].

*** From Table II it is seen that the 'retention factor'[12] for the introduction of a methoxy group into the 3-position of the aromatic ring ($f_{3\text{-}OMe}$) can be estimated to be approximately three and the retention factor for the introduction of a hydroxy group into the 4-position of phenylacetic acid ($f_{4\text{-}OH}$) is twenty-seven. The presence of both of these groups on the aromatic ring of phenylacetic acid (to yield 3-methoxy-4-hydroxyphenylacetic acid) would thus be expected to increase its retention time by a factor of eighty, or to six or seven times that of 3,4-dimethoxyphenylacetic acid.

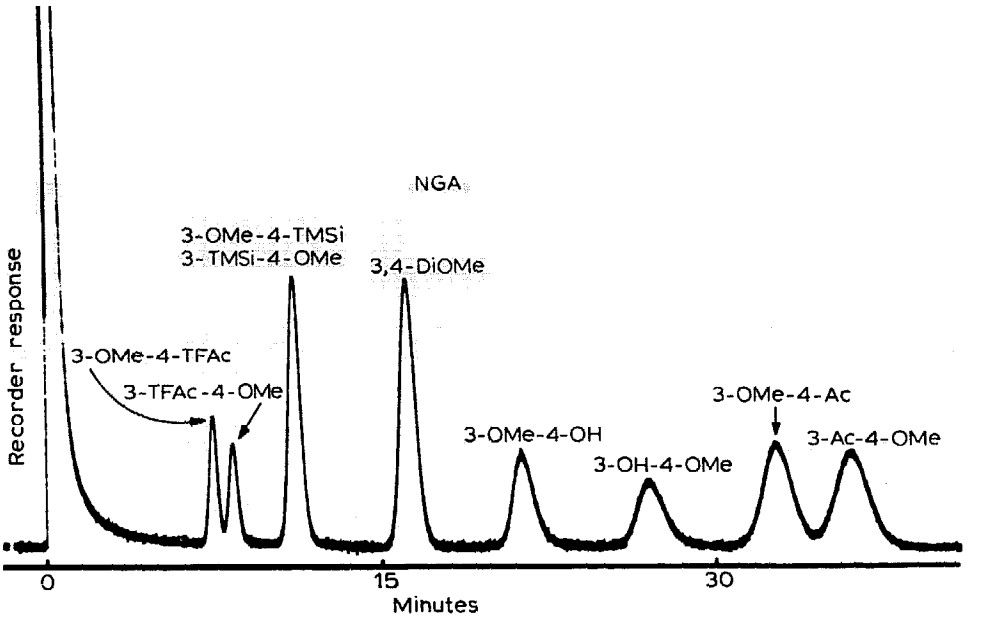

Fig. 1. Gas–liquid chromatographic separation of the methyl esters of a mixture of nine closely related 3,4-disubstituted phenylacetic acids with NGA illustrating the influence of positional isomerism and substituent groups upon retention behavior. The compounds (methylyl esters) are 3-methoxy-4-trifluoroacetoxyphenylacetic acid (3-OMe-4-TFAc), 3-trifluoroacetoxy-4-methoxyphenylacetic acid (3-TFAc-4-OMe), 3-methoxy-4-trimethylsilyloxy- and 3-trimethylsilyloxy-4-methoxyphenylacetic acids (3-OMe-4-TMSi and 3-TMSi-4-OMe, respectively), 3,4-dimethoxyphenylacetic acid (3,4-DiOMe), 3-methoxy-4-hydroxyphenylacetic acid (3-OMe-4-OH), 3-hydroxy-4-methoxyphenylacetic acid (3-OH-4-OMe), 3-methoxy-4-acetoxyphenylacetic acid (3-OMe-4-Ac) and 3-acetoxy-4-methoxyphenylacetic acid (3-Ac-4-OMe). Column conditions are given in Table I.

solute–solvent interaction results in a decrease in retention time. With F-60-Z the introduction of the 3-methoxy group into 4-hydroxyphenylacetic acid does result in a small increase in retention time, but the increase is much less than would be expected from comparison with the increase observed when a similar transformation is carried out on 4-methoxyphenylacetic acid. This can again be explained on the basis of intramolecular hydrogen bonding.

A possible explanation for the isomer separation pattern observed for the methoxyphenols is as follows: It is possible to correlate (by use of the Hammett expression or related approaches) the rates of reaction of *meta*- and *para*-substituted phenyl compounds, such as benzoic acids and methyl benzoates. This is because the electronic effects of substituents are conducted through the aromatic ring to the site of reaction. Correlation also exists, but to a lesser extent, for ring-substituted phenylacetic acids, indicating that increasing the number of atoms between the two interacting groups (introduction of a methylene group) decreases the electronic interaction. Because the *para*-methoxy group of methyl 3-hydroxy-4-methoxyphenylacetate should interact more strongly with the electron-attracting carbomethoxy group than does the *meta*-methoxy group with the carbomethoxy group in the isomeric compound, the latter methoxy would be expected to be more basic and thus more available for intramolecular hydrogen bonding with the neighboring phenolic hydroxyl group. On this basis it would be predicted that the phenolic hydroxyl group of methyl 3-methoxy-4-hydroxyphenylacetate should be more strongly intramolecularly hydrogen bonded than the phenolic hydroxyl group of methyl 3-hydroxy-4-methoxyphenylacetate; with

the result that the former compound should interact less strongly with the stationary phase and thus possess a shorter retention time than its positional isomer. If the basicity of the methoxy groups is indeed involved in determining the difference in retention behaviors between the two isomers, the effect should be even more dramatic with the corresponding methyl benzoates, where electronic interactions through the ring are more pronounced. The isomer separation would thus be expected to be even

TABLE I

Compound[a]	*Relative retention time*[b]	
	NGA	*F-60-Z*
3,4-Dimethoxyphenylacetic acid	1.00	1.00
3-Methoxy-4-hydroxyphenylacetic acid	1.32	0.92
3-Hydroxy-4-methoxyphenylacetic acid	1.66	1.12
3-Methoxy-4-trifluoroacetoxyphenylacetic acid	0.46	0.47
3-Trifluoroacetoxy-4-methoxyphenylacetic acid	0.52	0.50
3-Methoxy-4-trimethylsilyloxyphenylacetic acid	0.69	1.05
3-Trimethylsilyloxy-4-methoxyphenylacetic acid	0.69	1.03
3-Methoxy-4-acetoxyphenylacetic acid	2.03	1.91
3-Acetoxy-4-methoxyphenylacetic acid	2.24	2.08
3,4-Dimethoxybenzoic acid	0.80	0.80
3-Methoxy-4-hydroxybenzoic acid	0.98	0.64
3-Hydroxy-4-methoxybenzoic acid	1.55	0.90

[a] Run as the methyl esters, which were prepared by reaction (3 min) with an ethereal solution of diazomethane. Under these conditions O-methylation occurred to only a very slight extent (0.7% with 3-methoxy-4-hydroxyphenylacetic acid). The acetyl, trifluoroacetyl, and trimethylsilyl ether derivatives were prepared by allowing the parent methyl ester to react with the appropriate reagent (acetic anhydride–pyridine; trifluoroacetic anhydride–pyridine; or hexamethyldisilazane–trimethylchlorosilane) in ethyl acetate. The excess reagent was removed (nitrogen) and the derivative dissolved in a suitable volume of solvent for chromatography.

[b] Column conditions (NGA): 8% neopentylglycol succinate on 80–100 mesh Gas-Chrom P prepared according to HORNING, *et al.*[15]; 6 ft. × 4 mm glass U-tube; 170°; 12 psi. Column conditions (F-60-Z): a mixture of 4.9% F-60 (a methylsiloxane polymer containing a low percentage of *p*-chlorophenyl groups; Dow Corning Corp.) and 0.9% EGSS-Z (a copolymer from ethylene glycol, succinic acid and a methylphenyl-siloxane monomer, Applied Science Labs., Inc.) on 80–100 mesh Gas Chrom P prepared as above; 6 ft. × 4 mm glass U-tube; 150°; 10 psi. Retention times relative to that of 3,4-dimethoxyphenylacetic acid (16.5 min NGA; 12.0 min F-60-Z).

TABLE II

Compound[a]	*Relative retention time*[b]	
	NGA	*F-60-Z*
3,4-Dimethoxyphenylacetic acid	1.00	1.00
Phenylacetic acid	0.08	0.09
4-Methoxyphenylacetic acid	0.32	0.32
4-Trifluoroacetoxyphenylacetic acid	0.19	0.19
4-Trimethylsilyloxyphenylacetic acid	0.33	0.50
4-Acetoxyphenylacetic acid	0.86	0.76
4-Hydroxyphenylacetic acid	2.16	2.85

[a] Run as the methyl esters which were prepared as described in Table I. The preparation of the derivatized esters was also as described in Table I.

[b] Column conditions same as in Table I.

greater for these compounds, and, as can be observed in Table I, the separation factors are indeed considerably increased: to 1.58 with NGA and 1.41 with F-60-Z.

Derivative formation is widely used in GLC to increase the volatility of solutes, especially of hydroxy-substituted compounds with polar stationary phases[13]. Some typical changes in retention time when a polar group is converted to a less polar group are given in Table II. With NGA trifluoroacetylation leads to a ten-fold decrease in retention time, whereas methylation and trimethylsilylation reduce retention by a factor of roughly one-sixth. Acetylation leads to the smallest increase in volatility*. The decreases in retention time with the less polar F-60-Z are not as dramatic. Acetylation leads to only a small change in volatility, indicating that the decrease in polarity of the solute does not lead to a great enough decrease in solute-solvent interaction to more than slightly offset the increase in molecular weight. The trimethylsilyl ether is retained longer than the methyl ether (due to the molecular weight difference), and the greater volatility of the trifluoroacetate is again evident.

When a methoxy group is *ortho* to the *p*-hydroxy group, however, the magnitude of the change in volatility resulting from derivatization of the latter group is reduced. One may say that in 3-methoxy-4-hydroxyphenylacetic acid the phenolic hydroxyl group is already in a sense derivatized (tied up by hydrogen bonding), and acylation or etherification reduces the power of this phenol to bond with the stationary phase far less than do such transformations of the phenol group of 4-hydroxyphenylacetic acid. Trifluoroacetylation still leads to a decrease in retention time with both phases, but trimethylsilyl ether formation leads to an increase in volatility only with NGA (see Fig. 1); with F-60-Z the reduction in polarity brought about by this reaction is less of a factor in the determination of volatility than is the increase in molecular weight. Acetylation leads to an increase in retention time with both stationary phases.

It is clear from the data presented in Table I that for the isomeric pairs the general retention time relationship is 3-OMe-4-X < 3-X-4-OMe, where X is the substituent group at the 4-position**. Derivative formation is often employed in steroid GLC to increase differences in volatility between closely related and isomeric compounds[13,15]. Just the opposite is observed with the isomeric 3,4-disubstituted phenylacetic acids, however, for with these compounds the greatest separation is found with the underivatized methoxyphenols. Derivatization eliminates the possibility of intramolecular hydrogen bonding; separation decreases upon acylation, and trimethylsilylation leads to identical retention behavior with NGA (see Fig. 1) and an actual reversal of the 3-OMe-4-X < 3-X-4-OMe generalization with F-60-Z.

SUMMARY

The gas–liquid chromatography of a number of aromatic acids (as their methyl esters) was investigated with two stationary phases in order to study the effect of positional isomerism and substituent groups upon retention behavior. In the case of 3-methoxy-4-hydroxy- and 3-hydroxy-4-methoxy-substituted compounds the isomer separation pattern observed is held to be due to intramolecular hydrogen bonding.

* Fluoroamides and fluoroesters are known for their strikingly high volatilities when compared to the corresponding nonfluorinated derivatives[14].

** The same relationship is found for the two isomeric aromatic amine derivatives 3-methoxy-4-trifluoroacetoxy- and 3-trifluoroacetoxy-4-methoxy-N-trifluoroacetylphenylethylamine (separation factor 1.08 with both stationary phases).

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