

CHROM. 5841

PERFLUOROBENZENE DERIVATIVES AS DERIVATISING AGENTS FOR THE GAS CHROMATOGRAPHY OF PRIMARY AND SECONDARY AMINES USING ELECTRON CAPTURE DETECTION

A. C. MOFFAT* AND E. C. HORNING

Institute for Lipid Research, Baylor College of Medicine, Houston, Texas 77025 (U.S.A.)

AND

S. B. MATIN AND M. ROWLAND

School of Pharmacy, University of California, San Francisco, Calif. 94122 (U.S.A.)

(Received November 15th, 1971)

SUMMARY

A variety of perfluorobenzene derivatives of phenethylamine and N-methylphenethylamine were prepared and examined by gas chromatography using an electron capture detector and by gas chromatographic-mass spectrometric techniques. The structural requirement for good electron capturing properties was found to be the presence of a double bond between nitrogen or oxygen and the carbon atom adjacent to the perfluoroaromatic ring. The best derivatising agent for secondary amines was pentafluorobenzoyl chloride; for primary amines the choice was between pentafluorobenzaldehyde and pentafluorobenzoyl chloride.

INTRODUCTION

Recent studies in this laboratory^{1,2} have shown that pentafluorobenzaldehyde-amine condensation products have good gas chromatographic (GC) properties and are highly electron capturing molecules. Their sensitivity of detection by the electron capture detector is approximately 2×10^3 times that obtained with a flame ionization detector. Amounts of the amines down to 10 pg could easily be quantified. Similar results have been obtained by ZLATKIS AND PETTITT³, WILKINSON⁴, SALMON⁵ and MATIN AND ROWLAND⁶ using the pentafluorobenzoyl derivatives of a variety of amines. It is supposedly the perfluorophenyl system that confers this great electron capturing property to the molecule. We have therefore compared various reagents containing the perfluorophenyl nucleus to find the most suitable derivatising agents for primary and secondary amines for their detection and estimation by GC with electron capture detection.

* Present address: Home Office Central Research Establishment, Aldermaston, Reading, Berkshire, RG7 4PN, Great Britain.

EXPERIMENTAL

Reagents

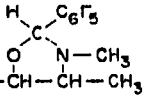
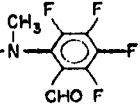
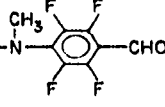
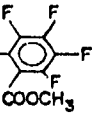
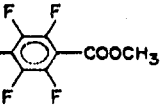
β -Phenethylamine was supplied by Pierce Chemical Company, N-methyl- β -phenethylamine by K & K Laboratories and *d*-pseudoephedrine by Sigma Chemical Company. All the fluorinated reagents were obtained from Peninsular Chemresearch (methyl pentafluorobenzoate was formed by the treatment of pentafluorobenzoic acid with diazomethane).

Preparation of derivatives

1 mg of amine was added to 0.2 ml of acetonitrile and 0.1 ml of reagent (or 20 mg of solid reagent in 0.1 ml of acetonitrile) in a small screw-capped (Teflon-lined) vial. The reagents were: pentafluorobenzaldehyde (with phenethylamine to give compound I (Table I), with N-methylphenethylamine to give VII and VIII, and with

TABLE I

METHYLENE UNIT (*MU*) VALUES AND RELATIVE ELECTRON CAPTURE DETECTION SENSITIVITIES OF SOME PERFLUOROBENZENE DERIVATIVES OF PHENETHYLAMINE AND N-METHYLPHENETHYLAMINE

Compound	<i>MU</i> value ^a	Sensitivity ^b
No. Structure		
I Ph-CH ₂ -CH ₂ -N=CH-C ₆ F ₅	16.81	100
II Ph-CH ₂ -CH ₂ -NH-C(=O)-C ₆ F ₅	18.81	78
III Ph-CH ₂ -CH ₂ -NH-C(=O)-CH ₂ -C ₆ F ₅	19.69	11
IV Ph-CH ₂ -CH ₂ -NH-CH ₂ -C ₆ F ₅	16.81	4.1
V Ph-CH ₂ -CH ₂ -N $\begin{cases} \text{CH}_2\text{-C}_6\text{F}_5 \\ \text{CH}_2\text{-C}_6\text{F}_5 \end{cases}$	21.38	34
VI 	18.45	3.0
VII Ph-CH ₂ -CH ₂ -N 	18.65	47
VIII Ph-CH ₂ -CH ₂ -N 	20.31	24
IX Ph-CH ₂ -CH ₂ -NH 	19.78	53
X Ph-CH ₂ -CH ₂ -NH 	21.34	22

^a 12 ft. \times 4 mm I.D. 5% SE-30.

^b Relative to compound I (on a C/mole basis).

pseudoephedrine to give VI), pentafluorobenzoyl chloride (with phenethylamine to give II), pentafluorophenylacetyl chloride (with phenethylamine to give III), pentafluorobenzyl bromide (with phenethylamine to give IV and V), and methyl pentafluorobenzoate (with phenethylamine to give IX and X). After 1 h at 60°, the mixture was diluted to 1 ml and 1 μ l was analyzed by GC (flame ionization detection) and GC-MS procedures. The solution was diluted with hexane for electron capture detection so that 1 μ l of the resultant solution gave a peak approximately equivalent to 10% of the detector standing current.

Gas chromatography

Analyses were made using a Barber-Colman 5000 instrument equipped with Keithley 417 picoammeters and Texas Instruments recorders. The columns were 12 ft. \times 4 mm I.D. glass W-tubes packed with 5% SE-30 on 80-100 mesh Gas-Chrom P, prepared according to the usual method of this laboratory⁷. Chromatographic conditions for flame ionization detection were: carrier (nitrogen) flow rate, 60 ml/min (column at 200°); air and hydrogen flow rates, 300 ml/min and 60 ml/min, respectively; injector zone temperature, 260°; detector temperature, 300°. Determination of methylene unit (*MU*) values⁸ were made with temperature programming from 150 to 300° at 2°/min. A ⁶³Ni (10 mCi) electron capture detector was used at 300° with a pulsed voltage (voltage, 50 V; pulse period, 300 μ sec; pulse width, 5 μ sec) and a carrier (argon-methane, 95:5) flow rate of 60 ml/min (column at 200°). Analyses were made isothermally, at temperatures so that the derivatives had retention times of 5-10 min. Electron capture detection sensitivities were calculated on a C/mole basis.

Mass spectrometry

Low resolution mass spectra were obtained using a LKB 9000 gas chromatograph-mass spectrometer with a 9 ft. \times 4 mm I.D. glass coil (1% SE-30 packing). The ion source temperature was 250°; current, 60 μ A; ionizing voltage, 70 eV; scanning time 3-6 sec. A CEC 21-110B instrument with GC inlet was used to obtain high resolution data.

RESULTS AND DISCUSSION

Structural formulae of the derivatives, their *MU* values and relative electron capture detection sensitivities are given in Table I.

When examined by combined GC-MS all the derivatives gave molecular ions with the expected *m/e* values.

The reactions of pentafluorobenzaldehyde with phenethylamine and pseudoephedrine, to give the Schiff's base (I) and the oxazolidine VI, were quantitative with the conditions used. Pentafluorobenzoyl chloride and pentafluorophenylacetyl chloride gave single products (II and III, respectively) in quantitative yield with phenethylamine.

When the pentafluorobenzyl bromide-phenethylamine reaction mixture was chromatographed, two peaks were observed (*MU* values 16.81 and 21.38). These peaks were due to N-pentafluorobenzyl and N,N-di(pentafluorobenzyl) derivatives of phenethylamine (IV and V, respectively). Each pentafluorobenzyl group added to the

phenethylamine molecule increased its *MU* value by approximately five units and *N,N*-di(pentafluorobenzyl)-phenethylamine had the largest *MU* value of all the derivatives studied. It is likely that the quaternary ammonium compound was also formed, although no evidence of this was observed. With reactions such as this (when multiple products were formed) the amount of each product present was calculated from its peak area after chromatography using flame ionization detection (assuming that the response factors were the same for each compound).

Pentafluorobenzaldehyde did not react with *N*-methylphenethylamine to give an enamine. Instead, two products were formed, with the elimination of HF in each case. The addition of methoxyamine hydrochloride to the reaction mixture increased the *MU* values of these derivatives from 18.65 and 20.31 to 19.35 and 21.04, respectively, indicating that the carbonyl functional group was still present. High resolution mass spectrometry showed the two compounds to have the same molecular formula, *viz.* $C_{16}H_{13}NOF_4$. Thus it appears that the carbonyl group of pentafluorobenzaldehyde activates the fluorine-containing ring sufficiently to allow a fluorine atom to leave. The two derivatives are the *o*- and *p*-isomers of the tertiary amine (VII and VIII). The difference in retention behaviour is probably due to hydrogen bonding of the oxygen atom of the *o*-compound (VII) to one of the hydrogen atoms attached to the methylene or methyl group on the nitrogen atom. This compound would be expected to have a shorter retention time than the corresponding *p*-isomer (VIII), which cannot form a hydrogen bond internally. Although these reactions were complete after heating for 1 h at 60°, they are much slower in rate than the condensation of the aldehyde and primary amine. Approximately 65% of the *p*-isomer was formed, reflecting the hindrance to *o*-reaction caused by the aldehyde group. Decafluorobenzophenone is even more hindered with respect to *o*-substitution because of the second ring. Only 8.3% of the *o*-isomer is formed under the same conditions (MOFFAT AND HORNING, unpublished results) and the carbonyl group of this product does not condense with methoxyamine hydrochloride even after 2 h at 80°.

Methyl pentafluorobenzoate also gave *o*- and *p*-substituted amines (IX and X). The yields were approximately the same as those for VII and VIII, and the difference in retention behaviour of the two isomers was also comparable. As might be expected from the addition of such a large group, the presence of the corresponding secondary amines was not observed.

The GC properties of all the derivatives were excellent, *e.g.*, Figs. 1 and 2. Comparison of the relative peak heights in the chromatograms clearly shows the different sensitivities of detection of the two compounds with the two detectors.

The most electron capturing of the derivatives examined was the pentafluorobenzaldehyde-phenethylamine Schiff's base (I), followed by pentafluorobenzoyl-phenethylamine (II)*. Separation of the double bond from the ring by a methylene group, as in pentafluorophenylacetylphenethylamine (III), causes a reduction in electron capturing properties by a factor of 7 (relative to II). When no double bond is present in the molecule, *e.g.*, as in IV and VI, the sensitivity of detection drops even further (to 4.1% and 3%, respectively, of that of the Schiff's base). Even when two

* While minor differences between laboratories were found in the relative electron capturing sensitivities of the pentafluorobenzamide and pentafluorobenzaldehyde-Schiff's base of a particular amine, in all cases their sensitivity was 20- to 60-fold greater than the corresponding pentafluorobenzyl derivative.

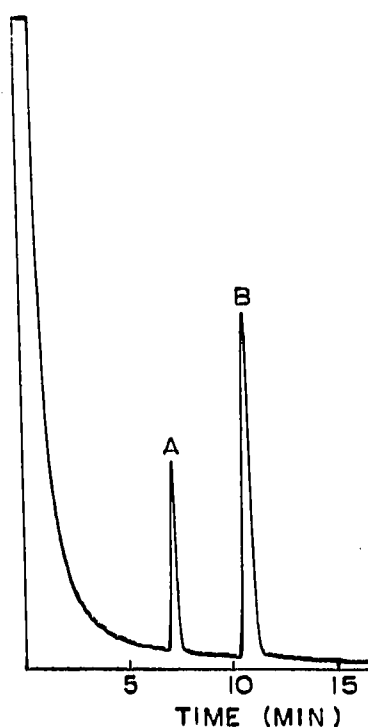


Fig. 1. Chromatogram of the reaction mixture of phenethylamine and methyl pentafluorobenzoate (equivalent to 1 μ g phenethylamine) using a 12-ft. 5% SE-30 column at 260° with flame ionization detection: A = compound IX; B = compound X.

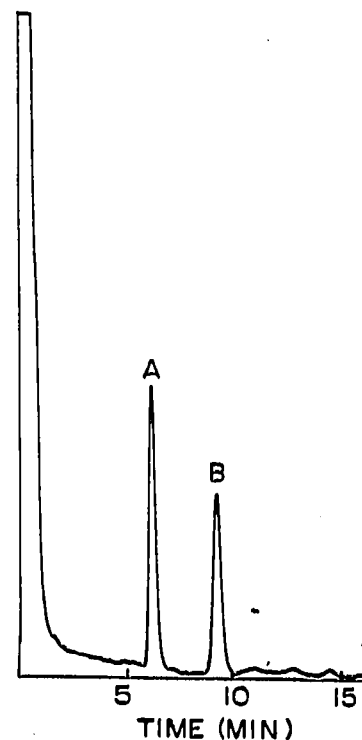


Fig. 2. Chromatogram of the reaction mixture of phenethylamine and methyl pentafluorobenzoate (equivalent to 100 pg phenethylamine) using a 12-ft. 5% SE-30 column at 260° with electron capture detection: A = compound IX; B = compound X.

pentafluorophenyl groups are in a molecule (V), although its sensitivity is increased by a factor of 8 compared to the monosubstituted amine, the sensitivity is still less than that of those compounds with a double bond on the carbon atom adjacent to the fluorine-containing ring. Thus, while the highly electronegative pentafluorophenyl group does confer some electron capture properties to the molecule, the presence of a polarizable carbon (C=O, C=N), adjacent to this aromatic ring, enhances the effect even further. This is consistent with the hypothesis that the perfluoroaromatic ring, through resonance with the polarizable carbon, provides a highly electron-delocalized system which then acts as a good electron capturing moiety. Once accepted, the electron can be stabilized in the aromatic ring or in the molecule as a whole in the excited state⁶.

Compounds VII to X still possess a polarizable carbon atom adjacent to the highly electron withdrawing tetrafluorophenyl group and therefore still retain considerable electron capture sensitivity. The reduced sensitivity of these compounds (relative to I), may be accounted for by the lone pair of electrons on the nitrogen atom feeding into the ring, thereby lowering the electron density and electron capturing potential of the system. Any factor which reduces this electron donation, such as steric hindrance produced by *ortho*-substitution (VII and IX), will enhance the electron capture response. Difference in the sensitivities of detection of various methoxy-substituted aromatic systems have been explained on similar grounds⁶.

From these results it can be seen that the presence of a perfluorinated aromatic ring in a molecule does not, in itself, give the molecule good electron capturing properties. Even two such rings in a molecule are not as effective as one which is adjacent to a C=O or C=N group. Of all the derivatives examined, the only derivatives that were formed quantitatively as single products and had good electron capturing properties were the Schiff's base from pentafluorobenzaldehyde and the pentafluorobenzoyl derivative. Pentafluorobenzaldehyde has a disadvantage as a derivatising agent in that it can condense with amines to form a variety of products, *e.g.*, Schiff's bases with primary amines, isomeric tertiary amines with secondary amines, oxazolidines with β -hydroxy secondary amines, and substituted tetrahydroisoquinolines with catecholamines². While in some cases multiple derivative formation may be useful, the best derivatising agent for detection by electron capture gas chromatography of secondary amines is pentafluorobenzoyl chloride and for primary amines the choice lies between pentafluorobenzaldehyde and pentafluorobenzoyl chloride.

ACKNOWLEDGEMENTS

The work was aided by Grant HE-05435 of the National Heart and Lung Institute, Grants GM-13901 and GM-16496 of the National Institute of General Medical Sciences and Grant Q-125 of the Robert A. Welch Foundation. We thank Miss P. F. CRAIN and Mr. K. J. LYMAN for help in obtaining the mass spectra. A.C.M. also thanks the Wellcome Trust for a travel grant.

REFERENCES

- 1 A. C. MOFFAT AND E. C. HORNING, *Anal. Lett.*, 3 (1970) 205.
- 2 A. C. MOFFAT AND E. C. HORNING, *Biochim. Biophys. Acta*, 222 (1970) 248.
- 3 A. ZLATKIS AND B. C. PETTITT, *Chromatographia*, 2 (1969) 484.
- 4 G. R. WILKINSON, *Anal. Lett.*, 3 (1970) 289.
- 5 J. A. SALMON, *Ph. D. Thesis*, University of London, 1971.
- 6 S. B. MATIN AND M. ROWLAND, *J. Pharm. Sci.*, in press.
- 7 E. C. HORNING, W. J. A. VANDENHEUVEL AND B. G. CREECH, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. XI, Interscience, New York, 1963, p. 69.
- 8 E. C. HORNING, M. G. HORNING, N. IKEKAWA, E. M. CHAMBAZ, P. I. JAAKONMAKI AND C. J. W. BROOKS, *J. Gas Chromatogr.*, 5 (1967) 283.

J. Chromatogr., 66 (1972) 255-260