A technique for the improvement of gas chromatographic properties of tertiary amines such as amitriptyline

As pointed out by VANDENHEUVEL *et al.*[6], the conversion of a compound to a less polar derivative usually improves its gas chromatographic properties. Separation of mixtures is frequently enhanced and quantitation is more straightforward, since peak tailing and irreversible adsorption are reduced. Various suitable derivatives of primary and secondary amines have been proposed but little has been reported on means to improve gas chromatographic properties of tertiary amines. A possibility that occurred to us was to remove the amino group by a Hofmann exhaustive methylation reaction prior to chromatography. FALES AND PISANO[3] refer to the potential application of an "on-column" Hofmann-type reaction for tertiary amines and METCALFE[4] analyzed a series of long-chain quaternary ammonium compounds by injection onto strongly alkaline columns. However, the peaks observed were those of tertiary amines and not the expected hydrocarbons.

The purpose of this report is to show how conversion of tertiary amines via Hofman exhaustive methylation to the corresponding olefins enhances separation of mixtures of amines and permits quantitative assay. Although the study is concerned mainly with amitriptyline (I), or 5-(3-dimethylaminopropylidene)-dibenzo-[*a*,*d*] [1,4]-cycloheptadiene, a drug of value in the treatment of mental depression, and related compounds, the technique should be useful with any tertiary amines capable of undergoing degradation to the hydrocarbons via Hofmann exhaustive methylation.

Experimental

Amines were chromatographed as the free base, prepared by extraction of an alkalinized aqueous solution usually of the hydrochloride salt with benzene or chloroform. All other materials were reagent grade and were used as obtained. Fresh solutions of all compounds were prepared daily.

Analyses were performed with an F & M Model 810 gas chromatograph equipped with a hydrogen flame ionization detection system. The column support, 100 to 120 mesh Chromosorb G, acid-washed and silanized, was obtained from Applied Science Laboratories, Inc. The stationary phase, 3% QF-1, was dissolved in chloroform and stirred with the required amount of solid support. The solvent was evaporated on a steam-bath overnight. The columns, 6 ft. × 6 mm glass coils, were used with flow rates of 50 ml/min for H_2 and He and 450 ml/min for air. Dibenzocycloheptenes were chromatographed at a column temperature of 240°; their Hofmann reaction products were chromatographed at 210°. The column temperature used for the mononuclear arylalkylamines was 170° while their Hofmann reaction products were run at 130°.

Exhaustive methylation (Hofmann reaction) of amines. The free amine (1 to 10 μg) in chloroform (0.6 ml) was added to a centrifuge tube having the tip drawn out (similar to the centrifuge combination tube supplied by the A. H. Thomas Co.). The solvent was evaporated in a stream of nitrogen and the residue taken up in 10 μl of benzene. Approximately 4 μl of methyliodide were next added and the solution allowed to stand at room temperature for 5 min. A small particle of moist silver oxide was added and the tube placed in warm water (70°) until the benzene had evaporated. Traces of

TABLE I

STRUCTURES AND RETENTION TIMES OF AMINES AND THEIR HOFMANN REACTION PRODUCTS

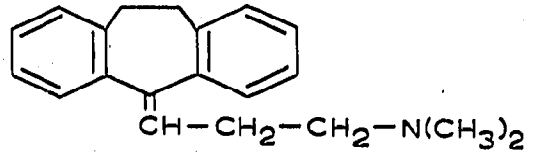
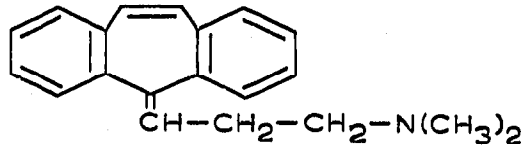
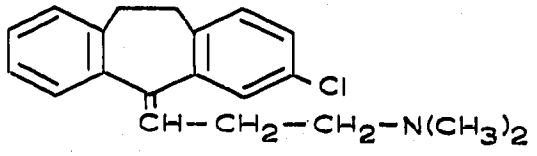
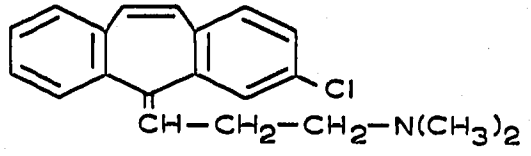
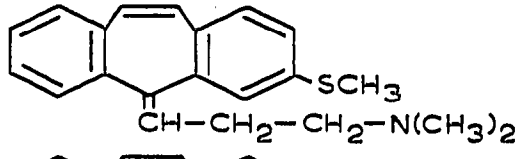
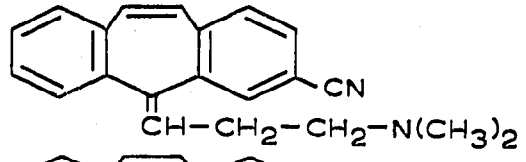
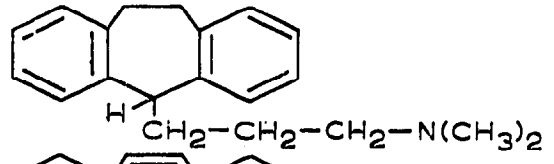
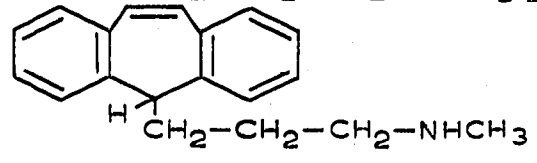
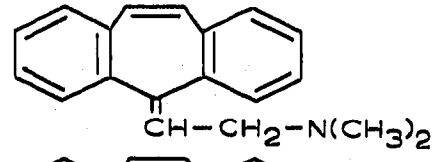
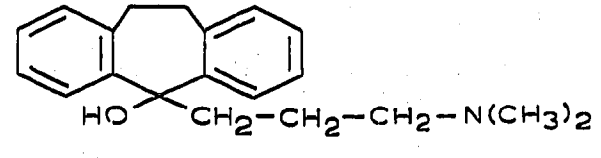
No.	*Compound*	*Retention time*	
		Amine	*Hofmann product*
I		2.1	2.5
II		2.5	3.2
III		3.5	4.4
IV		3.5	5.1
V		6.8,7.5	11.2
VI		10.6	16.0
VII		2.2	1.7,2.2
VIII		2.9	—
IX		4.3	—
X		9.8	1.7

TABLE II

RETENTION TIMES OF TERTIARY MONONUCLEAR ARYLALKYL AMINES AND THEIR HOFMANN REACTION PRODUCTS

Compound	*Retention times (min)*[a]	
	Amine	*Hofmann product*
5-(3,4-Dimethylphenyl)-N,N,1,5-tetramethylheptylamine	3.3	1.5[b]
4-(3,4-Dimethylphenyl)-N,N,4-trimethylpentylamine	6.0	1.8
3-(3,4-Dimethylphenyl)-N,N,1,3-tetramethylbutylamine	4.8	1.7
3-(3,4-Dimethylphenyl)-1,3-dimethylbutylamine	5.3	—[c]
N,N,2-Trimethyl-2-(3,4-dimethylphenyl)propylamine	2.1	2.8[d]
N,N,2-Trimethyl-2-(4-methylphenyl)propylamine	1.5	2.0[d]

[a] Column conditions were as given in the Experimental section.
[b] 170°.
[c] No peak appeared with 55 min.
[d] Product peak exhibited tailing.

water were removed in a gentle stream of nitrogen. The residue was dissolved in 5 to 10 μl of carbon disulfide or water and 1 μl injected into the chromatograph.

The structures and retention times for all compounds are shown in Table I and II.

Results and discussion

Separation of free amines. Fig. 1 shows the peak obtained on gas chromatography of amitriptyline and Fig. 2 shows the chromatographic separation of a mixture of I,

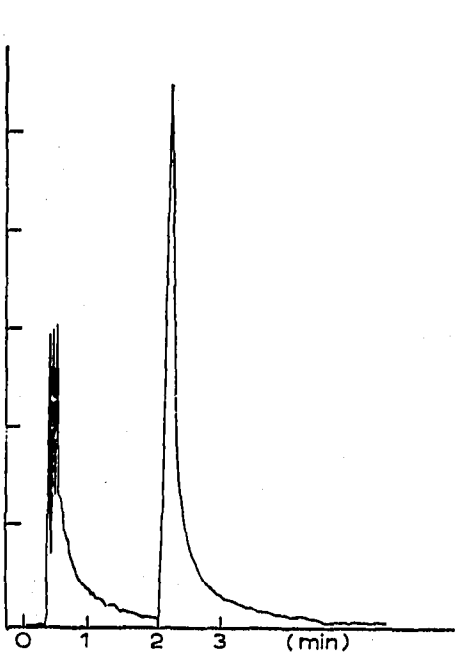


Fig. 1. Gas chromatogram of amitriptyline (I) as the free base.

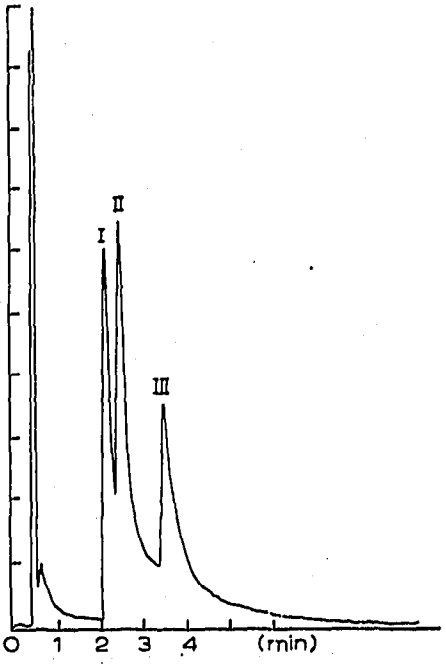
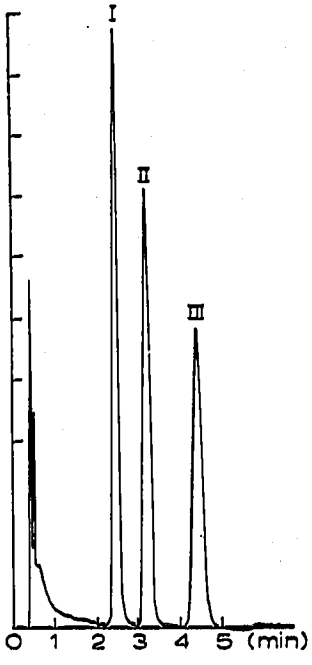


Fig. 2. Gas chromatographic separation of amitriptyline and two related compounds as the free amines. Structures of the compounds are given in Table I.



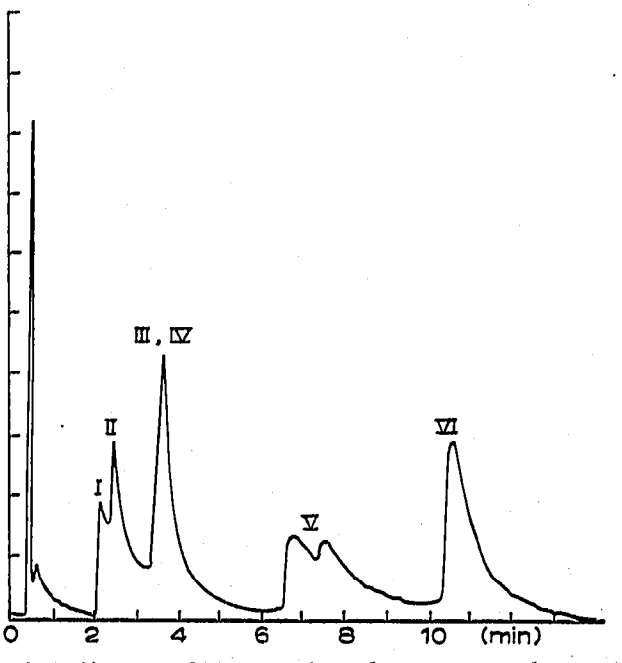


Fig. 3. Gas chromatographic separation of amitriptyline and two related compounds as their Hofmann reaction products. Structures of the compounds are given in Table I.

Fig. 4. Gas chromatographic separation of amitriptyline and five related compounds as the free amines. Structures of the compounds are given in Table I.

II and III. I and II were incompletely separated under these conditions; in addition, the peaks of all compounds exhibit a certain degree of asymmetry ("tailing").

Separation of Hofmann reaction products of amines. In view of the incomplete separation and peak tailing observed in chromatography of the free amines, they were subjected to Hofmann exhaustive methylation and degradation before chromatography. Fig. 3 shows the results obtained on chromatography of the Hofmann reaction products of I, II and III. As shown, the peaks are now highly symmetrical and well separated.

Fig. 4 shows the results of chromatographing an even more complex mixture of amines as the free bases. As shown, the peaks are again somewhat asymmetrical with pronounced tailing and are not completely resolved. Fig. 5 shows the same amine mixture chromatographed after subjection to the Hofmann reaction. As shown, the various peaks are now highly asymmetrical and completely resolved.

Not all amines in the series gave peaks under these conditions after subjection to Hofmann degradation. Only unchanged compound was seen, for example, on chromatography of VIII, a secondary amine which does not have a double bond at position 5. IX, similar to II except the side chain has only two carbon atoms, gave only one peak corresponding to unreacted IX.

Two peaks were observed on chromatography of V and may represent the *cis-trans* isomers of this compound. Only a single peak was seen, however, with the Hofmann reaction product, suggesting that the isomers in this case were not resolved.

Application of the foregoing technique to quantitative analysis of I is illustrated by the linearity of the standard curve obtained (Fig. 6) when varying amounts of I were chromatographed, using II as a mass internal standard[5].

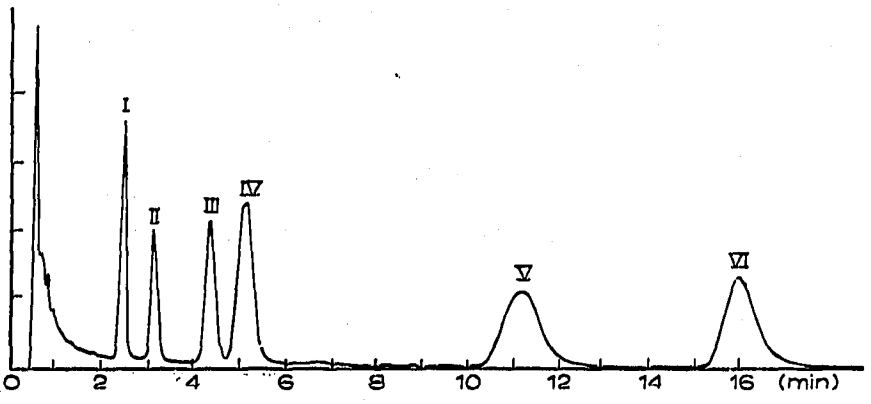


Fig. 5. Gas chromatographic separation of amitriptyline and five related compounds as their Hofmann reaction products. Structures of the compounds are given in Table I.

Tertiary amines of a series of mononuclear arylalkylamines were also subjected to gas chromatography before and after undergoing a Hofmann reaction. As shown in Table II, the results were somewhat varied, with improvement in gas chromatographic properties and shortening of retention times being noted in three of the six compounds studied.

Incorporation of alkali into the solid support may have improved the chromatographic properties and separation of the free amines. However, in view of the generally recognized preference for silicone pretreatment of the support to addition of alkali (FALES AND PISANO[3]), use of supports containing alkali was not explored. BECKETT AND ROWLAND[1] found that amphetamine gave pronounced tailing when chromatographed on a variety of columns, including polyethylene glycol and KOH on Celite. These authors noted improvement in the gas chromatographic properties when the Schiff base (acetone) was used in place of the free amine. BROCHMANN-HANSSEN AND

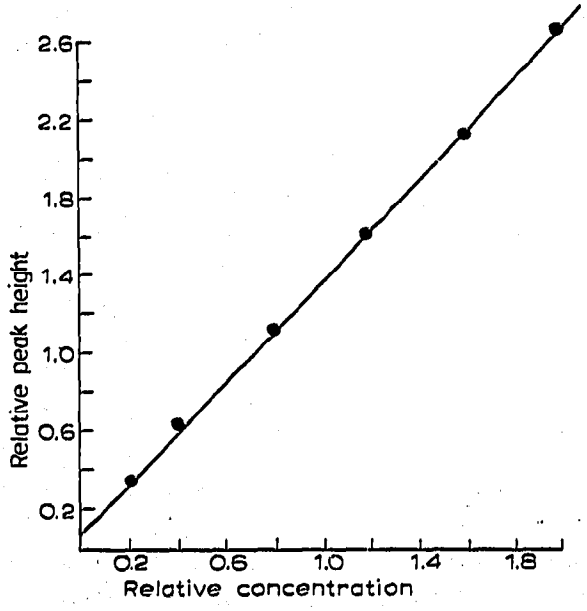


Fig. 6. Standard curve obtained on injection of mixtures containing 1, 2, 4, 6, 8 and 10 μg of 1 and 5 μg each of II after subjection to Hofmann exhaustive methylation. The ratios of peak heights of I/II are plotted *vs.* the concentration ratios of I/II.

SVENDSEN[2], on the other hand, reported no difficulty in separating propylhexedrine and methamphetamine on a column containing QF-1 on silanized chromosorb W.

The results described above indicate that conversion of tertiary amines to olefins by Hofmann exhaustive methylation prior to gas chromatography is a useful technique in cases where separation is incomplete or excessive tailing occurs. Quantitative application of the technique is illustrated, and has an inherent advantage in that the products have shorter retention times, thereby shortening analysis time. A further important application is in qualitative identification of an unknown amine by comparison of the retention time before and after exhaustive methylation with an authentic reference compound.

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Solid support as subtractor in gas chromatographic trace analysis*

Subtractive analytical techniques, in which certain classes of compounds are selectively removed from complex samples, have often been described in GLC[1–8]. To this end precolumns with a selective reagent have been used. In a previous communication it was stated that the selective adsorption of traces of polar compounds by the solid support of a nonpolar GLC column may be used in the analysis of air contamination[9]. Following up this work, a more generally applicable procedure for this kind of selective retention has been investigated for trace analysis. For this purpose a precolumn packed with an active solid support and positioned in front of a GLC partition column has been tested.

Experimental

The experiments were carried out with an Aerograph 1520 gas chromatograph equipped with a flame ionisation detector (F.I.D.). Precolumns of 20 cm × 1/8 in. O.D. packed with 80–100 mesh Chromosorb P were used for the selective retention of polar substances as well as those coated with 0.1 % of Apiezon L. The precolumn used was placed in the oven between the injector and the partition column.

Nitrogen was used as carrier gas and with a 75 lb/sq. in. pressure a flow of 15 ml/min was maintained. For separation a 3 m × 1/8 in. O.D. column was used