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CHARACTERISATION OF DRUGS CONTAINING TERTIARY AMINE GROUPS BY APPLICATION OF THE HOFMANN DEGRADATION REACTION AND GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

Drugs containing tertiary amine groups (*e.g.*, certain phenothiazine derivatives, imipramine, amitriptyline, etc.) may be tentatively identified by their retention times on gas–liquid chromatography. However, further confirmation tests often prove difficult because the tertiary amine group is not readily amenable to derivative formation. Experiments have been carried out using the HOFMANN degradation reaction to produce compounds having retention times which differ from those of the parent drug, thus offering a further criterion for identification purposes. It has been found possible to perform the final stages of this reaction within the injector port of a gas chromatograph.

The procedure involves alkylation of the drug with an alkyl halide to form the quaternary ammonium compound. Treatment of this with moist silver oxide yields the quaternary ammonium hydroxide, which, on injection into the gas chromatograph, decomposes to yield a compound with a new retention time.

This procedure has been successfully applied to drugs in pure solution and to extracts of these drugs from liver in cases of death due to overdosage. The work is being extended to include primary and secondary amines, and to quaternary ammonium compounds themselves.

INTRODUCTION

In toxicological analysis, the basic drugs, which, if present, may be extracted from aqueous alkaline solution by organic solvents consist mainly of amines. These amines may be primary, secondary or tertiary amines. In the case of primary and secondary amines, derivatives (*e.g.*, N-acyl derivatives) can easily be formed, the properties of which offer further criteria for identification purposes.

In many cases, such derivatives can be formed in a gas chromatograph, at the microgram level, by direct injection of the reaction mixture, thus giving rise to a new peak with a retention time (t_r), which usually differs from t_r of the original amine. Such procedures have already been described by BROCHMANN-HANSSEN AND SVENDSEN[1], ANDERS AND MANNERING[2], BECKETT AND ROWLAND[3], AND STREET[4].

However, many of the basic drugs are *tertiary* amines, which are not readily

amenable to derivative formation. This means that although the unchanged drug may be tentatively identified by its t_r, confirmation of identity is not possible without recourse to some other procedure such as colour formation following thin-layer chromatography. But such procedures often do not operate satisfactorily at the microgram level. The stimulus for the work described in this paper arose from the need to have a further parameter (other than a single t_r) for the gas–liquid chromatographic (GLC) identification of drugs containing a tertiary amine group.

In 1881, HOFMANN[5] demonstrated that methylation of piperidine (which is a secondary amine) with iodomethane followed by subsequent pyrolysis of the hydroxide of the resulting quaternary ammonium compound gave rise to an olefine and trimethylamine. We have used this degradation reaction for the analysis of certain drugs containing tertiary amine groups in the following way. The amines, in their free-base form, were treated with an iodoalkane and then excess of the reagent was removed by evaporation. The resulting quaternary ammonium iodide was dissolved in methanol and then solid silver oxide was added. The quaternary ammonium hydroxide, so formed, was injected directly into a gas chromatograph.

EXPERIMENTAL

Procedure

The details of the technique used are as follows: 5 ml of blood or 5 g of liver (or 20 g of liver, if necessary) are treated according to the procedure described by STREET[6] (pp. 72, 73), for basic alkaloids. To the residue from this extract, 1 ml of diethyl ether (purified as described by STREET[6]) and 1 ml of redistilled iodomethane are added. The tube is loosely stoppered with a glass stopper and held over a boiling-water bath so that the liquid in the tube is only just boiling. After 3 or 4 min (the mixture usually becomes yellow at this stage if any appreciable amount of N-methylation has occurred) the stopper is removed and the mixture evaporated carefully to dryness. The tube is cooled and 0.5 ml (or 0.2 ml) of methanol added to dissolve the residue. A knife-point of silver oxide is then added, the tube stoppered and the mixture shaken. When the excess silver oxide has settled (after about 1-min standing), a suitable aliquot part (say 5 μl) of the supernatant liquid is injected directly into the gas chromatograph.

Gas chromatographic details

The apparatus used was a Perkin-Elmer gas chromatograph Model 800 fitted with a flame ionisation detector. The carrier gas was oxygen-free nitrogen (flow-rate 30 ml/min). The column temperature was 200°; the injector port temperature, 300°; the detector temperature, 280°. The column used was as described by STREET[7], except that SE-30 was used instead of SE-52 as the liquid phase. The chart speed of the recorder was 1 in. in 4 min.

RESULTS AND DISCUSSION

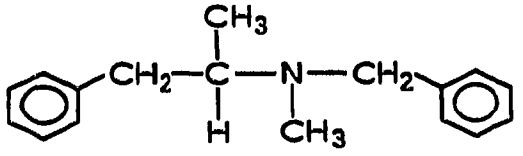
Depending on the nature of the drug being investigated, the results (for tertiary amines) seen on the chromatogram were of three types:

(1) The t_r of the peak was the same as that of the parent drug.

(2) The t_r of the new peak was shorter than that of the parent drug.

(3) Two new peaks were obtained — usually with t_r very close to each other — but with each t_r shorter than that of the parent drug.

Drugs in the type 1 classification have consisted, so far, of those drugs whose nitrogen atom is surrounded by bulky alkyl and/or aryl groups, so that under the conditions employed it has not been possible to form the quaternary ammonium compounds. A typical tertiary amine in this category is benzphetamine (I). It is



(I) BENZPHETAMINE

important to note here, of course, that in the analysis of an unknown substance, peaks may also be obtained of the type 1 category with compounds which are not amines. But then these compounds would most likely not display basic characteristics and so would not be present in the so-called basic extract.

Drugs which fall into the type 2 classification are exemplified by amitriptyline (N-methyl derivative shown) (II). The reaction here may be as follows:

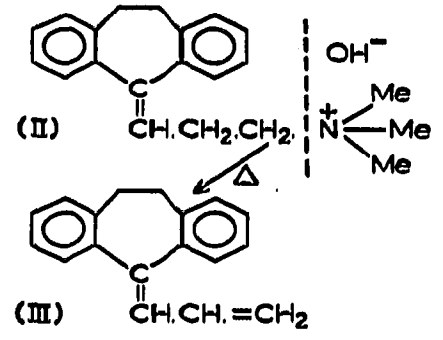


Fig. 1 compares the chromatogram obtained from amitriptyline itself and that obtained when amitriptyline is subjected to the HOFMANN treatment. Compound III

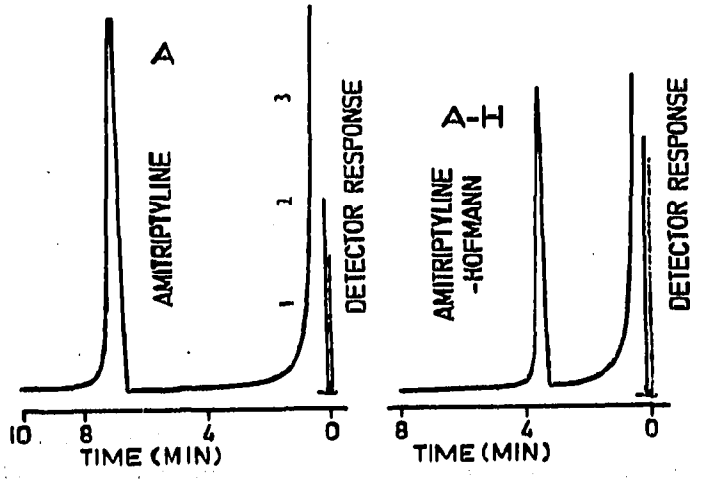
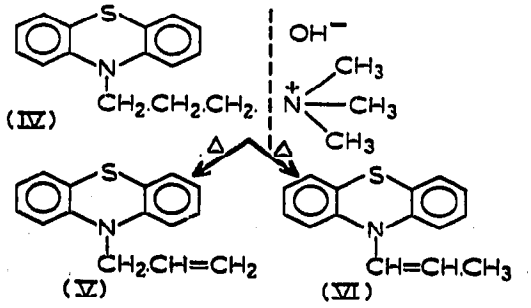


Fig. 1. Gas–liquid chromatograms showing relationship between the retention times of peaks obtained from (A) unchanged amitriptyline (10 μg), and (A–H) amitriptyline after subjection to HOFMANN treatment. For details see text. Column temperature, 200°.

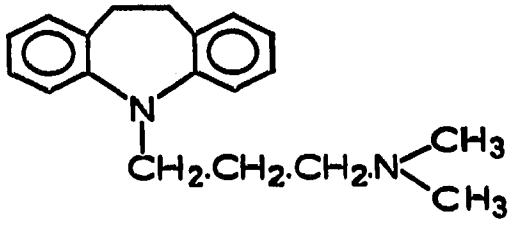
above may be responsible for the peak with the shorter retention time; the trimethylamine peak merging with the methanol peak. Identical results were obtained when iodomethane, iodoethane or iodopropane was used as the methylating agent, indicating that the added alkyl group is subsequently removed as part of the alkylamine.

Results which fit into the type 3 category are obtained by the HOFMANN treatment of certain phenothiazine derivatives such as promazine (N-methyl derivative shown) (IV) and chlorpromazine. In these cases, the degradation reaction may be as follows:



(IV) (V) (VI)

The two isomeric products V and VI would differ only in the position of the double bond in the 3-carbon chain attached to the ring nitrogen. This difference might account for the presence of two peaks with very similar retention times. Figs. 2 and 3 show the chromatograms obtained with promazine and chlorpromazine, respectively, when these drugs are subjected to the HOFMANN treatment.



(VII) IMIPRAMINE

It was felt that imipramine (VII) would give results, which would fit into category 3, *i.e.* give two new peaks. However, as is shown in Fig. 4, only one new peak is seen. It is possible, of course, that two new compounds are formed but are not resolved on the GLC column. It should be noted that, in the case of amitriptyline, the presence of the double bond in the parent drug will exclude formation of two products such as are obtained with the phenothiazine derivatives. In the cases of the phenothiazines and of imipramine, it is probable that the ring nitrogen does not become alkylated owing to the presence of the bulky aryl and alkyl groups surrounding this nitrogen atom.

It must be emphasised that, although peaks with 'new' retention times are formed with this procedure, the precise structure of the compounds responsible for these 'new' peaks has not yet been established.

However, this does not detract greatly from allowing the technique to be used for qualitative analytical purposes in certain cases and, indeed, it has been found of value in toxicological analysis of blood and of liver extracts, where it has provided a further parameter for identification purposes.

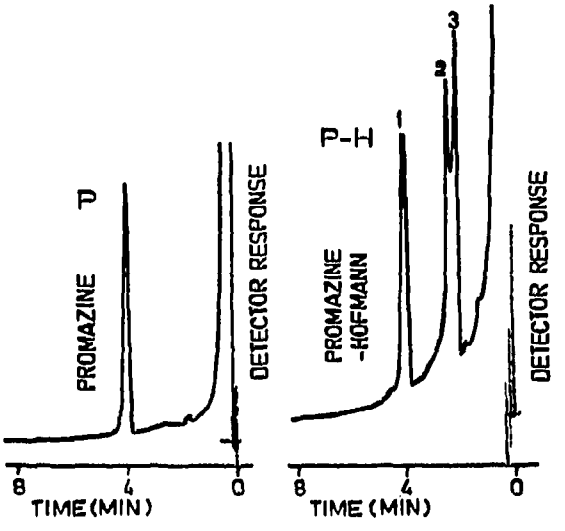


Fig. 2

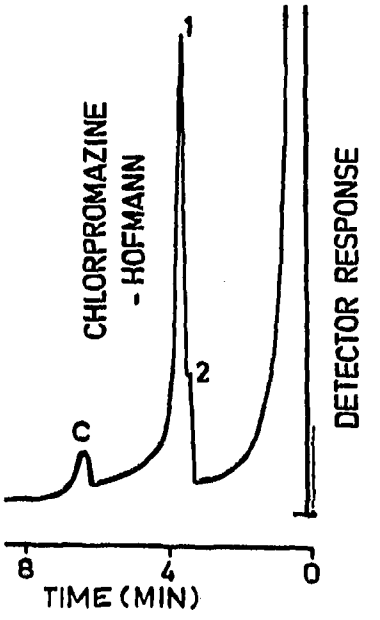


Fig. 3

Fig. 2. Gas–liquid chromatograms showing relationship between the retention times of peaks obtained from (P) unchanged promazine (10 μg), and (P–H) promazine after subjection to HOFMANN treatment. For details see text. Attenuation in (P) was × 2000, and in (P–H) × 1000. Column temperature, 230°. Note that in (P-H) two extra peaks, 2 and 3, are present, as well as unchanged promazine (1).

Fig. 3. Gas–liquid chromatogram showing the peaks obtained when chlorpromazine was subjected to the HOFMANN treatment. For details see text. Column temperature, 230°. Note the two incompletely resolved peaks 1 and 2. Unchanged chlorpromazine is seen at peak C.

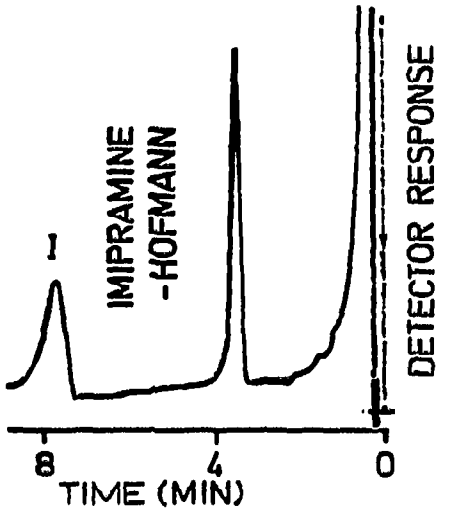


Fig. 4. Gas–liquid chromatogram of imipramine when subjected to the HOFMANN treatment. For details see text. Column temperature, 200°. Unchanged imipramine is seen at peak I.

We have also extended this work to cover some primary and secondary amines and also quaternary ammonium compounds themselves, but the data we have so far accumulated are not yet sufficient to warrant inclusion in this paper.

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