*Journal of Chromatography*, 194 (1980) 399–403
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,760

## Note

# Gas chromatography of phenolic amines, 3-methoxycatecholamines, indoleamines and related amines as their N,O-ethyloxycarbonyl derivatives

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(Received February 11th, 1980)

In general, the quantitative gas chromatographic (GC) analysis of amines as free bases at low concentrations is limited by adsorption and decomposition in the column and tailed peaks. In order to overcome these limitations, the amino groups and other functional groups if present in the molecule have been masked by different types of derivative formation reactions prior to GC analysis. The derivatives used include acetyl[1–3], trimethylsilyl[4], enamines[3,5], trimethylsilylenamines[6], trimethylsilylheptafluorobutyryl[7], trifluoroacetyl[8], pentafluoropropionyl[9], heptafluorobutyryl[10], *p*-tosylamides[11] and isothiocyanates[12,13].

During a study of the determination of amino acids by GC, we found that alkyl chloroformates are very useful as derivatization reagents for the micro-scale GC of amino acids[14,15]. Alkyl chloroformates react readily not only with the α-amino groups but also with other functional groups such as imino, phenolic hydroxyl, sulphydryl and imidazolic NH groups in aqueous alkaline media at room temperature to provide the corresponding compounds with N-, O- and S-substituted alkyloxycarbonyl groups.

The present study, based on this observation, was undertaken in order to explore the possibility of carrying out the GC of some phenolic amines, 3-methoxycatecholamines, indoleamines and related amines as their N,O-alkyloxycarbonyl derivatives. The results suggested that the N,O-ethyloxycarbonyl (EOC) derivative is the most suitable for the GC of these amines.

## EXPERIMENTAL

### *Reagents*

Amines were obtained, mostly as hydrochloride salts, from Sigma (St. Louis, MO, U.S.A.) or Nakarai Chemicals (Kyoto, Japan). All salts, prior to use, were dried in a vacuum desiccator over phosphorus pentoxide and dissolved in water to give a concentration of 100 μg/ml as the free base. Amines obtained as free bases were dissolved in a few drops of 5% hydrochloric acid and then made up to 100 μg/ml with water. A standard solution containing 100 μg/ml of each of the 12 amines tested (see Table I) was also prepared as described above and was used for the evalua-

0021-9673/80/0000–0000/$02.25 

tion of the GC separation. In the preparation of calibration graphs, 3,4-dimethoxyphenethylamine was used as the internal standard and the remaining 11 amines were classified into two groups, one containing synephrine and 5-methoxytryptamine and the other all other amines. All amine solutions were stored in capped glass bottles maintained at 4°C. Methyl, ethyl, isobutyl, *n*-butyl and *n*-amyl chloroformates stabilized with calcium carbonate were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Diisopropyl ether of reagent grade was further purified by distillation in an all-glass apparatus. All other chemicals were the purest available grades from standard commercial sources.

*Derivatization*

Volatile derivatives of amines were prepared in an aqueous alkaline medium based on the observations in a previous study[15]. An aliquot of the standard solution (10–100 μg of each amine) and 0.5 ml of the internal standard solution (100 μg/ml) were pipetted into a 10-ml polyethylene-stoppered vial. To this solution, 0.5 ml of 10% sodium carbonate solution was added, and the total volume was made up to 2 ml with water if necessary. Subsequently, 0.2 ml of ethyl chloroformate was added and the mixture was vigorously shaken for 10 min at room temperature. The resulting derivatives were extracted three times with 1.5 ml of diisopropyl ether. The ethereal layers, consisting of the derivatives together with excess of the reagent, were aspirated with a pasteur pipette, and the combined extracts were evaporated to dryness at 75°C under a stream of air in a draft chamber to ensure the removal of all excess of reagent. The residue was redissolved in 50–100 μl of diisopropyl ether and the solution was then dried over a small amount of anhydrous sodium sulphate. A 2–4-μl volume of the resulting solution was injected into the gas chromatograph. The other N,O-alkyloxycarbonyl derivatives were similarly prepared and analysed, except that 0.2 ml of methyl chloroformate and 0.1 ml of isobutyl, *n*-butyl and *n*-amyl chloroformates were used individually instead of ethyl chloroformate.

*Gas chromatography and mass spectrometry*

A Shimadzu 4CM dual-column gas chromatograph equipped with hydrogen flame ionization detectors, on-column injection ports and a linear temperature programmer was used.

The column packings were prepared using *n*-butanol–chloroform (1:1) as a coating solvent according to the solution coating technique[16]. Most of the work was carried out with a silanized glass column (0.5 m × 3 mm I.D.) packed with a two-component stationary phase (1.5% OV-17 + 0.2% SP-1000) on 100–120-mesh Uniport HP. The packed column was conditioned at 275°C for 15 h with a nitrogen flow-rate of 30 ml/min. The chromatographic conditions used to effect the separation of the N,O-EOC derivatives of amines are given in Table I.

For combined gas chromatography–mass spectrometry (GC–MS), a Shimadzu LKB 9000 instrument with the same type of column as used for GC analysis was employed.

*Calibration graphs*

A number of standards, each containing 50 μg of the internal standard and various amounts of amines (10–100 μg of each), were converted into the N,O-EOC

derivatives and analysed as described above. The peak-height ratios relative to the internal standard were plotted against the amount of amine derivatized.

## RESULTS AND DISCUSSION

Reaction conditions for the preparation of N,O-alkyloxycarbonyl derivatives of 12 amines were established on the basis of a previous investigation[15]. An initial effort was directed to testing the applicability of five alkyl chloroformates which were commercially available and inexpensive. Each of the amine derivatives prepared by using these reagents was first chromatographed individually in order to evaluate its GC behaviour and properties in parallel with the investigation of GC columns suitable for the separation of amines. Of five derivatives examined, the N,O-EOC derivative proved to be the most useful for the analysis of the 12 amines tested in terms of GC separation, volatility and absence of peaks derived from reagents. This derivative was therefore selected for subsequent studies.

Amines containing a $\beta$-alcoholic hydroxyl group showed a tendency to give peaks of poor shape on non-selective phases such as OV-17 and OV-1. The serotonin derivative showed a longer retention time on a selective phase (SP-1000) and could not be eluted with the temperature programme used. However, all of the peaks eluted from an SP-1000 column were sharp and symmetrical. The best result was obtained with a column with a mixed-stationary phase (1.5% OV-17 + 0.2% SP-1000) and this was therefore adopted in subsequent work. Further, the other four derivatives of

TABLE I

COMPARISON OF THE RETENTION TIMES OF FIVE KINDS OF N,O-ALKYLOXYCARBONYL DERIVATIVES OF TWELVE AMINES

GC conditions: 1.5% OV-17 + 0.2% SP-1000 on Uniport HP (100–120 mesh); 0.5 m × 3 mm I.D. glass column; nitrogen flow-rate, 60 ml/min; temperature programme, 100–275°C at 8°C/min and held at 275°; injection and detector temperature, 280°C. Retention times are given in minutes and seconds.

| *Amine* | *Derivative** | | | | |
|---|---|---|---|---|---|
| | *MOC* | *EOC* | *iso-BOC*** | *n-BOC*** | *n-AOC*** |
| Phenethylamine | 3-12 | 3-59 | 5-34 | 6-12 | 7-19 |
| $\beta$-Hydroxyphenethylamine | 7-40 | 8-20 | 9-40 | 10-20 | 11-14 |
| 3,4-Dimethoxyphenethylamine | 9-55 | 10-30 | 11-50 | 12-29 | 13-22 |
| Tyramine | 10-46 | 12-02 | 14-37 | 15-46 | 17-22 |
| Synephrine | 13-12 | 14-14 | 16-28 | 18-07 | 19-04 |
| 3-Methoxytyramine | 13-12 | 14-22 | 16-28 | 18-07 | 19-04 |
| Tryptamine | 14-20 | 14-54 | 15-59 | 16-28 | 17-19 |
| Octopamine | 14-41 | 15-41 | 17-43 | 18-47 | 20-14 |
| Metanephrine | 15-16 | 16-10 | 18-06 | 19-01 | 20-31 |
| 5-Metoxytryptamine | 16-48 | 17-24 | 18-17 | 18-47 | 19-35 |
| Normetanephrine | 16-48 | 17-32 | 19-19 | 20-14 | 21-41 |
| Serotonin | 20-24 | 21-12 | 22-53 | 24-11 | 26-37 |

* MOC = methyloxycarbonyl; EOC = ethyloxycarbonyl; BOC = butyloxycarbonyl; AOC = amyloxycarbonyl.

** Retention times of the peaks derived from reagents are as follows: *iso*-BOC, 8-44 and 11-11; *n*-BOC, 9-23 and 11-50; *n*-AOC, 10-26 and 12-50 min-sec.

each amine also showed reasonable peak heights on this column. The retention times of five derivatives are compared in Table I. None of the isobutyloxycarbonyl, *n*-butyloxycarbonyl and *n*-amyloxycarbonyl derivatives of serotonin could be eluted with the temperature programme used. Relatively large peaks derived from the reagents were observed when isobutyl, *n*-butyl and *n*-amyl chloroformates were used. This may be due to the difficulty of complete removal of excess of reagents with lower volatility. A typical gas chromatogram for the N,O-EOC derivatives of 12 amines is shown in Fig. 1. The separation of two pairs (3-methoxytyramine–synephrine and 5-methoxytryptamine–normetanephrine) could not be achieved.

The structures of all of the N,O-EOC derivatives prepared by the procedure described above were elucidated by GC–MS. A molecular ion peak ($M^+$) which is consistent with the structure postulated was observed for each derivative. The GC–MS study indicated that, as expected, the amino and phenolic hydroxyl

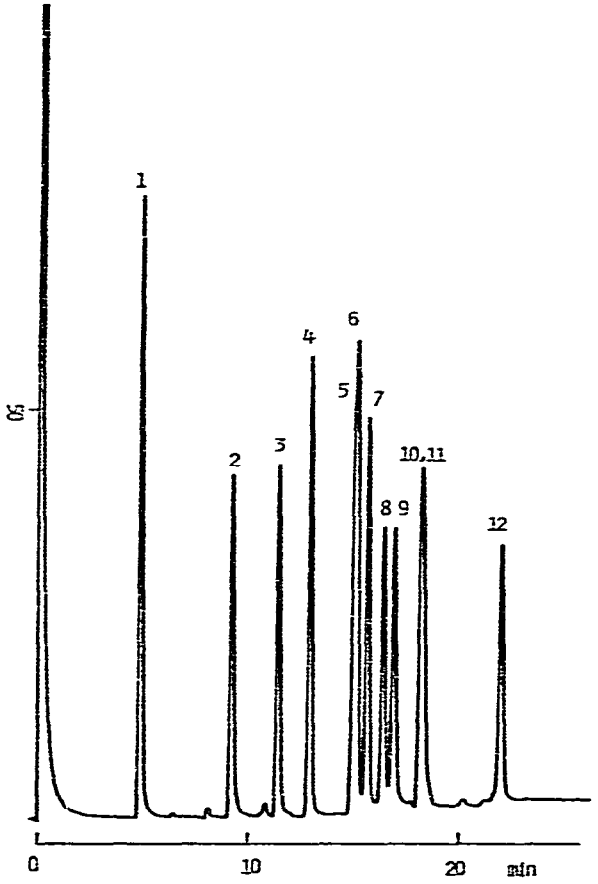


Fig. 1. Gas chromatogram obtained from a known mixture of 12 amines subjected to ethyloxycarbonylation and gas chromatography. Each peak represents *ca.* 0.5 μg of amine. 1 = Phenethylamine; 2 = β-hydroxyphenethylamine; 3 = 3,4-dimethoxyphenethylamine (internal standard); 4 = tyramine; 5 = synephrine; 6 = 3-methoxytyramine; 7 = tryptamine; 8 = octopamine; 9 = metanephrine; 10 = 5-methoxytryptamine; 11 = normetanephrine; 12 = serotonin. FID sensitivity, $10^2$ (×$10^6$ ohm); range, 16 (×0.01 V); chart speed, 0.5 cm/min. Other GC conditions as in Table I.

groups are substituted with ethyloxycarbonyl groups but the β-alcoholic hydroxyl groups are not.

In order to assess the conversion yield of the N,O-EOC derivatives in the derivatization procedure, pure reference standards of tyramine and octopamine were synthesized. The average yields ($n = 4$) obtained when these representative amines were derivatized together with all other amines were 96.7% for tyramine and 92.1% for octopamine.

The standards were also used to check the stability at room temperature of the N,O-EOC derivatives of amines by injecting the same at two intervals. The results showed that there was no significant degradation over at least 1 week.

A calibration graph for each amine in the range of 10–100 μg was prepared using, for convenience, 3,4-dimethoxyphenethylamine as the internal standard. The linearity for each amine was satisfactory for quantitative determination. However, when diethyl ether was used instead of diisopropyl ether as the extraction solvent, the reproducibility of the linearity for amines containing a β-alcoholic hydroxyl group was inadequate, probably owing to the inconsistent efficiency of extraction.

In conclusion, it should be noted that the recommended derivatives are easily prepared in aqueous alkaline medium and are stable, unlike most derivatives developed previously. We propose a GC method which, with its rapidity and simplicity, has a real advantage over the methods presently available. This method has been used in our laboratory for the routine determination of tyramine and related amines in various foods and urines.

ACKNOWLEDGEMENT

This work was supported in part by a grant for scientific research from the Ministry of Education, Science and Culture to one of us (S.Y.).

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