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GAS–LIQUID CHROMATOGRAPHIC PROPERTIES OF CATECHOLAMINES, PHENYLETHYLAMINES AND INDOLALKYLAMINES AS THEIR PROPIONYL DERIVATIVES

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SUMMARY

The gas chromatographic properties of the biogenic amines, catecholamines, phenylethylamines and indolalkylamines as their propionyl derivatives were studied. These derivatives are readily formed in an aqueous medium. Propionylated amines are more stable than their parent compounds and increasingly lipophilic, so that they can be extracted quantitatively into an organic solvent.

The propionyl derivatives of the biogenic amines show good gas chromatographic properties. They can be well separated on OV-101 and OV-17 silicones. Care must be taken of certain interactions of the compounds during the chromatographic procedures. Pre-treatment of the column with thionyl chloride inhibits decomposition of β-O-propionylated catecholamines and prevents their interference with other amines.

Propionylation is a useful means for the isolation and determination of a wide range of biogenic amines from biological materials by gas chromatography.

INTRODUCTION

In biological fluids, the concentration of biogenic amines is normally very low and very sensitive methods must therefore be used for their qualitative and quantitative analysis. In this respect, gas–liquid chromatography has been widely used for the separation and determination of catecholamines, phenylethylamines and indolalkylamines of various origin. For the gas chromatographic analysis of polar compounds such as these biogenic amines, their derivatization is essential. Most investigators have used trimethylsilyl[1–3], trifluoracetic[4,5] or pentafluorpropionyl[5–7] derivatives of the amines. These derivatives are volatile and have good chromato-

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graphic properties. However, they are stable only in the absence of water[5]. For their analysis in biological fluids, the amines have to be transferred into an organic solvent before derivatization. During this procedure, especially at an alkaline pH, polar amines such as noradrenaline and dopamine may decompose, sometimes resulting in a loss of up to 50%[8-10].

In a preceding paper[11], we considered acylated amine derivatives (propionyl-, *n*-butyryl-, isobutyryl- and pivaloylamines), which can be prepared in aqueous solution and which are stable. They also are increasingly lipophilic so that they can be extracted quantitatively into ethyl acetate or other organic solvents. Of these acyl derivatives, propionylated amines appear to be the most advantageous. In this investigation we have studied the gas chromatographic properties of 23 propionylated catecholamines, phenylethylamines and indolalkylamines. Special emphasis was directed towards the stability of the derivatives in gas chromatography and towards any possible interactions or interferences between the various compounds during analytical procedures.

MATERIALS AND METHODS

Substances used

The biogenic amines used were as follows: N,N-dimethyltyramine (hordenin), N,N-dimethyl-5-methoxytryptamine and N-methyl-5-hydroxytryptamine, obtained from Aldrich, Beerse, Belgium; D,L-metanephrine hydrochloride and D,L-normetanephrine hydrochloride, obtained from Calbiochem, Frankfurt, G.F.R.; N-methylphenylethylamine, L-noradrenaline hydrochloride, phenylpropylamine, bufotenin hydrogenoxalate, N-methyltryptamine and 5-hydroxytryptamine hydrogenoxalate, obtained from Fluka, Neu-Ulm, G.F.R.; L-adrenaline, 3,4-dimethoxyphenylethylamine and tryptamine hydrochloride, obtained from Koch-Light, Colnbrook, Great Britain; 3,4-dihydroxyphenylethylamine hydrochloride (dopamine), tyramine hydrochloride, phenylethylamine, 3-methoxy-4-hydroxyphenylethylamine and 5-methyltryptamine obtained from Serva, Heidelberg, G.F.R.; *p*-methoxyphenylethylamine and 5-methoxytryptamine, obtained from Sigma, Munich, G.F.R.; and N,N-dimethyltryptamine and N-methyltyramine, kindly donated by Dr. Rimek, Institute of Pharmacy, University of Bonn, Bonn, G.F.R.

All reference compounds were obtained in the highest purity available from commercial sources and used without further purification.

Abbreviations

The abbreviations used for the amines in the figures are listed in Table I.

Reagents

Propionic anhydride (puriss.) and thionyl chloride (puriss.) were obtained from Fluka.

Preparation of propionylated amines

A 5-μmole amount of the amines is dissolved in 1.0 ml of 0.1 *N* hydrochloric acid and the solution is saturated with solid sodium carbonate. Between intervals of constant shaking for 5 min, 0.05 ml of propionic anhydride are added three times. The propionylated amines are then extracted three times with 1.0 ml of ethyl acetate.

TABLE I

ABBREVIATIONS FOR AMINES

Amine	*Abbreviation*	*Amine*	*Abbreviation*
Phenylethylamine	PE	Tryptamine	TR
N-Methylphenylethylamine	MPE	N-Methyltryptamine	MTR
N,N-Dimethyltyramine	DMTY	Dopamine	DA
Phenylpropylamine	PP	5-Methoxytryptamine	MOTR
p-Methoxyphenylethylamine	MOPE	Normetanephrine	NMN
N,N-Dimethyltryptamine	DMT	Metanephrine	MN
5-Methoxydimethyltryptamine	MODMT	Noradrenaline	NA
3,4-Dimethoxyphenylethylamine	DMPEA	Adrenaline	ADR
Tyramine	TY	5-Methyltryptamine	5MTR
N-Methyltyramine	MTY	Serotonin (5-hydroxytryptamine)	HT
3-Methoxy-4-hydroxyphenylethylamine	MOHPE	N-Methylserotonin	MHT
Bufotenin	BUFO		

The combined organic extracts are evaporated to dryness at 25–30° under a stream of dry air, the residue is dissolved in 0.2 ml of pyridine–propionic anhydride (3:1, v/v) and the solution is heated for 15 min at 100° in PTFE-capped vials. After cooling to room temperature, excess of pyridine and propionic anhydride is evaporated. The propionylated amine derivatives are then dissolved in 1.0 ml of acetonitrile to give solutions ready for gas chromatographic analysis.

RESULTS AND DISCUSSION

Comparing different acyl derivatives of biogenic amines, Kauert *et al.*[11] found that propionic anhydride readily reacts with primary and secondary amino groups and with hydroxyl groups. In preliminary experiments, we found that quantitative derivatization takes place even in an aqueous medium if the amines have no or only one phenolic hydroxyl group (in addition to the amino groups). For quantitative propionylation of all functional groups of biogenic amines such as catecholamines, phenylethylamines and indolalkylamines, it is neccessary to carry out the derivatization reaction under anhydrous conditions in the presence of pyridine and propionic anhydride. The reaction of propionylation of tyramine and bufotenin is shown in Fig. 1.

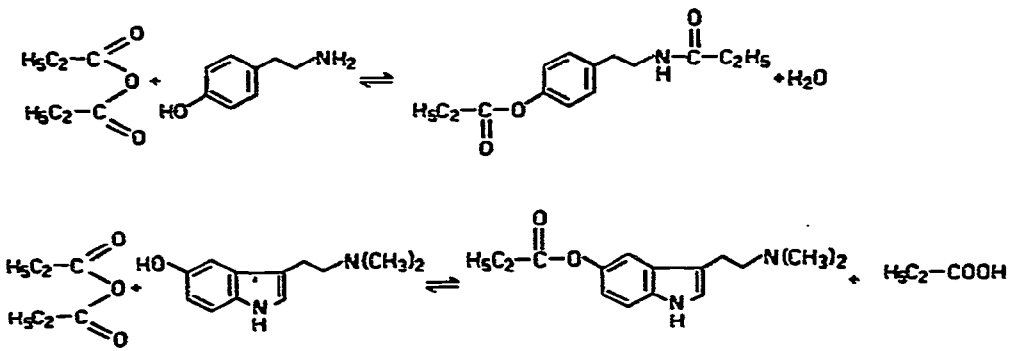

Fig. 1. Acylation of tyramine and bufotenin with propionic anhydride.

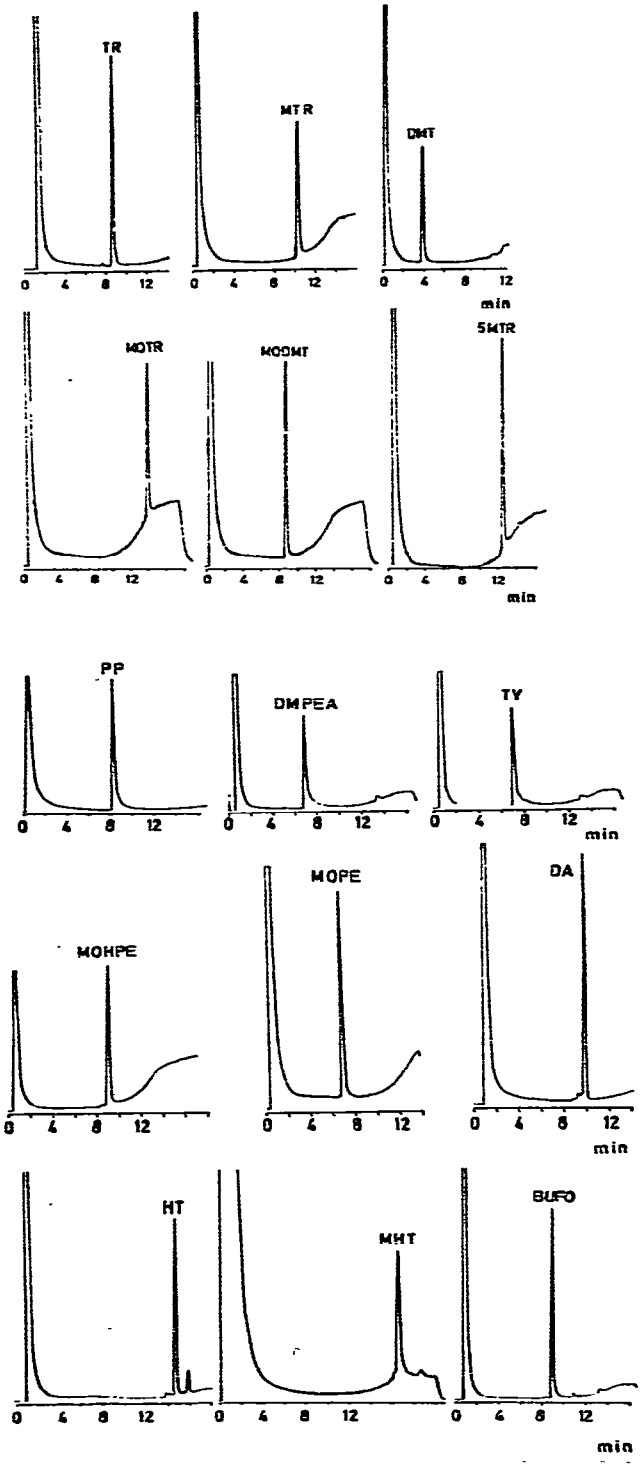

Fig. 2. Gas chromatographic assay of phenylethylamines, catecholamines and indolalkylamines as propionyl derivatives.

If quantitative propionylation of the various biogenic amines occurs, only a single peak for each should appear in the gas chromatogram. In Fig. 2, gas chromatograms of 15 propionylated amines are shown, clearly indicating that each substance is eluted in a single peak. The only exception is 5-hydroxytryptamine (which will be discussed later).

Gas chromatographic analysis of amines containing β-hydroxyl groups

We found that biogenic amines with a β-hydroxyl group (adrenaline, noradrenaline, metanephrine, normetanephrine) were not eluted in a single peak when chromatographed as propionyl derivatives. As shown in Fig. 3 (left), noradrenaline appeared as three peaks. In other gas chromatographic separations we found that the number of peaks may vary under different conditions. This indicates that the derivatives are destroyed during the gas chromatographic procedure. In order to prevent destruction, we tried to transform the β-hydroxycatecholamines into more stable derivatives. Kauert[12] was successful in forming oxazoline derivatives by the use of thionyl chloride. Accidentally we found that treating the gas chromatographic column with small amounts of thionyl chloride resulted in deactivation of the column. When the propionylated catecholamines are then injected and chromatographed, they appear as single peaks and show no signs of destruction (see Fig. 3, right).

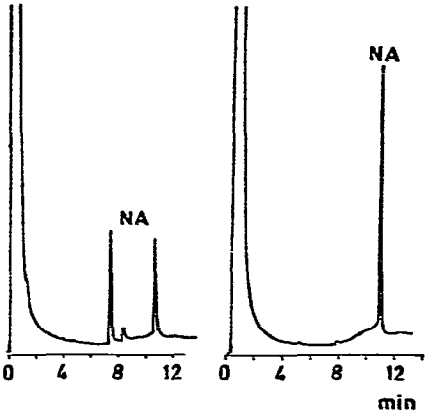

Fig. 3. Gas chromatographic assay of noradrenaline on OV-101 without (left) and with (right) pretreatment of the column with thionyl chloride.

Deactivation of the gas chromatographic column was also obtained with other chemicals such as silylating reagents, propionic acid anhydride and N-methyl-bistrifluoracetamide, but deactivation was not as complete as with thionyl chloride.

We were interested in establishing the cause of reactions that occur during the gas chromatographic separation of the amines within the column. The following can be stated: (1) only those biogenic amines which have a β-O-propionyl group are not eluted as a single peak; (2) all reagents that were able to prevent destruction of these amines react with hydroxyl groups; and (3) glass, the support material and silicones (used as the stationary phase) contain hydroxyl (silanol) groups. We concluded that a propionyl group is transfered from the β-hydroxyl position of the catecholamine to a silanol group (Fig. 4).

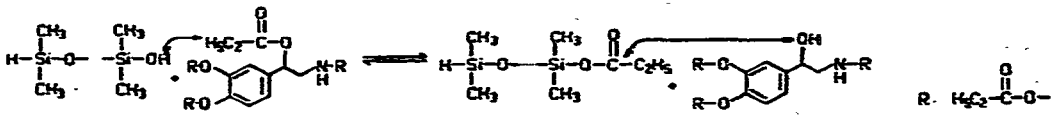

Fig. 4. Proposed interaction of a silanol group with a β-O-propionylated catecholamine (NA).

Pre-treatment of the column with thionyl chloride chlorinates the silanol groups[13] according to the following equation:

$$\text{–Si–OH} + SOCl_2 \rightarrow \text{–Si–Cl} + HCl + SO_2$$

By this reaction, the column is deactivated for an exchange with β-O-propionyl groups, so that all catecholamines can be chromatographed without any problems (Fig. 5).

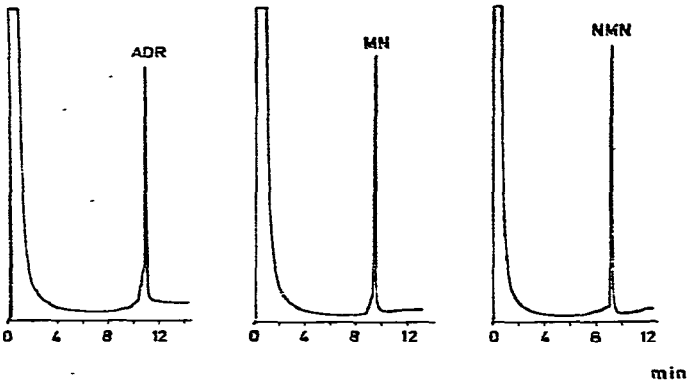

Fig. 5. Gas chromatographic assay of β-O-propionylated catecholamines after deactivation of the column with thionyl chloride.

Gas chromatographic analysis of 5-hydroxytryptamines

Further investigations of the gas chromatographic properties of propionylated biogenic amines have shown that 5-hydroxylated tryptamines such as serotonin and N-methyl-5-hydroxytryptamine behave differently when chromatographed alone (Fig. 6, I) or with propionic anhydride (Fig. 6, II) or together with a catecholamine such as adrenaline (Fig. 6, III). As can be seen in Fig. 6, II and III, an additional peak (B) can be detected.

Mass spectrometric analysis showed that the additional peak (B) represents a tripropionylated hydroxytryptamine with the third propionyl group at the indole-N position. The donor of the third propionyl group may be propionic anhydride as well as β-O-propionyladrenaline, whereas the indolalkylamine is the acceptor. Catalyst of the reaction is again the material of the column, because when it is deactivated with thionyl chloride as described above, the propionyl transfer is inhibited (Fig. 6, IV).

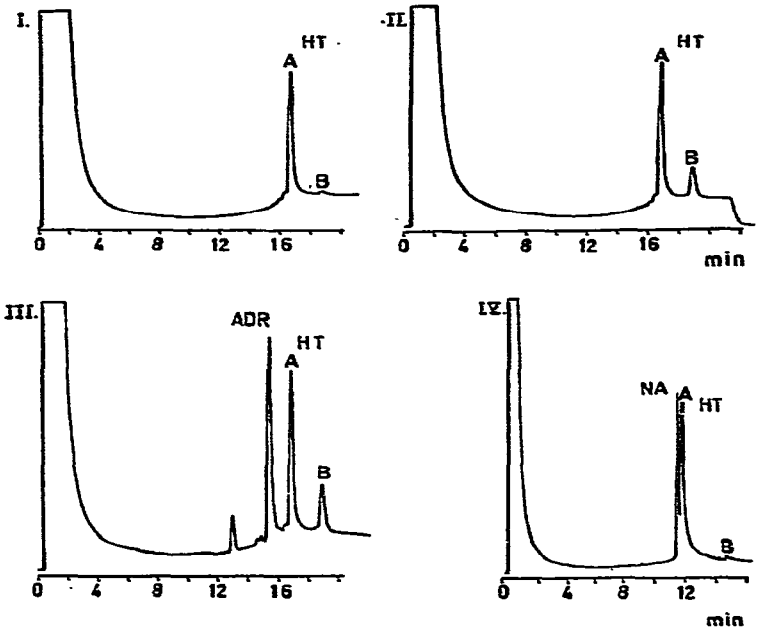

Fig. 6. Gas chromatographic assay of 5-hydroxytryptamine. I, HT dissolved in acetonitrile; II, HT dissolved in propionic anhydride; III, HT + ADR dissolved in acetonitrile; IV, HT + NA dissolved in acetonitrile, column deactivated with thionyl chloride.

It was further found that the effect of deactivation by thionyl chloride is not permanent. Injection of small amounts of water into the column will restore the silanol groups according to the equation

$$-\underset{|}{\overset{|}{Si}}-Cl + H_2O \rightarrow -\underset{|}{\overset{|}{Si}}-OH + HCl$$

and the column becomes active again. Trace amounts of water are present in any sample. In order to prevent reactivation, we injected 1–2 μl of ethyl acetate containing 0.5% of thionyl chloride before each gas chromatographic run, which ensured that the propionylated amines are eluted in single peaks.

Gas chromatographic analysis of mixtures of biogenic amines

In studying the gas chromatographic properties of biogenic amines as their propionyl derivatives, further experiments were performed to find the optimal conditions for the separation of amines in a complete mixture. For these experiments, an equimolar mixture containing 21 propionylated amines (5 μm/ml) was prepared. A 5-nmole (1-μl) amount was injected into the column. Because the propionylated amines have high boiling points, stationary phases with high thermal stability (maximum temperature *ca.* 300°) must be used. Various silicone phases with different polarity were tested, and the silicones OV-17 and OV-101 proved to be the most suitable. Gas chromatograms obtained on these two phases are shown in Figs. 7 and 8.

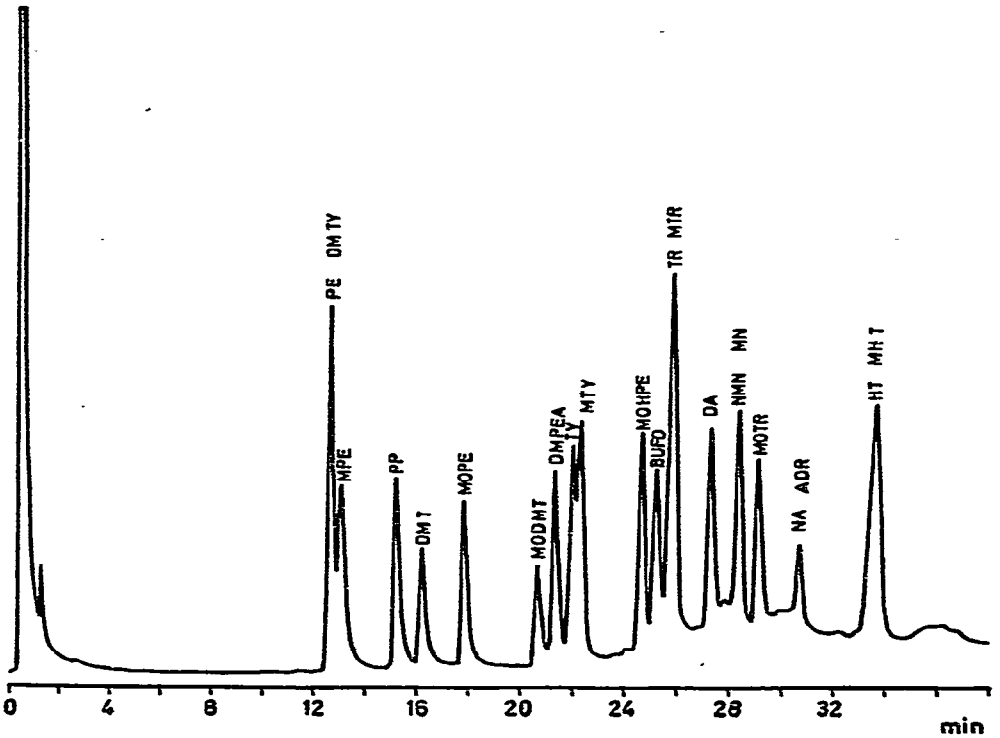

Fig. 7. Gas chromatographic separation of 21 biogenic amines as their propionyl derivatives. Stationary phase, 3% OV-101 on Chromosorb Q (80–100 mesh).

The retention times of the derivatives increase with the number of propionyl groups that are bound to the amines. A complete separation of all amines could not be obtained on packed columns, but this will be possible if capillary columns are used.

CONCLUSION

The properties of propionylated amines, in comparison with those of other derivatives, especially silyl, trifluoracetyl and pentafluorpropionyl derivatives, have considerable advantages. Propionylation is performed in aqueous solution; the resulting derivatives are more stable than the basic amines and increasingly lipophilic, so that they can be extracted quantitatively from the aqueous medium; the propionylation step leads to uniform derivatives that are eluted in a single gas chromatographic peak and that can be well separated; and storage of the propionylated amines for several weeks at room temperature does not lead to measurable decomposition.

Hence propionylation provides a useful means not only for the gas chromatographic separation and determination but also for the isolation of a wide range of catecholamines, phenylethylamines and indolalkylamines. Preliminary analyses of human urines have given very useful results[14].

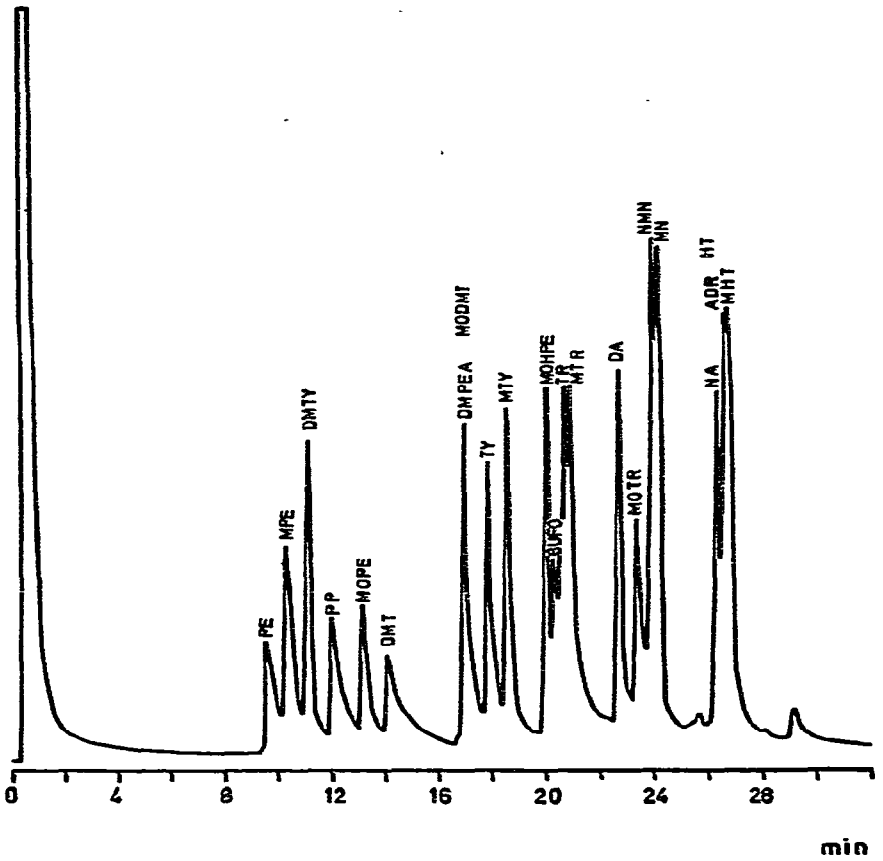

Fig. 8. Gas chromatographic separation of 21 biogenic amines as their propionyl derivatives. Stationary phase, 3% OV-17 on Chromosorb Q (80–100 mesh); glass column, 1.9 m × 2 mm I.D; oven temperature, 100–280°, increased at 6°/min; injection port temperature, 270°; detector temperature, 300°; flow-rate of carrier gas (helium), 30 ml/min.

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