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Separation and Identification of Sympathomimetic Amines by Gas-Liquid Chromatography

By E. BROCHMANN-HANSSEN and A. BAERHEIM SVENDSEN

A number of commonly used sympathomimetic amines have been subjected to gas chromatographic analysis on low-loaded columns. With the exception of certain isomers, most compounds can be separated on a column of silicone rubber SE-30. Many of the amines investigated react with ketones and the products produce sharp, symmetrical peaks on the gas chromatogram. Ephedrine and pseudoephedrine can be separated and identified on the basis of the difference in the rate of reaction with acetone. Monohydroxyphenols show strong adsorptive effects as the free bases but can readily be gas chromatographed as the acetone derivatives. The dihydroxyphenols are converted to the triacetyl derivatives which are treated with hexamethyldisilazane prior to the gas chromatographic analysis.

OF THE HUNDREDS of sympathomimetic amines which have been synthesized or isolated from natural sources, about 25 find widespread use in medical practice (1). Many methods for their identification have appeared in the literature (2–9). The official products are usually identified by color reactions, precipitation reactions, or on the basis of the melting ranges of the free amines, their salts, or prepared derivatives. Although these methods often lack specificity, they are usually satisfactory when the problem is to confirm the identity of a single compound which is available in sufficient amount. When mixtures of several amines are involved, as is often the case in pharmaceutical preparations, a separation is usually necessary before the components can be identified. In biological work, the problem is further complicated by the limited amounts available and by the presence of amines which are normal products of the animal body or formed by decomposition of biological material. Another complication stems from the fact that the metabolic products of certain sympathomimetic amines are themselves used as drugs (10, 11).

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Paper chromatography in combination with the use of several different spray reagents has recently been found to be a valuable method for problems in toxicology involving sympathomimetic amines (12).

In a preliminary communication, the authors showed that a number of widely used sympathomimetic amines could be separated by gas-liquid chromatography (13). The details of this study, which has been extended to include a larger number of compounds, are reported in the present paper.

EXPERIMENTAL

A Barber Colman model 15 gas chromatograph equipped with an argon β-ionization detector (radium 226) was used for the experimental work. The colums were glass U-tubes, 6 to 8 feet in length and having an inner diameter of 3 mm. The solid support materials were Gas-Chrom P, 100 to 140 mesh, and Chromosorb W, 60 to 80 mesh, both of which were washed with concentrated hydrochloric acid and methanolic potassium hydroxide and treated with hexamethyldisilazane (14). The stationary phases were applied by means of a solution in toluene or butanone as described by Horning, *et al.*, (15). The amines were introduced with a Hamilton microliter syringe as 1.0 μl. of a 0.5 to 1.0% solution of the free bases in chloroform, acetone, or butanone. A number of the amines were found to

react with the ketone solvents to produce reaction products which gave sharp, symmetric peaks on the chromatograms. The catechol amines, epinephrine and levarterenol, were first converted to the triacetyl derivatives (16) and then treated with an excess of hexamethyldisilazane in tetrahydrofuran, either at room temperature overnight or by refluxing for about 2 to 3 hours.

RESULTS AND DISCUSSION

The retention values of 16 sympathomimetic amines and/or their derivatives are listed in Table I. Ephedrine and pseudoephedrine do not separate as the free bases on silicone rubber SE-30, Fig. 1. However, pseudoephedrine reacts relatively rapidly

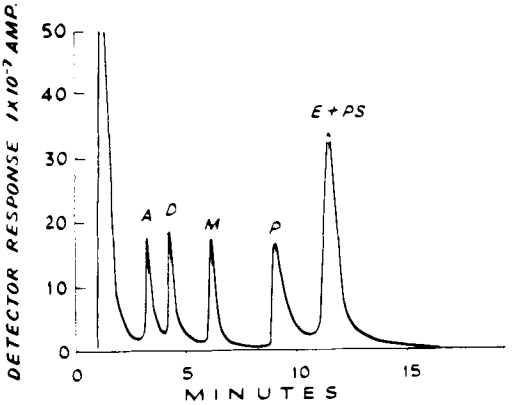

Fig. 1.—Gas chromatogram of six sympathomimetic amines on silicone rubber SE-30, 1.15%. A, amphetamine; D, methamphetamine; M, mephentermine; P, phenylpropanolamine; E, ephedrine; and PS, pseudoephedrine. Operating conditions; *cf.* Table I, footnotes[a], [c].

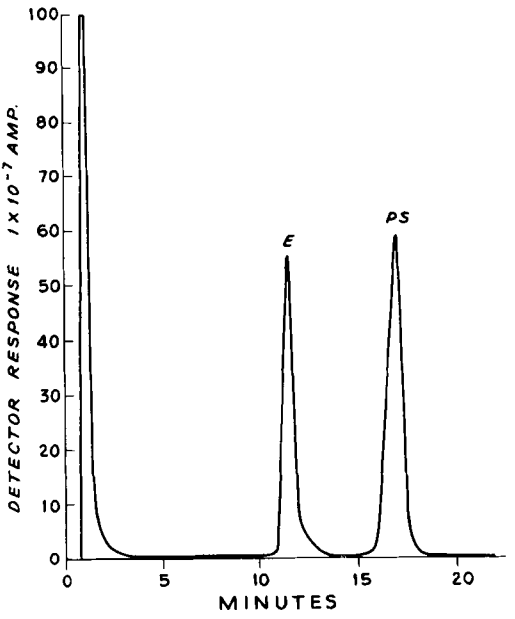

Fig. 2.—Gas chromatogram of an acetone solution of ephedrine (E) and pseudoephedrine (PS) after 3 hours at room temperature. Operating conditions, *cf.* Table I, footnotes[a], [c].

with acetone, while the reaction between acetone and ephedrine is very slow and incomplete. The reaction rate was studied gas chromatographically on the basis of the peak areas corresponding to the free amine and its ketone derivative. The relative rates of reaction of ephedrine and pseudoephedrine are expressed semiquantitatively in Table II. The two amines could easily be separated and/or identified by preparing an acetone solution and allowing it to stand for 2 to 3 hours prior to the gas chromatographic analysis, Fig. 2. Whereas all

TABLE I.—RETENTION TIMES OF SYMPATHOMIMETIC AMINES ON SILICONE RUBBER SE-30, 1.15%[a]

Compound	Column Temp., °C.	Retention Time, min. Base	Acetone Deriv.	Butanone Deriv.
Amphetamine	82[b]	7.2	16.9	28.5
Cyclopentamine		5.6	...	...
Mephentermine		16.7	...	...
Methamphetamine		10.6	...	...
Propylhexedrine		10.3	...	...
Tuaminoheptane		1.9	4.9	8.0
Amphetamine	104[c]	3.3	6.2	9.5
Cyclopentamine		2.6	...	...
Ephedrine		11.4	17.6	30.0
Mephentermine		6.1	...	...
Methamphetamine		4.6	...	...
Phenylpropanolamine		9.1	13.4	23.5
Propylhexedrine		4.0	...	...
Pseudoephedrine		11.6	16.4	27.0
Tuaminoheptane		with solvent	2.3	3.3
Benzphetamine	135[d]	15.0	...	...
Hydroxyamphetamine		2.9	5.0	7.0
Metaraminol		...[f]	9.5	14.0
Phenylephrine		9.0[g]	9.5	13.7
Synephrine		9.1[g]	9.5	14.2
Epinephrine[h]	179[e]	13.4	...	...
Levarterenol[h]		12.4	...	...

[a] Column length, 8 ft.; i.d., 3 mm.; solid support, Gas-Chrom P, 100 to 140 mesh. [b] Inlet pressure, 25 lb. p.s.i.; flow rate, 33 ml./min. [c] Inlet pressure, 21.5 lb. p.s.i.; flow rate, 22.5 ml./min. [d] Inlet pressure, 31 lb. p.s.i.; flow rate, 30 ml./min. [e] Inlet pressure, 30 lb. p.s.i.; flow rate, 25 ml./min. [f] No elution peak observed. [g] Adsorptive effects caused a badly tailing peak; retention time dependent on sample size. [h] Triacetyl trimethylsilyl ether.

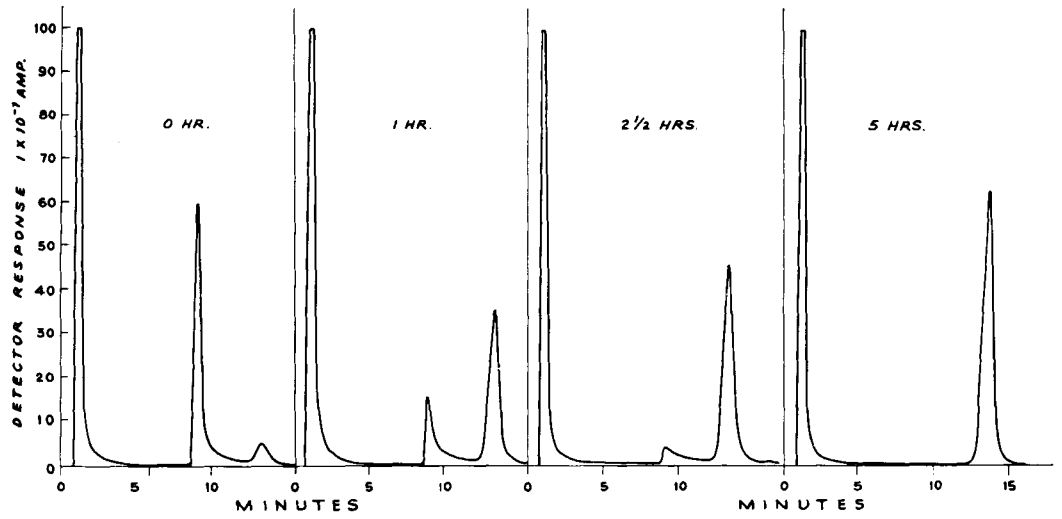

Fig. 3.—Gas chromatograms of an acetone solution of phenylpropanolamine. Formation of acetone derivative. *cf.* Table I, footnotes[a, c].

of the primary amines investigated reacted with ketones, this was not the case with all of the secondary amines. The rate of the reaction between phenylpropanolamine and acetone is illustrated in Fig. 3. As would be expected, benzphetamine, being a tertiary amine, gave no reaction with ketones. The reaction between ketones and certain primary amines has been studied by Bergel and Lewis (17, 18). According to these authors, the reaction involves an addition followed by loss of water to form an azomethine. This is accompanied by a dramatic increase in the specific rotation of optically active amines and amino acids. With secondary amines no such loss of water can occur; the reaction is probably a simple addition forming an amino alcohol. Butanone, because of the bulkier ethyl group, reacted more slowly than acetone. Methamphetamine and propylhexedrine had retention times that were very close and could not be separated on the silicone rubber column, but they were separated without difficulty on the more selective fluorosilicone fluid, Fig. 4.

Hydroxyamphetamine could easily be separated from the nonphenolic as well as from the other phenolic amines. Metaraminol could be distinguished from phenylephrine and synephrine on fluorosilicone fluid, but not on silicone rubber, Tables I and III. The isomeric phenylephrine and synephrine could not be separated on either stationary phase.

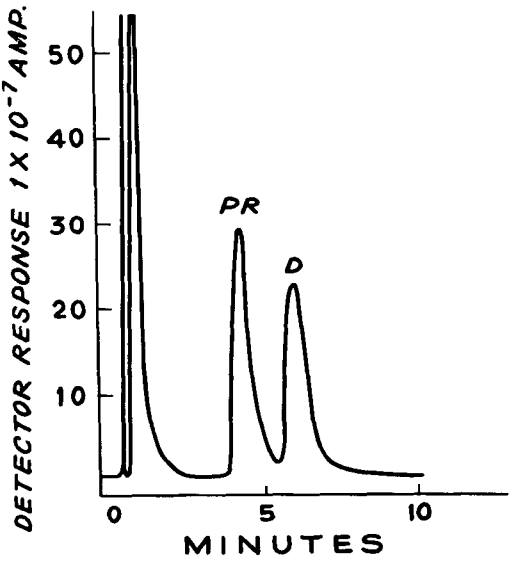

Fig. 4.—Gas chromatographic separation of propylhexedrine (PR) and methamphetamine (D). Stationary liquid, fluorosilicone fluid QF 1-0065, 2.8% on Chromosorb W, 60–80 mesh. Temp. 82°; inlet pressure, 20 lb. p.s.i.; flow rate 50 ml./min. Column, 6 ft. in length, 3 mm. i.d.

Table II.—Reaction of Ephedrine and Pseudoephedrine with Acetone as a Function of Time[a]

Reaction Time, hr.	Ephedrine 22° Eph.	Eph./Ac.	Ephedrine 56–57° Eph.	Eph./Ac.	Pseudoephedrine 22° Ps.E.	Ps.E./Ac.	Pseudoephedrine 56–57° Ps.E.	Ps.E./Ac.
0	++++	−	++++	...	++++	−	++++	...
1/4	++++	−	++++	Trace	+++	+	++	++
1	++++	−	+++	+	++	++	+	+++
2	++++	−	+++	+	+	+++	Trace	++++
5	++++	−	++	++	Trace	++++	−	++++
10	++++	−	+	+++	−	++++	...	...
24	+++	+	...	...				

[a] Ten milligrams of amine was dissolved in 1 ml. of acetone. Heating at 56–57° was carried out under reflux in micro equipment. The solutions were gas chromatographed under conditions described in Table I, footnotes[a, c].

The catechol amines are rather insoluble in most organic solvents and are, furthermore, firmly adsorbed on the low-loaded columns used in this work. Epinephrine and levarterenol were, therefore, converted to the triacetyl derivatives by treatment of an aqueous solution with acetic anhydride and sodium bicarbonate (16). Gas chromatographic analysis of triacetylepinephrine produced three peaks indicating that decomposition had taken place. This decomposition was probably due to the free alcoholic hydroxyl group which in the flash heater caused alcoholysis and transesterification resulting in a mixture of diacetyl-, triacetyl-, and tetraacetylepinephrine. Similar decomposition reactions have been observed by gas chromatography of acetylated morphine (19). Attempts to acetylate epinephrine completely did not entirely eliminate this problem. However, if the secondary alcohol group of triacetylepinephrine was converted to a trimethylsilyl ether, the resulting product gave a single, symmetrical peak. Levarterenol, after acetylation and treatment with hexamethyldisilazane, could be differentiated from epinephrine on the basis of the retention time, Table I.

TABLE III.—RETENTION TIMES OF SOME PHENOLIC AMINES ON FLUOROSILICONE FLUID, QF 1-0065[a]

Compound	—Retention Time, min.— Base[b]	Acetone Deriv.
Metaraminol	...	6.7
Phenylephrine	...	5.7
Synephrine	...	5.7

[a] Column, 6 ft. × 3 mm. i.d.; 2.8% QF 1-0065 (Dow Corning) on Chromosorb, 60–80 mesh; column temp., 138°; inlet pressure 30 lb. p.s.i. [b] No elution peaks were observed for the free amines.

CONCLUSION

With the exception of phenylephrine and synephrine, all the sympathomimetic amines studied could be separated and identified. The reaction with ketones was very useful as a means of separating and identifying certain amines which followed each other very closely as the free bases. The ketone derivatives, furthermore, gave nontailing peaks that should be suitable for quantitative work. Certain phenolic amines were difficult to gas chromatograph on low-loaded columns because of adsorptive effects. The monophenolic amines investigated gave good chromatograms as the ketone derivatives. The catechol amines gave best results by acetylation and treatment with hexamethyldisilazane prior to the gas chromatographic analysis.

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Synthesis of Diphenic Acid Derivatives

By WILLIAM D. ROLL† and GUSTAV E. CWALINA

Five new derivatives of diphenic acid were prepared for the purpose of studying their antibacterial, antihyperglycemic, antispasmodic, and/or local anesthetic activity: *o,o'*-bis(2-nitro-1,3-dihydroxypropyl)biphenyl disodium salt; *o,o'*-bis(3-benzoxy-2-benzamido-1-hydroxypropyl)biphenyl; *o,o'*-bis(β-dichloroacetamidoethyl)diphenate; N,N'-bis(benzenesulfonyl)-*o,o'*-diphenoylurea, and *m,m'*-bis(N-carboxymethyl)diphenamide.

VARIOUS types of derivatives of diphenic acid were synthesized for the purpose of making them available for pharmacological testing as possible antibacterial, antihyperglycemic, antispasmodic, and/or local anesthetic agents.

Roberts and Johnson (1) reported that diethylaminoethyl diphenate and diethylaminoethyl imidoester of diphenanilide possessed local anesthetic activity. Case and Koft (2) synthesized a similar compound, diethylaminoethyl 5,5-diaminodiphenate, which had marked local anesthetic activity. A number of substituted amine esters and amides of diphenic acid were prepared by Demers and Jenkins (3) for evaluation as possible antispasmodics and antihistamines. Compound VII of our derivatives contains an amide-ester linkage.

Woolley (4) has suggested that analogs of phenylalanine might function as antimetabolites

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