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Separation and Identification of Tryptamine-Related Indole Bases by Gas Chromatographic Methods

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INTRODUCTION

Methods for the isolation and identification of naturally occurring organic bases have been studied by chemists and pharmacologists for many years. Following the development in 1960 (1) of procedures for the separation of steroids by gas-liquid chromatography, it was demonstrated (2) that many alkaloids of relatively high molecular weight and structural complexity could be separated in the same way. Nevertheless, gas chromatographic methods are not yet widely used in studies of organic bases. The apparent reason for the relative slowness of evolution of these techniques in this area lies in difficulties often encountered when amines of complex structure are separated under ordinary chromatographic conditions. Partial or complete decomposition, trailing of peaks, and incomplete separation of closely related substances are not unusual effects. These difficulties are almost certainly due to inadequate or unsatisfactory chromatographic techniques rather than to any basic limitation imposed by the structure or properties of many of the compounds which have been studied.

The present status of work in this area was summarized by Brochmann-Hanssen (3) in 1962. Phenolic amines present special problems, and the use of trimethylsilyl ethers as derivatives was recommended for these compounds by Brochmann-Hanssen and Svendsen (4). However, Fales and Pisano (5) showed that a number of biologically important phenolic amines could be separated without derivative formation. Amines used as tranquilizers have been separated in a number of laboratories (6–8) and steroidal amines have also been studied by gas chromatographic methods (9). A few additional applications of gas chromatography to organic base separations are described in the review by Brochmann-Hanssen (3).

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The study described in this paper arose from earlier work by Holmstedt (10) on the physiologically active agent of *epená,* a South American snuff reported to produce hallucinations. An isolation procedure suitable for the separation of phenolic amines of the indole series (11) was applied to the snuff and the basic fraction was found to give a strong positive Ehrlich test. A paper chromatographic separation of the mixed bases indicated that the major component was not *N,N*-dimethyltryptamine or 5-hydroxy-*N,N*-dimethyltryptamine (bufotenin). These substances had been found earlier in another hallucinogenic snuff used in South America and the Caribbean region (11). The amount of snuff available for the investigation did not permit a classical study, involving the isolation of individual indole bases followed by characterization of each base by physical and chemical methods. A study was therefore made of gas chromatographic methods for distinguishing several different kinds of indole amines related to tryptamine, and the results were used to solve the problem of the structure of the chief indole base of *epená.*

MATERIALS AND METHODS

Relative retention times were obtained with a Barber-Colman model 10 instrument and with an EIR model AU-8 instrument. Both were equipped with argon ionization detection systems with radium foil sources. The columns were 6 ft × 4 mm glass U-tubes. The preparation of the column packings followed the usual practice at this laboratory (12). The support was Gas Chrom P, 80 to 100 mesh, inactivated with dichlorodimethylsilane. Coating was carried out by a filtration technique. The phases were: (*1*) a mixture of 7% of F-60 (a methyl *p*-chlorophenylsiloxane polymer, Dow Corning Corp.) and 1% EGSS-Z (a copolymer from ethylene glycol, succinic acid, and methyl phenyl siloxane monomers, Applied Science Laboratories, Inc.); and (*2*) 10% NGS (neopentylglycol succinate). The "flash heater" was kept 30–40°C above the column temperature, and the detection cell was held at 240°C. Samples (1-μl volume) were injected in tetrahydrofuran or acetone solution. Trimethylsilyl ethers were prepared by reaction with hexamethyldisilazane (13) in tetrahydrofuran or acetone solution.

The isolation of an organic base fraction from *epená* was carried out by a procedure known to be satisfactory for phenolic amines in the indole series (11). The crude extract gave a strong positive test with Ehrlich's reagent. A gas chromatographic analysis of the mixture is shown in Fig. 2 (Extract E).

RESULTS AND DISCUSSION

The primary objective of the present study was to determine the nature of the major indole base of *epená,* but in order to solve the problem it

was necessary to determine conditions suitable for the gas chromatographic separation and identification of indole amines substituted in various ways. Very little information was available at the start of the work about the behavior in gas chromatography of indole bases related to tryptamine, but it was considered likely that gas chromatographic methods would be entirely satisfactory for identification purposes. Fales and Pisano (5) described the separation of several indole bases, and Brochmann-Hanssen and Svendsen (4) demonstrated that trimethylsilyl ethers were suitable derivatives for the separation of phenolic alkaloids.

The biological hydroxylation of indoles is known to occur in at least three positions, leading to 4-hydroxy-, 5-hydroxy-, and 6-hydroxyindole derivatives. In order to compare the gas chromatographic behavior of positional isomers of hydroxyl-substituted *N,N*-dimethyltryptamines, the four possible isomers were chromatographed directly and as the trimethylsilyl ethers. Two columns were employed. One contained a liquid phase consisting of 7% F-60 and 1% EGSS-Z, and the other contained 10% NGS. The order of elution of the positional isomers (for the free phenolic amines) with both columns was found to be 4-, 7-, 5-, and 6-substituted. When these amines were converted to trimethylsilyl ethers, the order of elution with the F-60-Z column was found to be 7-, 4-, 5-, and 6-substituted. The 4-substituted ether was relatively unstable and

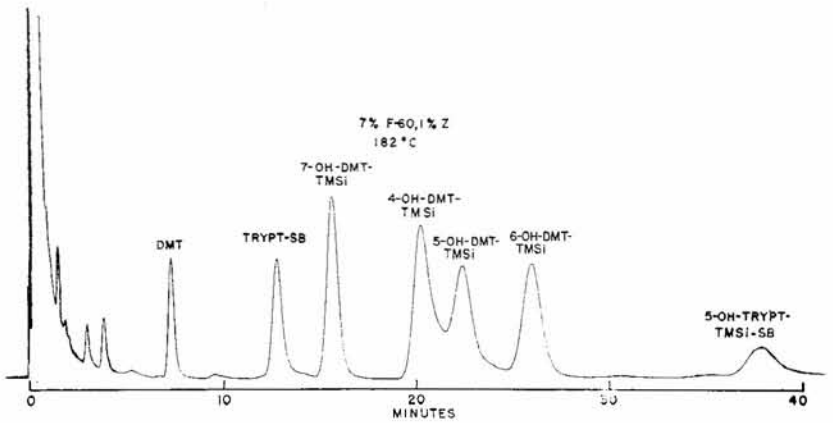

FIG. 1. Gas chromatographic separation of a mixture of closely related tryptamine derivatives. The compounds are *N,N*-dimethyltryptamine (DMT); the acetone condensation product (Schiff base) of tryptamine (TRYPT-SB); 7-trimethylsilyloxy-*N,N*-dimethyltryptamine (7-OH-DMT-TMSi); 4-trimethylsilyloxy-*N,N*-dimethyltryptamine (4-OH-DMT-TMSi); 5-trimethylsilyloxy-*N,N*-dimethyltryptamine (5-OH-DMT-TMSi); 6-trimethylsilyloxy-*N,N*-dimethyltryptamine (6-OH-DMT-TMSi); acetone condensation product of 5-trimethylsilyloxytryptamine (5-OH-TRYPT-TMSi-SB). Column conditions: 6 ft × 4 mm glass column; 7% F-60 and 1% EGSS-Z on 80–100 mesh Gas Chrom P; 182°C; 18 psi.

did not survive chromatography conditions with an NGS column. The elution order for the other trimethylsilyl ethers was the same for NGS and for F-60-Z. The separation factors for these compounds were relatively great. Figure 1 shows a separation of the four possible positional isomers. In accordance with the observation of Brochmann-Hanssen and Svendsen (4) it was found that trimethylsilyl ethers were good derivatives, although the relative instability of the 4-substituted ether was an obvious disadvantage for extensive chromatographic work. Acetyl derivatives were not investigated, since the ethers provided a satisfactory means of separating positional isomers.

Naturally occurring compounds related to tryptamine contain a variety of side chain structures. A differentiation of primary, secondary, and tertiary amine structures might be made either on the basis of a direct separation or through the separation of derivatives. Terminal (unhindered) primary amines condense readily with acetone, and a simple

TABLE 1
RELATIVE RETENTION TIMES FOR INDOLE BASES RELATED TO TRYPTAMINE

Compound	F-60-Z,[a] 182°C	NGS,[b] 216°C
Anthracene	1.00[c]	1.00[d]
N,N-Dimethyltryptamine	1.05	1.68
N,N-Diethyltryptamine	1.71	2.14
4-Trimethylsilyloxy-*N,N*-dimethyltryptamine	2.89	—
5-Trimethylsilyloxy-*N,N*-dimethyltryptamine	3.19	3.21
6-Trimethylsilyloxy-*N,N*-dimethyltryptamine	3.70	3.74
7-Trimethylsilyloxy-*N,N*-dimethyltryptamine	2.23	1.72
5-Trimethylsilyloxy-*N,N*-diethyltryptamine	5.10	3.96
Tryptamine	1.00	—
Acetone condensation product of tryptamine	1.86	3.26
5-Methoxytryptamine	2.74	—
Acetone condensation product of 5-methoxytryptamine	4.50	9.50
5-Methoxy-*N,N*-dimethyltryptamine	2.69	5.10
Serotonin	3.10	—
Acetone condensation product of serotonin	8.74	—
Trimethylsilyl ether of acetone condensation product of serotonin	5.29	— NGS[e], 227°C
4-Hydroxy-*N,N*-dimethyltryptamine	3.46	7.22
5-Hydroxy-*N,N*-dimethyltryptamine	5.77	15.0
6-Hydroxy-*N,N*-dimethyltryptamine	5.92	15.9
7-Hydroxy-*N,N*-dimethyltryptamine	4.41	11.9
5-Hydroxy-*N,N*-diethyltryptamine	8.11	—

[a] Conditions: 6 ft × 4 mm glass U-tube; 7% F-60 and 1% EGSS-Z; 182°C; 18 psi.
[b] Conditions: 6 ft × 4 mm glass U-tube; 10% NGS; 216°C; 19 psi.
[c] Anthracene time, 7.0 min.
[d] Anthracene time, 6.6 min.
[e] Anthracene time, 4.9 min; 21 psi.

method for recognizing a primary amine of this kind is to use an acetone solution of the amine for a gas chromatographic separation (3, 5, 8). The data in Table 1 show this effect for tryptamine, for 5-methoxytryptamine, and for serotonin. For a phenolic amine (serotonin) the reaction leading to a trimethylsilyl ether may be carried out in acetone solution, and the product under these circumstances will be the corresponding trimethylsilyl ether-acetone condensation product. The formation of this type of derivative is illustrated for serotonin in Fig. 1. Primary amines may be lost entirely on NGS columns, but acetone condensation products usually have a normal behavior under these conditions. *N*-Substitution usually leads to altered retention times when methyl or ethyl groups are introduced. In this work the chief consideration was that of distinguishing primary from tertiary amines, and the data in Table 1 and Fig. 1 show effective separations for amines with $—CH_2CH_2NH_2$, $—CH_2CH_2N(CH_3)_2$, and $—CH_2CH_2N(C_2H_5)_2$ side chains.

The indole bases of the South American snuff were isolated by a procedure suitable for use with phenolic amines related to tryptamine (11). The extract gave a positive Ehrlich test. A direct gas chromatographic separation gave the results shown in Fig. 2. The minor components had

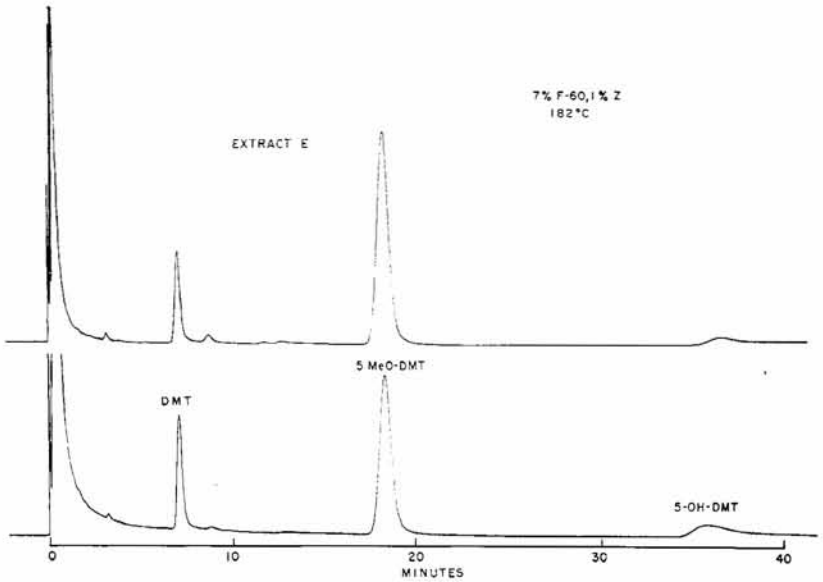

FIG. 2. Comparison of gas chromatographic analysis of indole base fraction of extract of South American snuff *epená* (Extract E) with gas chromatographic analysis of synthetic mixture of *N,N*-dimethyltryptamine (DMT), 5-methoxy-*N,N*-dimethyltryptamine (5-MeO-DMT), and 5-hydroxy-*N,N*-dimethyltryptamine (5-OH-DMT). Column conditions same as for Fig. 1.

retention times corresponding to *N,N*-dimethyltryptamine and to bufotenin, but the major alkaloid did not correspond to reference substances which were at hand. The absence of a reactive primary amino group was established by the failure to form an acetone condensation product. The absence of a phenolic group was indicated by a failure to form a trimethylsilyl ether. Inspection of the retention time data suggested that a methoxy-*N,N*-dimethyltryptamine structure was likely. Through the courtesy of Dr. Irvine Page an authentic sample of 5-methoxy-*N,N*-dimethyltryptamine was used for comparison, and identity was established by gas chromatographic techniques with the columns used for Table 1. The compound was also prepared by methylation (diazomethane) of bufotenin. A mixture of bases was made to correspond approximately to the composition seen for the natural mixture of indole bases, and a comparison of the gas chromatography records for the two mixtures is shown in Fig. 2.

5-Methoxy-*N,N*-dimethyltryptamine was found earlier by classical isolation methods in *Piptadenia peregrina* Benth. (14) and in *Dictyloma incandescens* D. C. (15). The use of gas chromatography provides a rapid means for identifying this compound in mixtures of biological origin; when only very small samples are available gas chromatographic procedures may well prove to be the best way to study mixtures of indole bases of the tryptamine group.

SUMMARY

The possibility of separating and identifying a number of indole bases related to tryptamine by gas chromatographic techniques has been investigated. Methods were found for the separation of positional isomers of phenolic amines and related compounds in this series. The major indole base of *epená*, a South American snuff with reported hallucinogenic properties, was found to be 5-methoxy-*N,N*-dimethyltryptamine.

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