GAS CHROMATOGRAPHIC ANALYSIS OF SOME PSYCHOACTIVE INDOLE BASES

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WITH the many methods available for the isolation and identification of indoles, recently summarized by Hanson (1965), one might rightly ask what position gas liquid chromatography (GLC) occupies in this context.

I will not attempt here to point out the general applicability of GLC to the separation and determination of biologically occurring compounds. Excellent general surveys are already available (Burchfield and Storrs, 1962; Szymanski, 1964). Instead I will summarize some of the research carried out in our department in recent years concerned with the identification of psychoactive tryptamines in South American drugs. By presenting the sequence of events leading to the final identification of some of these indole bases I think the audience will get a good idea of the usefulness of gas liquid chromatography as compared to other methods. The result of this research with regard to details of botany, ethnography and pharmacology has already been published (Wassén and Holmstedt, 1963; Holmstedt, 1965). We are concerned here mostly with the analytical procedures.

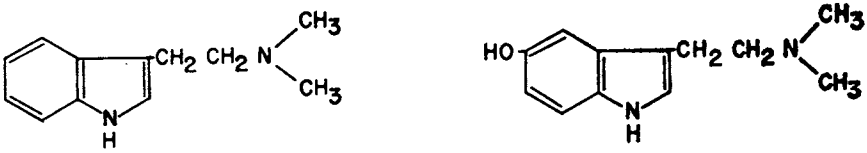
About 10 years ago E. C. Horning and co-workers isolated from the seed of *Piptadenia peregrina*, a leguminous plant, indole alkaloids which were identified by means of paper chromatography, colour reactions, fluorescence and infrared spectra (Stromberg, 1954; Fish *et al.*, 1955; Fish and Horning, 1956). They found the seed to contain dimethyltryptamine (DMT) and bufotenine (5-OH-DMT) and also the N-oxides of those two compounds (Fig. 1).

A snuff made of the seeds of *Piptadenia peregrina* has been used by South American Indian tribes to produce hallucinations and the interest of Horning and co-workers arouse from these properties of the drug. As you know, synthetic DMT is now used experimentally by psychiatrists to produce short-lasting states of illusions and hallucinations (Szara *et al.*, 1957, 1961; Böszörményi and Grunecker, 1957).

Ethnological and botanical explorations in recent years indicate, however, that *Piptadenia peregrina* is not the main constituent in all snuffs used by the South American Indians. Powders of other botanical origin have, for instance,

been collected by Schultes (1954) who found them to be prepared from two species of Myristicaceae (*Virola calophylla* Warburg and *V. calophylloidea* Markgraf). A specimen of the powder used for inhalation by the Waica tribe was placed at the author's disposal by courtesy of Mr. Georg J. Seitz of Rio de Janeiro, and it was judged interesting to compare this to the Paricà powder previously analysed.

The Waica Indians belong to a group of ethnologically related tribes called the Yanonàmi who inhabit the region between the Rio Negro and Rio Branco in north-west Brazil and who have been thoroughly investigated by Zerries (1964) and Becher (1960). Their intoxicating snuff is called *epená* and its main constituent is species of *Virola*. It is a fine brownish-grey powder which may very well have been sifted. Primarily, and during all stages of the analysis, it gives a positive Ehrlich reaction indicating the presence of indoles.



DMT

5-OH-DMT
BUFOTENINE

FIG. 1. Formulas for N,N-dimethyltryptamine (DMT) and 5-hydroxy-N,N-dimethyltryptamine = bufotenine (5-OH-DMT).

During the course of work with this native drug both bioassay and conventional biochemical techniques were used. Eventually, it was found that gas chromatography provided the best means of identification of the components in the alcaloid fraction. With regard to the techniques they have all been completely described and can be found in Holmstedt *et al.* (1964), Horning *et al.* (1964) and Holmstedt (1965). No detailed analytical description will therefore be given here.

For isolation of the organic basis the drug was treated according to the procedure described by Fish *et al.* (1955). The isolation procedure was followed in detail and the steps followed with tests using Ehrlich's reagent. The main constituents were further isolated by preparative paper chromatography run descendingly in 20% KCl. Two spots giving positive reaction with Ehrlich's reagent were identified in this way, a slow-running one giving a dark blue colour and a fast-running one giving a red-blue colour. For the sake of convenience we called these fractions A and B respectively.

The fractions A and B were compared paper chromatographically to a large number of synthetic tryptamine derivatives in several solvent systems. Because of the occurrence of bufotenine in similar plant material attention was first focused on this compound. The oxidation product of bufotenine as

described by Fish *et al.* (1955) was also put on paper. An experiment of this kind is shown in Fig. 2. It will be seen that neither bufotenine nor bufotenine-N-oxide are identical with the other compounds investigated.

When all the tryptamines available to us during this stage of the investigation were compared to fractions A and B, two of them had Rf values com-

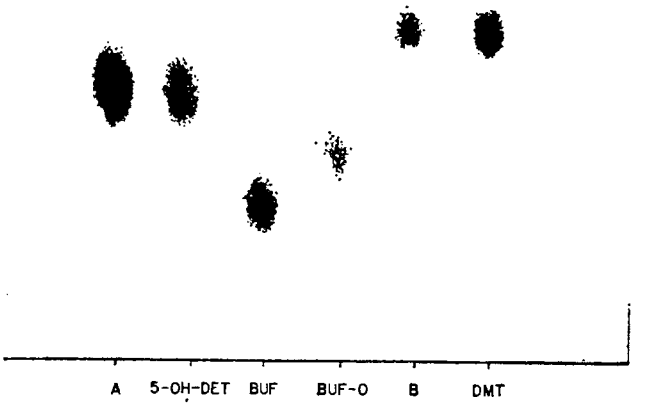


FIG. 2. Paper chromatogram. Solvent *n*-butanol–acetic acid–aqua (120:30:50). Time 16 hr

Rf values:

Fraction A	0·72
5-OH-N,N-diethyltryptamine (5-OH-DET)	0·72
Fraction B	0·78
N,N-dimethyltryptamine (DMT)	0·78
Bufotenine (BUF)	0·60
Bufotenine oxide (BUF-O)	0·65

From HOLMSTEDT, *Arch. Internat. Pharmacodyn.* (1965).

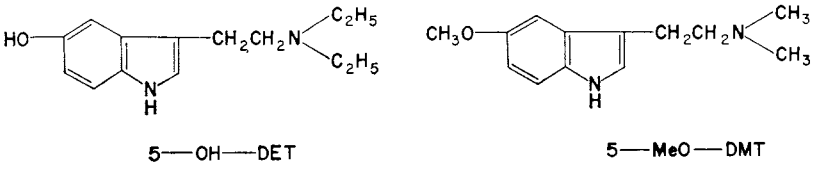


FIG. 3. Chemical formulas of 5-hydroxy-N,N-diethyltryptamine and 5-methoxy-N,N-dimethyltryptamine.

parable to those of the fractions. They were respectively 5-hydroxy-N,N-diethyltryptamine (5-OH-DET) and N,N-dimethyltryptamine (DMT) (Fig. 3). The Rf values in three solvent systems are presented in Table 1. No other compounds available at the time showed any similarity with fractions A and B in this respect.

The paper chromatograms thus gave no evidence for the presence of bufotenine in fractions A and B, but strongly indicated the identity of the fractions with 5-OH-DET and DMT respectively.

The occurrence of DMT in the drug came as no surprise, since it had been isolated from similar material before but the presence of a N,N-diethyl-substituted amine (5-OH-DET) presented problems. Not many alcaloids containing ethyl groups exist and to the author's knowledge none containing

TABLE 1

Substance	Rf value Solvent n-butanol, acetic acid glacial, water, 120:30:50	Rf value Solvent n-propanol ammonia 5:1	Rf value Solvent t-butanol, water, formic acid 207:87:6
Fraction A	0·71	0·90	0·73
5-OH–N,N-diethyl-tryptamine base	0·71	0·89	0·72
Fraction B	0·80	0·91	0·69
N,N-dimethyl-tryptamine (hydrogen oxalate) DMT	0·80	0·91	0·69

From HOLMSTEDT, *Arch. Internat. Pharmacodyn.* (1965).

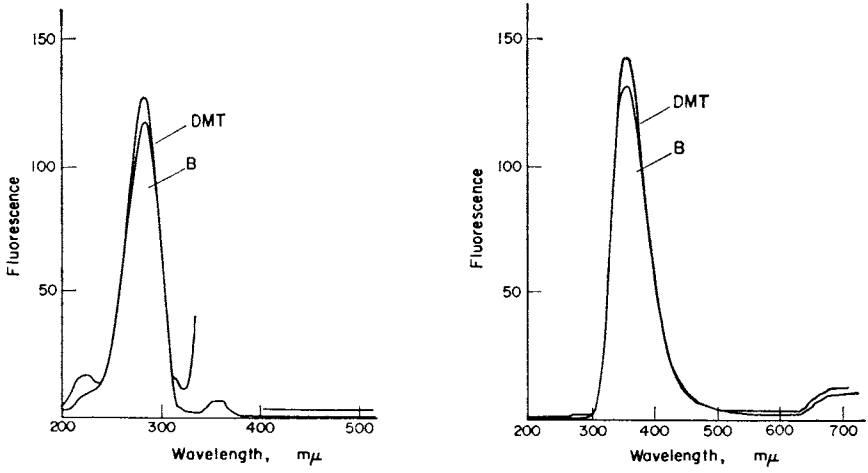


FIG. 4. Comparison of N,N-dimethyltryptamine (DMT) and fraction B (B). Medium aqua. Left activation spectrum. Right fluorescence spectrum at activation maximum 278 mμ. Fluorescence peak at 350 mμ.
From HOLMSTEDT, *Arch. Internat. Pharmacodyn.* (1965).

a diethyl group. For all practical reasons ethyl groups may be considered biosynthetical rarities (Jackson *et al.*, 1962). Because of this in the following work we concentrated our efforts upon solving the identity of fraction A.

Thin-layer chromatography disclosed one difference from the paper

chromatography in that synthetic 5-OH-DET gave a yellow colour instead of a blue one. It was, however, not possible to achieve anything but a group separation of the tryptamines in the *epená* extract. The spot given by the extract had an Rf value between those of DMT and 5-OH-DET and a blue colour. No trace of the yellow tinge of 5-OH-DET could be refound in the group-separated extract which made us somewhat suspicious.

Next we tried spectrophotofluorometry. Activation and fluorescence spectra of fractions A and B, 5-OH-DET and DMT were run and recorded (Figs. 4 and 5). These spectra were run in water solutions with both fractions and the test substances and the magnification of the Aminco–Bowman instrument

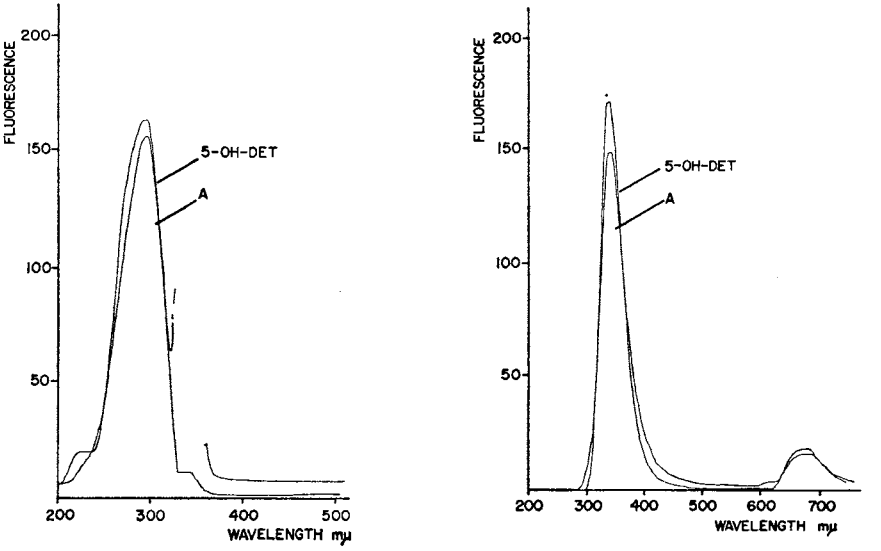


FIG. 5. Comparison of 5-hydroxy-N,N-diethyltryptamine (5-OH-DET) and fraction A (A). Medium aqua. Left activation spectrum. Right fluorescence spectrum at activation maximum of 295 mμ. Fluorescence peak at 340 mμ.

was adjusted to give approximately the same heights of the spectra. As seen from the curves the spectra are superimposable and for all practical reasons identical. It is well known that fluorescence spectra of 5-substituted indoles when run in 3 N HCl show a shift of the peak to higher wavelengths (Udenfriend, 1962). This occurred also with our fraction A as will be seen from Fig. 6 where in acid solution a peak is obtained in the region of 540 mμ. This at least gave us the valuable information that the compound represented by fraction A was substituted in the 5-position, since the shift described is not known to occur with indoles substituted in the 4, 6 or 7.

For bioassay of serotonine-like activity a modification of the method of Vane (1957, 1959) was used. Serotonine (5-HT) was served as a standard and bufotenine was included in the experiments. Fraction A was compared

both to a standard solution of 5-OH-DET and a solution run on paper in 20% KCl under conditions identical to those of fraction A. The concentration of the supposed 5-OH-DET in fraction A was calculated spectrophoto-

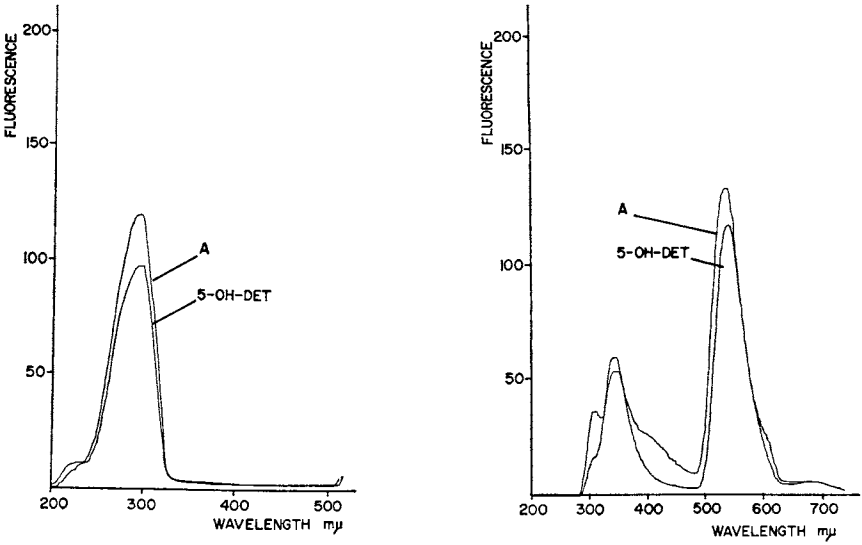


FIG. 6. Comparison of 5-hydroxy-N,N-diethyltryptamine (5-OH-DET) and fraction A (A). Medium 3 N HCl. Left activation spectrum. Right fluorescence spectrum at activation maximum 295 mμ.

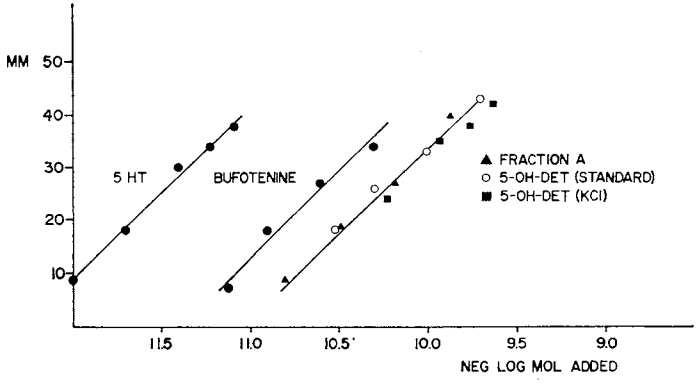


FIG. 7. Bioassay of tryptamine derivatives. Abscissa neg. log. mol. added. Ordinate contraction height in mm. Modified rat fundus strip preparation according to Vane. 5 HT = 5-hydroxytryptamine. Explanation see text. Volume of test bath 5 ml. Volume of active substance added 0·25 ml.

fluorimetrically based upon comparison with standard solutions of known concentration. As seen from the curve, Fig. 7, the dose response curves of fraction A and 5-OH-DET are identical, whether the latter one is run on

paper or not. In no case did the curve for bufotenine coincide with other curves. Judged from the data accumulated so far 5-OH-DET could therefore very well be identical with component A in the extract in spite of its "unlikely" chemical structure.

At this stage I went to Houston to work with Evan Horning and one of the problems we decided to tackle was the identity of fraction A for which we thought, as it turned out rightly, GLC would be the ideal method. A study was therefore first made of GLC methods for distinguishing several different kinds of indole amines related to tryptamine. This necessitated the determination of conditions suitable for the GLC separation and identification of indole amines substituted in various ways. Very little information was available at the start of the work about the GLC behaviour of indole bases related to tryptamine. Much work was therefore devoted to the preparation of suitable columns, packings and coating. The details will be found in Holmstedt *et al.* (1964), and the general procedures have recently also been summarized by Fales and Pisano (1964). The latter authors described in 1963 the separation

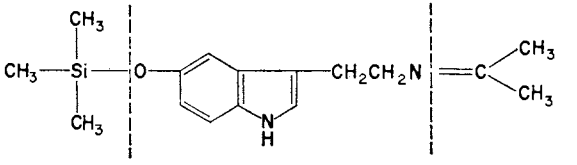


FIG. 8. Serotonine treated with hexamethyldisilazane in acetone. Left part formation of trimethylsilylether. Right part formation of eneamine (Schiff's base).

of several indole bases, and Brochmann-Hanssen and Svendsen (1962) demonstrated that trimethylsilyl (TMSi) ethers were suitable derivatives for the GLC separation of phenolic alkaloids.

The trimethylsilylations carried out with hexamethyldisilazane in solution serves to block OH groups in the compound, although increasing molecular weight lowers the boiling point of the compounds. It has been extensively used in gas chromatographic analysis of steroids (Luukainen *et al.*, 1961). We took advantage of this method also with the amines (Fig. 8, left part). Another reaction which was taken advantage of was the formation of eneamines (Schiff bases) (Fig. 8, right part). These condensation products of amines with acetone were not isolated; they are formed readily and are best used in gas chromatographic work without isolation. The completeness of the reaction can be followed in each instance by disappearance of the free amine.

If acetone is used during the extraction procedure it must be with the knowledge that it reacts with primary amines with the elimination of water to form Schiff's bases. This behaviour can often be utilized to determine whether an amine is primary or secondary since the latter do not commonly

react in this manner. The Schiff's bases elute slightly later than the parent amines on non-polar phases and exhibit less tailing due to conversion of the NH_2 to an N-isopropylidene group (Fig. 8, right part). The formation of trimethylsilylethers and Schiff's bases represents only two of the reactions

TABLE 2
RELATIVE RETENTION TIMES FOR INDOLE BASES RELATED TO TRYPTAMINE

Compound	F-60-Z,[a] 182°C	NGS,[b] 216°C
Antracene	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
Serotonine	3·10	—
Acetone condensation product of serotonine	8·74	—
Trimethylsilyl ether of acetone condensation product of serotonine	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.

From HOLMSTEDT *et al.*, *Analyt. Biochem.* (1964), and HORNING *et al.*, *Analyt. Chem.* (1964).

to which the indoles can be subjected. Later on, other derivatives have also been used successfully (Horning *et al.*, 1964).

The relative retention times for indole bases related to tryptamine and their derivatives will be found in Table 2. Naturally occurring compounds related to tryptamine may contain a variety of side chain structures. N-substitution usually leads to altered retention times when methyl (or ethyl) groups are introduced. Because of the data from the paper chromatography one could

suspect the presence of ethyl groups. One chief consideration was therefore the effective separation of compounds with $—CH_2CH_2N(CH_3)_2$ and $—CH_2CH_2N(C_2H_5)_2$ side chains. Figure 9 conclusively demonstrates the longer retention time of compounds with a $—CH_2CH_2N(C_2H_5)_2$ side chain.

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 GLC behaviour of positional isomers of hydroxyl-substituted N,N-dimethyltryptamines, the possible isomers were chromatographed directly and as the TMSi ethers. Two columns were employed (see

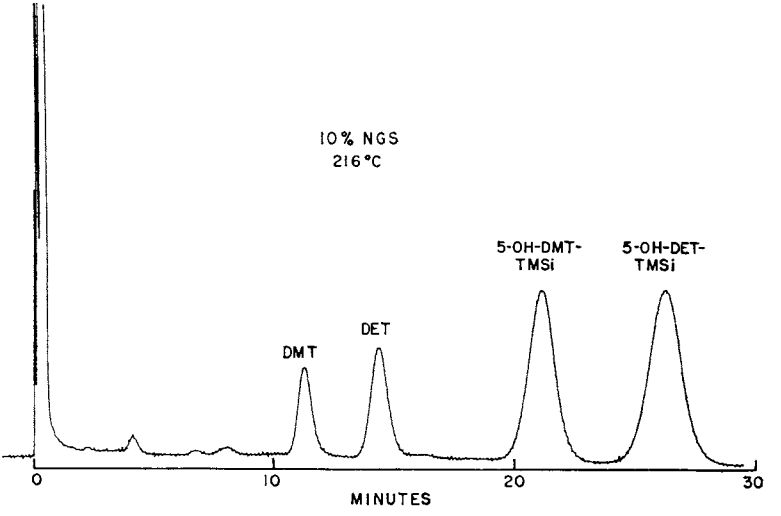


FIG. 9. Separation of N,N-dimethyltryptamine (DMT), N,N-diethyltryptamine (DET), 5-trimethylsilyloxy-N,N-dimethyltryptamine (5-OH-DMT-TMSi) and 5-trimethylsilyloxy-N,N-diethyltryptamine (5-OH-DET-TMSi). Conditions: 10% NGS on 100- to 120-mesh Gas Chrom P, 216°C, 19 psi; argon ionization detection system.
From HORNING *et al.*, *Analyt. Chem.* (1965).

Table 2). The order of elution of the position 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 TMSi ethers, the order of elution (with the F-60-Z column) was found to be 7-, 4-, 5- and 6-substituted (Fig. 10). The 4-substituted ether was relatively unstable, but could still be determined. Its parent compound psilocine is noted for its instability even under less stringent chemical conditions. The excellent separation powers of the method is apparent from Fig. 10 and this can be compared to Fig. 11 where a fluorescence analysis fails to distinguish between DMT + 5-OH-DMT + 5-HT in a synthetic mixture. The great advantage of gas chromatography in this case is that it

allows the identification also of position isomers with identical molecular weight.

We then proceeded to the analysis of the *epená* extract by direct GLC separation. The results were those in Fig. 12 and Fig. 13, upper parts. The minor components had retention times corresponding to N,N-dimethyltryptamine and to bufotenine, but the major alkaloid did not correspond to reference substances which were at hand. The synthetic derivatives containing $—CH_2CH_2N(C_2H_5)_2$ side chains had much longer retention times than any of the components of the drug.

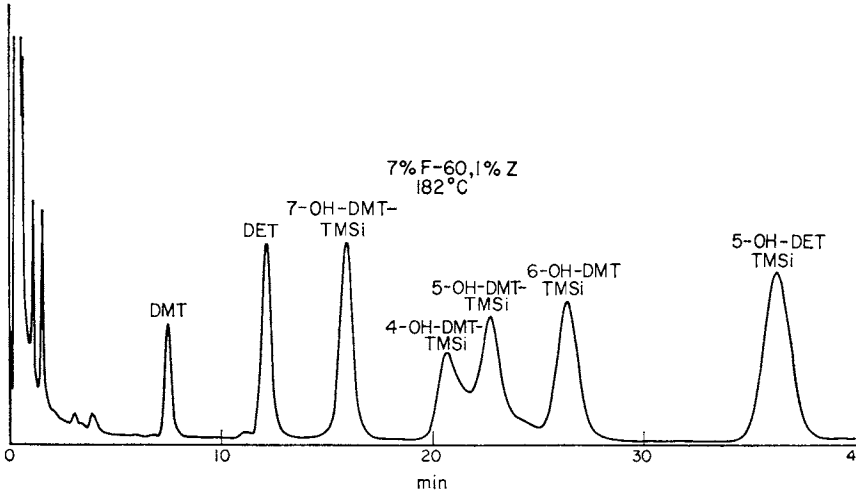


FIG. 10. Separation of tryptamine-related indole bases. The compounds are N,N-dimethyltryptamine (DMT), N,N-diethyltryptamine (DET), 7-trimethylsilyloxy-N,N-dimethyltryptamine (7-OH-DMT-TMSi), 4-trimethylsilyloxy-N,N-dimethyltryptamine (4-OH-DMT-TMSi), 5-trimethylsilyloxy-N,N-dimethyltryptamine (5-OH-DMT-TMSi), 6-trimethylsilyloxy-N,N-dimethyltryptamine (6-OH-DMT-TMSi), and 5-trimethylsilyloxy-N,N-diethyltryptamine (5-OH-DET-TMSi). Conditions: 7% F-60, 1% EGSS-Z, on 100- to 120-mesh Gas Chrom P, 182°C, 18 psi; argon ionization detection system.
From HOLMSTEDT, *Arch. Internat. Pharmacodyn.* (1965).

When the extract was treated with acetone, this did not bring about any change in the retention time, indicating the absence of a reactive primary amine group in the side chain. Again when the same extract was submitted to a trimethylsilylation with hexamethyldisilazane this did not change the position of the peaks, providing information that no phenolic group was present as had originally been suspected when previous analytical methods strongly indicated that 5-OH-DET was identical to fraction A. Inspection of the retention time data suggested that a methoxy-N,N-dimethyltryptamine structure was likely for the major component. 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) had previously been investigated by Gessner and

Page (1962) pharmacologically, but we had had no opportunity to include it among our test compounds. A telephone call to Dr. Page's department brought us an authentic sample of the synthetic product within 24 hr. Identity was established by GLC techniques with the major component of the extract. A mixture of bases was made to correspond approximately to the

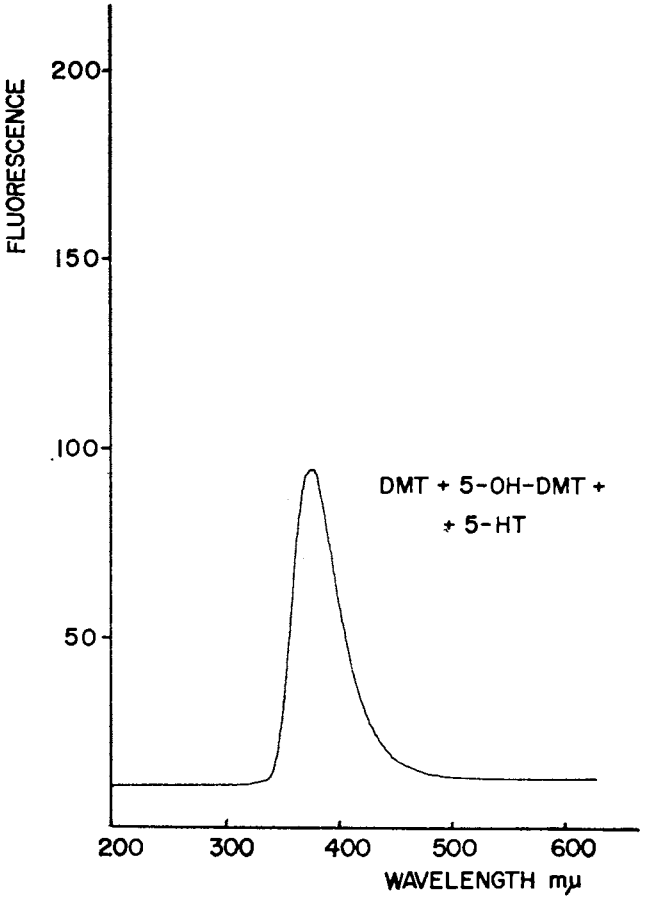


FIG. 11. Fluorescence spectrum of a mixture of N,N-dimethyltryptamine (DMT), 5-hydroxy-N,N-dimethyltryptamine (5-OH-DMT) and serotonine (5-HT). Activation at 290 mμ.

composition seen for the natural mixture of indole bases, and comparison of the GLC records for the two mixtures is demonstrated in Fig. 12 and Fig. 13.

Thus, we finally succeeded to prove that our original fraction A was not 5-hydroxy-N,N-diethyltryptamine but 5-methoxy-N,N-dimethyltryptamine or methylated bufotenine.

This exposition could be brought to a conclusion here, except for the fact that it appeared to me necessary to compare also with previously used methods 5-MeO-DMT and 5-OH-DET, even in the face of the overwhelming evidence that our original fraction A was identical with the former. Fluorescence spectra of the three and a bioassay of 5-MeO-DMT and 5-OH-DET were therefore made and are presented in Fig. 14 and Fig. 15. As seen, completely identical results were obtained which on the one hand provided a consolation for our previous mistake of 5-OH-DET being the compound identical with

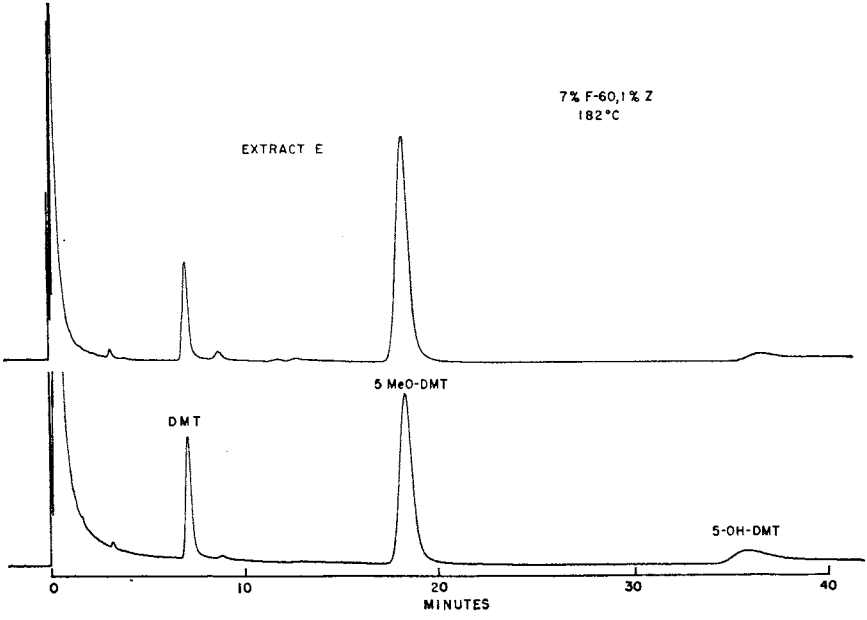


FIG. 12. Comparison of the gas chromatographic analysis of the indole base fraction of an extract of the South American snuff *epená* (EXTRACT E) with a gas chromatographic analysis of a synthetic mixture of N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) and 5-hydroxy-N,N-dimethyltryptamine (5-OH-DMT). Column conditions: 6 ft × 4 mm glass column; 7% F-60 and 1% EGSS-Z on 80- to 100-mesh Gas Chrom P, 182°C, 18 psi.
From HOLMSTEDT *et al.*, *Analyt. Biochem.* (1964).

fraction A. On the other hand, it serves well to demonstrate that although fluorescence and bioassay at the moment are more sensitive than gas chromatography, their power to distinguish between compounds with the related structures is much lower than that of GLC. When the former methods are used with biological extracts, a mixture of compounds may be quantitated instead of a single component.

5-MeO-DMT is only one of the many indoles found in plants (Stowe, 1958; Cerletti, 1960). GLC provides the easiest and most rapid way of

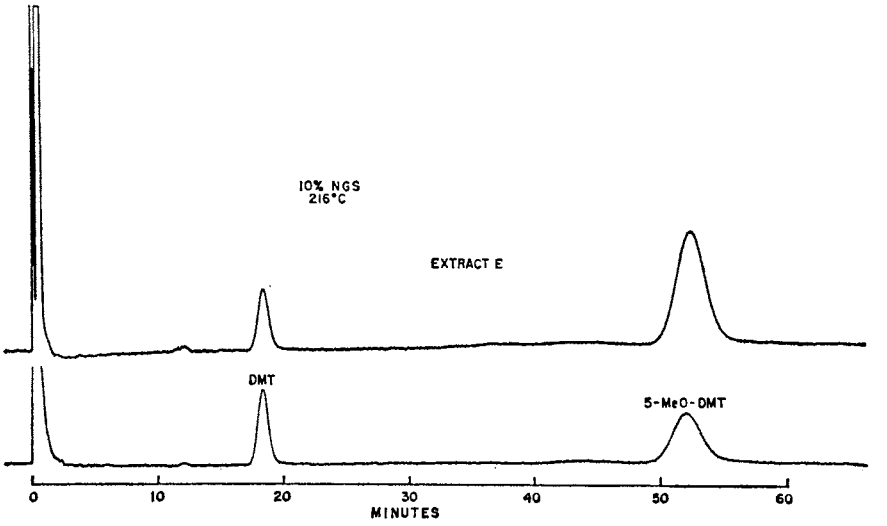


FIG. 13. Comparison of gas chromatographic separation of bases for: upper panel, extract (E) from *epená*, and, lower panel, mixture of reference samples of N,N-dimethyltryptamine (DMT) and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT). Conditions: same as for Fig. 9.
From HORNING *et al.*, *Analyt. Chem.* (1964).

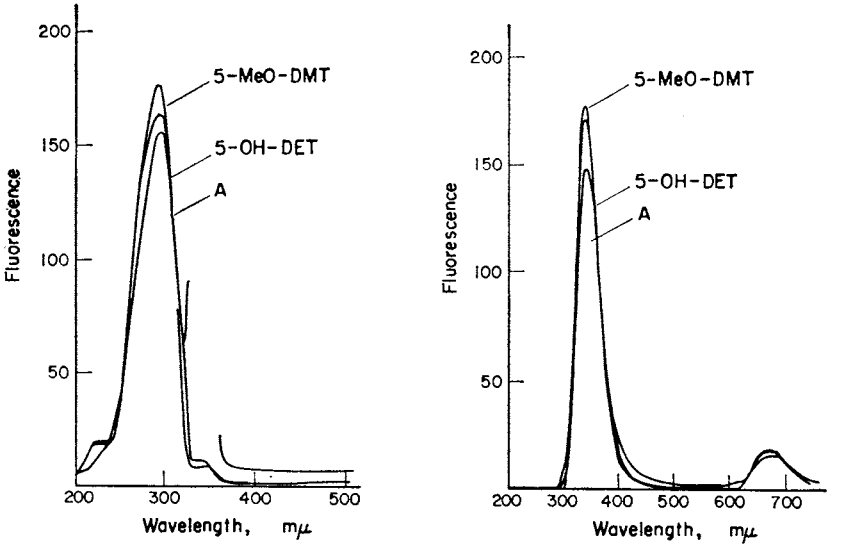


FIG. 14. Comparison of 5-MeO-DMT, 5-OH-DMT and fraction A. Conditions as in Fig. 5.
From HOLMSTEDT, *Arch. Internat. Pharmacodyn.* (1965).

determination of these compounds in various plant materials, and it can be expected to play a large role in the future for identification of hitherto unknown structures, especially since only small samples of the original material are needed. Work is at present on the way in our laboratory, where parts such as root, leaf, bark of reputed psychoactive plants are investigated. An example of this is the rapid identification of harmine derivatives in species of Banisteriopsis.

In my opinion, the experience gained by GLC analysis of indoles in plants will provide us with a very good background for future work with other

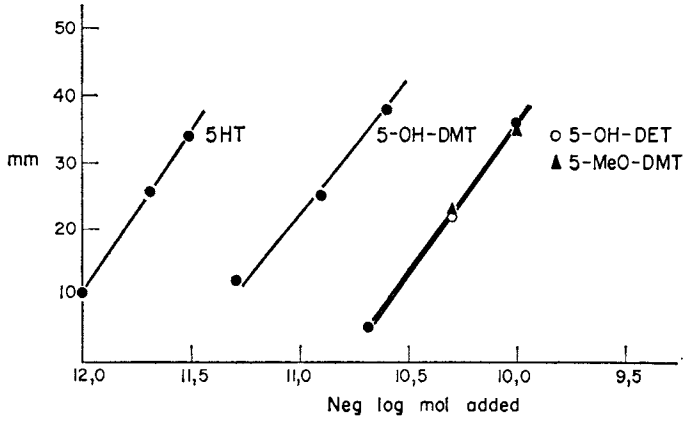


FIG. 15. Bioassay of tryptamine derivatives. Conditions as in Fig. 7. From HOLMSTEDT, *Arch. Internat. Pharmacodyn.* (1965).

sources of tryptamines such as urine and brain. For current analytical gas chromatography columns, only a very small volume may be applied (1–10 μl); hence, high indole concentrations must be achieved for suitable detection. This is in contrast to spectrophotofluorometric techniques whereby indoles may be detected at the 0·1 μg level in 1 ml of fluid. However, in situations where high concentrations can be obtained, gas liquid chromatography permits not only qualitative resolution, but quantitative estimation as well. A final outstanding feature which I hope has been well documented is the ability for gas liquid chromatography to resolve closely related compounds.

Along with the development of better extraction and concentration procedures an increasing sensitivity of the gas chromatographic systems may be expected.

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