to the oxime, which was found to melt in part around 95°; on further heating the sample resolidified and remelted at 194°.

The oxime base of III is mentioned in the literature[2] but no details are given. A sample, prepared from authentic III and recrystallized from dilute ethanol, melted in part around 95°, resolidified and remelted at 197°. Its mixture with the oxime of the methylation product of IV melted at 195–196°.

Anal. Calcd. for $C_{18}H_{22}N_2O_4$: N, 8.48. For a monohydrate: N, 8.04. Found: (sample dried *in vacuo* at 100°): N, 8.08.

For further proof of identity, the infrared spectra, in KBr discs, of the methylation product of IV, and of the oxime derived from it, were taken[8] and compared with those of authentic samples of III and its oxime. Complete agreement was found.

(8) We are indebted to Dr. S. Lieberman, Columbia University College of Physicians and Surgeons, N. Y., for permission to use the Perkin Elmer 21 spectrophotometer.

RICHMOND HILL, N. Y.

[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, U. S. PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION AND WELFARE]

Piptadenia Alkaloids. Indole Bases of *P. peregrina* (L.) Benth. and Related Species

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Four indole bases have been found to occur in the seeds and pods of *P. peregrina* (L.) Benth. and *P. macrocarpa* Benth. The pods contain N,N-dimethyltryptamine, and the seeds contain bufotenine, bufotenine oxide and N,N-dimethyltryptamine oxide. In addition, a 5-hydroxyindole base of unknown structure was found in *P. macrocarpa* seeds. The identification of these compounds was accomplished by isolation through paper chromatography, and comparison with authentic samples involving R_f data, ultraviolet spectra and fluorescence analysis. The synthesis of these two oxides, hitherto unknown as components of plant or animal metabolism, is described.

One of the most interesting customs of certain Indian tribes of South America and the Caribbean is the use of a ceremonial snuff inhaled through a bifurcated tube fitting the nose. The use of this snuff during the late fifteenth century was described by Ramon Pane, and a number of later writers have also described its preparation and use.[1,2] All accounts stress the power of the snuff to produce a kind of intoxication during which visions were reputed to occur; excessive doses apparently induced a violent temporary derangement. Seeds of *Piptadenia peregrina* (L.) Benth. and possibly *P. macrocarpa* Benth. were reportedly used for the preparation of the snuff, and the isolation of bufotenine from *P. peregrina* seeds of Puerto Rican origin was described recently.[3]

Further work on the indole bases of *Piptadenia* species has now been carried out. Because of current interest in the effects of 5-hydroxyindoles on the central nervous system, it was hoped that some direct means could be found to establish a connection between the suggested source of the snuff, *Piptadenia* seeds, and authentic snuff. Fortunately, the Smithsonian Institution collection includes under Cat. No. 387781 a Piaroa snuff box of relatively recent origin (1949); the box was found to contain a few milligrams of snuff, and this was used for comparison with "synthetic" laboratory snuff samples prepared in a way that corresponded as closely as possible to the descriptions of Humboldt and Spruce.[2,4] Using paper chromatography in a propanol–ammonia system, with Ehrlich's reagent as a spray, one indole area of major intensity and four additional lighter areas were found for the authentic snuff. With laboratory snuff from *peregrina* seeds of Puerto Rican origin, the area of major intensity was duplicated (bufotenine), and two additional areas were also identical in R_f value, in color of the sprayed area, and in relative intensity of the color. Two faint areas present for the South American snuff were not observed for the laboratory samples. This evidence indicates that the origin of the authentic snuff was undoubtedly *Piptadenia* seeds, although the species could not be identified with certainty.

Earlier tests on leaves, bark and seeds of *P. peregrina* indicated that organic bases were present only in the seeds. These observations were extended through examination of the seeds and seed pods of *P. peregrina*, *P. macrocarpa* and *P. paniculata* from several areas (Brazil, Florida, Puerto Rico). With the exception of *paniculata*, all seed samples gave very strong alkaloid tests, while the pods gave tests of varying strength (Table I). Preliminary experiments indicated that the indole bases, including bufotenine, were readily extractable with alcohol or with a tetrahydrofuran–chloroform–ammonia mixture and could be separated by paper chromatography for purposes of identification. A combined seed–seed pod sample of *P. macrocarpa* from Florida was chosen for detailed examination.

TABLE I

PRECIPITATION TESTS FOR ALKALOIDS

Species	Source	Seeds	Pods
P. peregrina (L.) Benth.	Puerto Rico, 1954	+++	+
P. peregrina	Puerto Rico, 1955	+++	+++
P. peregrina	Brazil, 1955	+++	+++
P. macrocarpa Benth.	Brazil, 1955	+++	+++
P. macrocarpa	Florida, 1955	+++	+
P. paniculata Benth.	Brazil, 1955	+	*a*

[a] Not received.

(1) The distribution of modern tribes following this custom is shown by J. E. Cooper in "Handbook of the South American Indian," Bureau of American Ethnology, Bulletin 143, U. S. Government Printing Office, Washington, D. C., 1949, Vol. 5, pp. 536–539.

(2) Summaries of several early accounts are in Safford, *J. Washington Acad. Sci.*, **6**, 547 (1916). Spruce's description of snuff preparation ("Notes of a Botanist on the Amazon and Andes," Ed. by A. R. Wallace, the Macmillan Co., Ltd., London, 1908, Vol. II, pp. 426–430) differs from that of Humboldt in the omission of calcined calcium carbonate.

(3) V. L. Stromberg, THIS JOURNAL, **76**, 1707 (1954).

(4) We are indebted to Dr. Herbert W. Krieger, Curator, Division of Ethnology, Smithsonian Institution, for advice and help in securing the authentic snuff sample.

The general procedure for identification of the bases involved a paper chromatographic separation (Whatman 3 MM, propanol–ammonia) of the principal components. Five distinct zones were eluted with ethanol, and these were employed for detailed identification work. Each component was chromatographed separately and in mixture with a reference compound. Three or four solvent systems were employed. The R_f values are in Table II. A pure sample was eluted in each case from a separate chromatographic run, and these were used for ultraviolet absorption spectra determinations and for fluorescence analysis spectra. These data were compared with corresponding spectra for reference compounds (Tables III and IV). In this way four indole components were identified as bufotenine, N,N-dimethyltryptamine, bufotenine oxide and N,N-dimethyltryptamine oxide. A fifth indole (E) remained unidentified, but its properties (discussed in the Experimental section) indicated a 5-hydroxyindole structure. It was not identical with serotonin, N-methylserotonin, bufotenidine or dehydrobufotenine.

TABLE II

PAPER CHROMATOGRAPHIC DATA

R_f VALUES FOR SOLVENT SYSTEMS

	I	II[a]	III[b]	IV[a]
N,N-Dimethyltryptamine (A)	0.86[a]	0.92	0.67	0.71
Bufotenine (B)	.82[b]	.89	.62	.51
N,N-Dimethyltryptamine oxide (C)	.56[a]	.70	.76	.72
Bufotenine oxide (D)	.35[b]	.46	.72	.62
Unknown (E)	.27[b]		.68	.49

[a] S & S 507, unwashed. [b] Whatman 3 MM, washed.

TABLE III

ULTRAVIOLET SPECTRA DATA

Compound	Maxima, mμ 1	2	3
N,N-Dimethyltryptamine	274	283	291
(A)	275	283	291
N,N-Dimethyltryptamine oxide	274	282	290
(C)	274	282	291
Bufotenine	279	301	314[a]
(B)	279	301	315[a]
Bufotenine oxide	278	301	314[a]
(D)	278	302	314[a]

[a] Shoulder.

TABLE IV

FLUORESCENCE ANALYSIS DATA

Compound	Position of maxima, mμ Exitation	Emission
N,N-Dimethyltryptamine (A)	283	350
N,N-Dimethyltryptamine oxide (C)	283	349
Bufotenine (B)	300	340
Bufotenine oxide (D)	308	330
(E)	305	335

The validity of these identifications may be estimated by examination of the data for bufotenine and N,N-dimethyltryptamine. The first compound previously was isolated in crystalline form from *P. peregrina*. The solvent systems and papers employed gave excellent resolution and good reproducibility for indole bases; in this case the R_f values and colors were identical for the natural and authentic reference compounds. The ultraviolet and fluoresence analysis curves are not adequate in themselves for the establishment of structure (for example, N,N-dimethyltryptamine and its oxide give almost identical ultraviolet absorption curves and fluorescence analysis curves), but these data, in conjunction with the paper chromatographic behavior, leave no doubt about the identification of the amines. The identification of the two N-oxides posed a greater problem, since these oxides were unknown until the present work. However, synthetic samples of the oxides were prepared by peroxide oxidation of the corresponding bases, and these synthetic substances were found to be identical with two of the Piptadenia components. In separate experiments it was found that reduction of both natural and synthetic oxides to the parent bases was readily accomplished by zinc–acetic acid, and these chemical data, together with the spectra of the oxidized forms, served to establish the N-oxide structure.

While the occurrence of N-oxides in plants is not unusual, separate experiments were undertaken to determine whether the oxides were indeed present in both seeds and pods, or whether they were formed during the isolation procedure. The rather surprising observation was made that the pods of both *P. peregrina* and *P. macrocarpa* contained only N,N-dimethyltryptamine; bufotenine and the two oxides were not present. It was further found that N,N-dimethyltryptamine oxidizes rather easily in solutions exposed to air, and that consequently the isolation of its oxide after extensive experimental manipulation may be an artifact. Comparable experiments demonstrated the presence of bufotenine oxide and dimethyltryptamine oxide, along with bufotenine, in both *peregrina* and *macrocarpa* seeds. Since the oxidation of bufotenine was never observed in the absence of a specific oxidizing agent, it may be presumed that this oxide is indeed present in the seeds. The unidentified indole base (E) (Tables II, IV) was also found in Brazilian *macrocarpa* seeds, but it was not observed as a component of the pods.

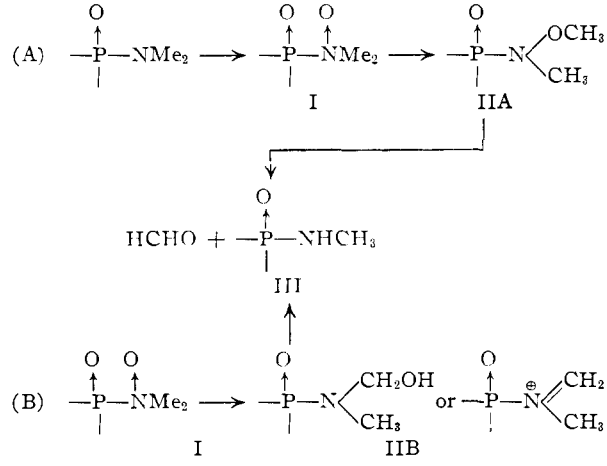
These determinations raise several interesting questions of comparative biochemistry. A recent investigation by Page[5] of the indole bases in normal human urine indicated the presence of serotonin, N-methylserotonin, bufotenine and two additional unidentified indole bases. Titus and Udenfriend,[6] working with toads, established the presence of serotonin along with bufotenine in the secretion of the parotid gland, and in separate studies Udenfriend developed the hypothesis that tryptophan is converted to serotonin *via* 5-hydroxytryptophan, and that the end product of this metabolic sequence is 5-hydroxyindoleacetic acid. The presence of bufotenine in such varied sources as *Piptadenia* seeds, toads, certain mushrooms and human urine indicates a ubiquitous nature. This compound is evidently associated with serotonin as a product of tryptophan metabolism, but its metabolic route of synthesis and its function are unknown. It may possibly be derived from serotonin by methylation, yet the distribution of N,N-dimethyltryptamine,

(5) F. M. Bumpus and I. H. Page, *J. Biol. Chem.*, **212**, 111 (1955).
(6) E. Titus and S. Udenfriend, *Federation Proc.*, **13**, 411 (1954).

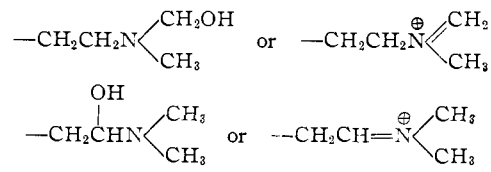
bufotenine and the two amine oxides in *Piptadenia* seeds and pods indicates that a common substance was supplied to the growing seed pod, and that a peroxidic $(OH)^{\oplus}$ type of oxidation leading to bufotenine and to bufotenine oxide occurred in the seed. Serotonin seems less likely to be this common substance than N,N-dimethyltryptamine or tryptamine.

These data point to the existence of a new pathway of tryptophan metabolism. The part sequence N,N-dimethyltryptamine → bufotenine → bufotenine oxide is suggested by the distribution pattern. The immediate precursor of N,N-dimethyltryptamine and the relationship of (E) to this sequence is unknown. Current speculations on the role of tryptophan metabolites in the central nervous system stress the possible role of serotonin; in view of Page's isolation of bufotenine from normal human urine it may be of equal importance to study the related problems involved in bufotenine synthesis and metabolism.[7]

In connection with the present isolation of two amine oxides as components in tryptophan metabolism, two recent notes should be cited. Recent studies by Tsuyuki, Stahmann and Casida[8] have shown that octamethylpyrophosphoramide (an insecticide) may be oxidized enzymatically and by chemical agents to a potent anticholinesterase (I). This metabolic transformation product is unstable in both acid and alkali by reason of its rearrangement to a less active but more stable substance (IIA) which in turn may be hydrolyzed easily to formaldehyde and heptamethylpyrophosphoramide (III). This reaction was interpreted as follows (A)



It is more likely that the reaction proceeds as in (B), since the carbinolamine structure IIB as well as the corresponding anhydronium base structure, are in fact the expected products of amine oxide rearrangements; this was pointed out by Wenkert[9] in a general review of the biogenesis of certain kinds of alkaloids. The significance of Stahmann's observation lies in the fact that it offers a possible explanation for the general reaction of N-dealkylation often observed in studies of drug metabolism, and it also may occur for normal metabolites. For example, the possible products from a similar reaction with bufotenine oxide and N,N-dimethyltryptamine oxide would have the part structures



We are currently studying oxide rearrangements in this case to determine whether additional metabolic components can be found which might arise in this way. We also hope to examine the generality of this possible biosynthetic pathway of N-dealkylation.

Experimental[10]

Examination of Snuff.—Several samples of laboratory snuff were prepared from *P. peregrina* seeds of Puerto Rican origin, using the descriptions of Humboldt and Spruce as a guide.[2] They were (1) a coarsely ground sample of seeds was roasted at 175° for 40 minutes, followed by mortar grinding, (2) preparation (1) was repeated with addition of calcium carbonate before heating, and (3) a fermented sample of seeds was dried and ground. A few milligrams of each snuff sample was taken for comparison with the same amount of authentic snuff obtained from a snuff box now in the Smithsonian Institution. The solids were ground thoroughly with about 0.5 ml. of ethanol; the solutions were placed on S. & S. 589R paper and developed in a propanol–ammonia system. Relatively good resolution was obtained. One area of major intensity corresponding to bufotenine was present for all samples. Two additional areas were common to the laboratory and authentic snuff chromatograms. Two faint areas present for the authentic snuff were not present for the laboratory samples. The colors of the common areas were identical. Ehrlich's reagent was used as a spray.

Alkaloid Tests (Table I).—Qualitative tests for the presence of organic bases were carried out as described for another problem.[11]

Isolation of Organic Bases.—A 450-g. sample of seed pods and seeds of *P. macrocarpa* (Florida, 1955) was ground in a Wiley mill, and the powder was stirred with a mixture of 1950 ml. of chloroform, 1050 ml. of tetrahydrofuran and 225 ml. of ammonium hydroxide for 1 hour at 40°. After removal of the solids, the organic solution was separated and washed well with 8 *N* ammonium hydroxide solution. The total volume was reduced to about 50 ml. by distillation under reduced pressure; this was diluted with chloroform, and the organic solution was washed well with 2 *N* hydrochloric acid solution. The aqueous extracts were combined, made basic with solid sodium carbonate solution, and extracted repeatedly with chloroform until a negative Ehrlich reagent test occurred for the aqueous solution. The chloroform solutions were combined and dried with magnesium sulfate. Removal of the solvent provided 1.4 g. of a crude, black gum which gave strong positive tests with Ehrlich reagent and with a nitrous acid–α-nitroso-β-naphthol reagent.[12] In the case of this plant material, the seeds were found to average 15% by weight of the total, and since the alkaloid precipitation tests for the pods were weak, it may be concluded that the organic base content of the seeds is approximately 1.5–2.0%. This is similar to the 1.6% estimate made for *P. peregrina* seeds.

Small samples of the crude mixture were subjected to ascending paper chromatography on washed Whatman 3 MM paper. The samples were placed on the paper with

(7) One of the unidentified indoles reported by Page for normal human urine had an average R_f value of 0.44 in a butanol–ammonia system. The R_f value found here for bufotenine oxide in the comparable propanol–ammonia system was 0.35–0.37.

(8) H. Tsuyuki, M. A. Stahmann and J. E. Casida, *Biochem. J.*, **59**, iv (1955).

(9) E. Wenkert, *Experientia*, **10**, 346 (1954).

(10) Melting points were taken on a Kofler stage. Ultraviolet data were obtained with a Cary spectrophotometer.

(11) W. H. Tallent, V. L. Stromberg and E. C. Horning, THIS JOURNAL, **77**, in press (1955).

(12) S. Udenfriend, H. Weissbach and C. T. Clark, *J. Biol. Chem.*, in press.

ethanol–chloroform. A propanol–ammonia system was used for development, and the entire width of the paper was utilized. Five zones were cut, at R_f values determined by strip spraying with Ehrlich reagent. Each zone was eluted with ethanol. The five solutions obtained in this way were used for further paper chromatographic work. Comparisons were made with reference samples in three or four solvent systems (Table II). For the determination of spectra, each eluted sample was carried through a separate purification on washed Whatman 3 MM paper. The pure samples obtained in this way were in ethanol solution; these solutions were used for ultraviolet spectra determinations and for fluorescence analysis spectra, after suitable adjustment of volume.

A number of separate experiments were carried out on the seeds and seed pods of most of the specimens in Table I. It was found that alcohol extracts of the seeds or pods could be used directly for paper chromatographic work, but the separations were generally incomplete, and best results were obtained when a preliminary solvent exchange procedure of the usual kind for the isolation of an organic base was employed. The short procedure was helpful in determining that bufotenine oxide was present after a minimum amount of experimental manipulation. Separate experiments on pods of *P. peregrina* and *P. macrocarpa* using a preliminary extraction procedure showed that only N,N-dimethyltryptamine was present. Work on the seeds alone indicated the presence of bufotenine, bufotenine oxide, N,N-dimethyltryptamine oxide and an indole of unknown structure (E).

Table II.—Five zones derived from *P. macrocarpa* seeds and pods were eluted separately to provide eluates (A) to (E). Each solution was subjected to separate comparison with a reference compound; this was done in the usual way, with concurrent development of the reference compound, a mixed sample, and a sample of natural origin. Solvent systems were (I) propanol–1 *N* ammonium hydroxide (5:1); (II) methyl ethyl ketone–*t*-butyl alcohol–diethylamine–water (40:40:4:20); (III) *n*-butyl alcohol–acetic acid (10:4) saturated with water; (IV) *t*-butyl alcohol–water–formic acid (207:87:6). The time for development, at room temperature, varied from 16 to 64 hr. depending on the paper and the system. The papers employed are noted in the table. The spray was a 0.5% solution of *p*-dimethylaminobenzaldehyde in 1 *N* hydrochloric acid.

Ultraviolet Spectra (Table III).—The spectra of eluted samples of natural origin (A-D) were compared with curves for authentic reference compounds at approximately the same concentration (as judged by optical density) and in the same solvent (ethanol). Wave lengths of the principal absorption bands are in the table.

Fluorescence Analysis Spectra (Table IV).—The instrument employed for this work was designed by Dr. R. L. Bowman of the Laboratory of Technical Development, National Heart Institute. After establishing the wave length required for maximum emission, the exciting light was held at this wave length, and the emitted light was scanned. Measurements were within ± 5 mμ for exciting and emitted light. For comparison with reference standards, approximately equal concentrations of the reference compound and the eluted samples were employed in the same solvent (ethanol), and under these conditions identical maxima were found for each pair (A-D).

N,N-Dimethyltryptamine Oxide.—A solution of 50 mg. of N,N-dimethyltryptamine in 2 ml. of ethanol was treated with 2 ml. of a solution prepared from 1 ml. of 30% hydrogen peroxide in 9 ml. of ethanol. After 2 hr. crystallization was induced by the addition of ether and chilling. The granular oxide was removed and recrystallized from ethanol–ether. It proved to be hydrated, and the melting points of independently prepared samples were not wholly reproducible. An analytical sample, m.p. 123–128°, had a composition indicative of approximately one molecule of water of hydration. All samples were examined by paper chromatography, and only one area of constant R_f value was observed in each case.

Anal. Calcd. for $C_{12}H_{16}N_2O \cdot H_2O$: C, 64.84; H, 8.16; N, 12.60. Found: C, 64.27; H, 7.55; N, 12.51.

The oxide yielded a **picrate** melting at 178–183° after recrystallization from ethanol.

Anal. Calcd. for $C_{18}H_{18}N_5O_8$: C, 50.00; H, 4.20; N, 16.20. Found: C, 50.26; H, 4.33; N, 15.90.

In separate experiments it was found that a drop or two of hydrogen peroxide, evaporated on the paper after spotting with N,N-dimethyltryptamine, provided a simple means of checking R_f values for the oxide in comparison with those for N,N-dimethyltryptamine. This was duplicated with compound (A) to provide further confirmation of its identity. It was further found that a reduction with zinc dust and acetic acid of the oxide returned the parent base in good yield, as estimated by the intensity of the color after paper chromatography. This reaction was carried out with both synthetic samples and (C), and this provides further confirmation of the identity of (C).

Bufotenine Oxide.—The preparation of this oxide followed that of N,N-dimethyltryptamine oxide. The freshly prepared oxide, after washing with ether and drying at room temperature, melted at 211–214°. A sample was taken for analysis, and proved to be free of water of hydration. This material gave only one area of constant R_f value, identical with (D). Recrystallization or drying at 80° *in vacuo* gave samples of varying melting point.

Anal. Calcd. for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.50; H, 7.40; N, 12.49.

Attempts to prepare the picrate and flavianate gave unsatisfactory results.

Separate oxidation–reduction experiments similar to those described for N,N-dimethyltryptamine oxide were carried out for bufotenine oxide and (D). This provided additional evidence for the structure of (D).

Compound (E).—The fluorescence analysis data for (E) indicated a 5-hydroxyindole structure for the compound. This was confirmed by a positive color test with nitrous acid–α-nitroso-β-naphthol reagent. The R_f values (Table II) indicated that (E) was not serotonin, methylserotonin, bufotenidine or dehydrobufotenine.

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