[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA AT LOS ANGELES, LOS ANGELES, CALIFORNIA]

Studies in the Simulated Biosynthesis of *Strychnos* and *Mitragyna* Alkaloids[1]

BY JAMES B. HENDRICKSON AND RICARDO A. SILVA[2]

RECEIVED FEBRUARY 27, 1961

2-Hydroxytryptamine and two phenylacetaldehydes have been prepared and found to react spontaneously at room temperature in neutral aqueous media; the product also cyclizes with formaldehyde and its stereochemistry is elucidated. This reaction is considered to be a model for the central condensation reaction in strychnine biosynthesis and that of other *Strychnos* and *Mitragyna* alkaloids; the stereochemistry of this condensation is discussed and the view advanced that no enzymatic mediation is requisite to the success of this condensation *in vivo*.

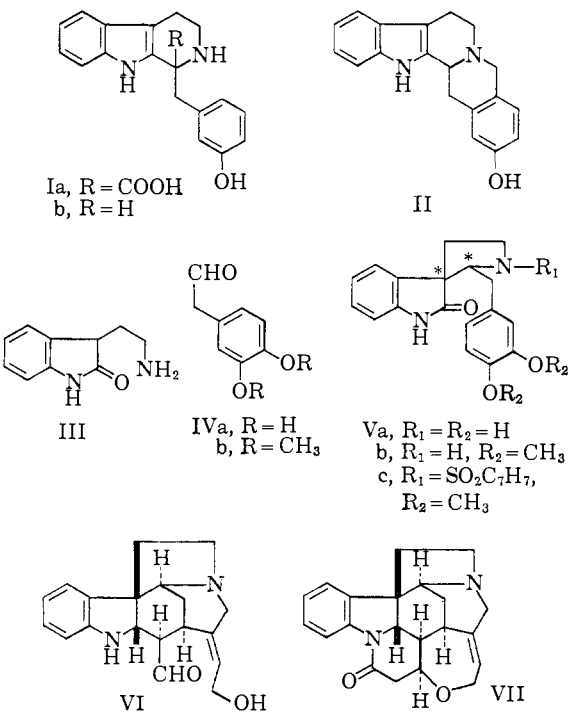
Introduction

Owing to the structural dissimilarity and complexity of the alkaloids compared to the central metabolites (amino acids, sugars, etc.) of the plants as well as the lack of any known metabolic function for the alkaloids, it has been a central feature of biogenetic theories that the major construction of the alkaloid molecule occurs in a single *spontaneous* reaction between intermediates known to be present in the plant cell. Accordingly, it has been necessary to demonstrate that the postulated biogenetic reactions are in fact spontaneous under conditions which may reasonably obtain in the plant cell; the dramatic success of several of these reactions[3,4] itself lends added credence to the theories. The intent of the present work has been to provide such demonstration of a biogenetic reaction for the *Strychnos* family of alkaloids.[5,6]

The major body of indole alkaloids appears to be unified by a central biogenetic plan[5] in which a tryptamine condenses with an aldehyde (*cf.*, a hydroxyphenylacetaldehyde) at either the α- or β-indolic positions, yielding the yohimbine and strychnine families, respectively. That this scheme was tenable for the yohimbine alkaloids was demonstrated by Hahn and Werner,[4] who showed that tryptamine would condense spontaneously with *m*-hydroxyphenylpyruvic acid in 87% yield under conditions reasonably present in the plant: aqueous *p*H 4.2–6.0, 25°, ten days. The acid Ia was easily decarboxylated to Ib, which condensed rapidly with formaldehyde under comparable conditions to yield hexadehydroyohimbol (II).

The very success of this experiment, however, raises some difficulties for the comparable demonstration of a spontaneous condensation leading to the *Strychnos* family, for it is clear that simple mixing of the components yields only the yohimbine skeleton by α-condensation on the indole. To circumvent this difficulty, it has been postulated[5,6] that the natural precursor possesses an α-substituent on the indole to block α-condensation and so force condensation into the β-position. A reasonable precursor then might well be the oxindole analog, 2-hydroxytryptamine (III), for oxindoles occur among the indole alkaloids, notably in the *Mitragyna* species (*cf.*, rhyncophylline[7]), which possess all the other structural features of the *Strychnos* family. As oxindoles are noted for their anionoid activity at the β-position,[8] III seemed a suitable candidate for condensation with the phenylacetaldehyde component, IVa, to produce Va, which may then react with the formaldehyde equivalent, undergo a Woodward fission and condense at the oxindole carbonyl to yield the skeleton of the Wieland–Gumlich aldehyde, VI, which appears to be the precursor of strychnine, VII, as well as the many *Strychnos* alkaloids of curare.[9]

Unlike the condensation to yield the yohimbinoid precursor Ib, the β-condensation yielding V generates two new sites of asymmetry (starred), neither of which is subject to later epimerization. Hence the relative stereochemistry at these sites must be



fixed in the spontaneous condensation, so that, if the biogenetic postulate is correct, V must possess at these two carbons the same stereochemistry as that in strychnine itself, which is known to have the

(1) This work was supported by a generous grant from the National Science Foundation.

(2) Abstracted in part from the Ph.D. thesis of Ricardo A. Silva, UCLA, 1961.

(3) R. Robinson, *J. Chem. Soc.*, **111**, 762 (1917), and ref. 9 and 12.

(4) G. Hahn and H. Werner, *Ann.*, **520**, 123 (1935).

(5) J. B. Hendrickson, in "The Alkaloids," VI, ed. R. H. F. Manske, Academic Press, Inc., New York, N. Y., 1960, pp. 206 ff.

(6) R. B. Woodward, *Nature*, **162**, 155 (1948).

(7) J. C. Seaton and Leo Marion, *Can. J. Chem.*, **35**, 1102 (1957).

(8) P. L. Julian, E. W. Meyer and H. C. Printy in "Heterocyclic Compounds," Vol. 3, ed. R. C. Elderfield, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 1 ff.

(9) A. R. Battersby and H. F. Hodson, *Quart. Rev.*, **14**, 77 (1960).

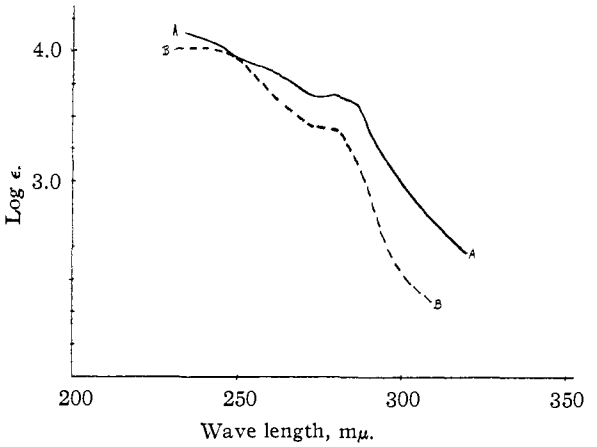
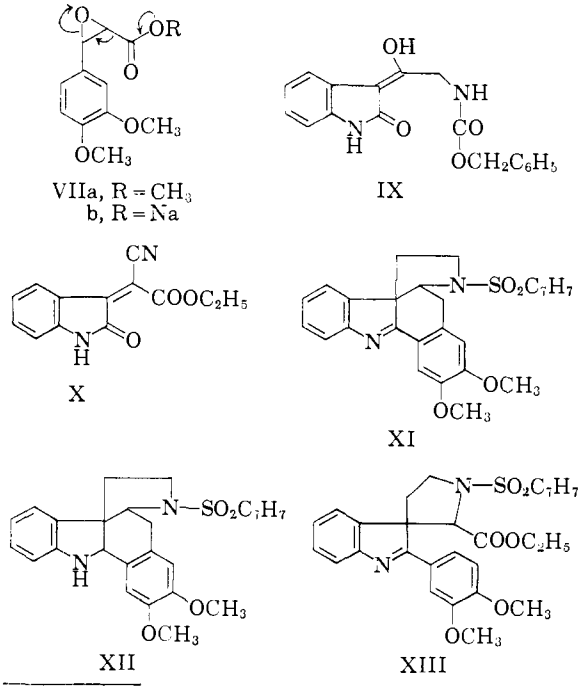


Fig. 1.—A (———), coupling product XIIb; B (---------), composite curve of 3,3-dimethyloxindole + 3,4-dimethoxyphenylacetic acid.

absolute stereochemistry VII.[10] With these considerations in mind we set out to ascertain the ease of formation of V under "physiological conditions" and to compare its stereochemistry with that of strychnine.[11]

Results

The aldehyde selected for condensation with oxytryptamine (III) was 3,4-dimethoxyphenylacetaldehyde (IVb); 3,4-dimethoxybenzaldehyde was condensed in a Darzens condensation with methyl chloroacetate to yield the glycidic ester VIIIa which precipitated the salt VIIIb on treatment with cold methanolic alkali. The salt underwent a



(10) A. F. Peerdeman, *Acta Cryst.*, **9**, 824 (1956); it should be noted that the reverse, and incorrect, absolute stereochemistry is reproduced in ref. 5.

(11) Since this work was begun, similar condensations were reported in a brief note by Y. Ban and T. Oishi, *Chem. and Ind. (London)*, 349 (1960), although they did not examine the spontaneity or stereochemistry of their examples.

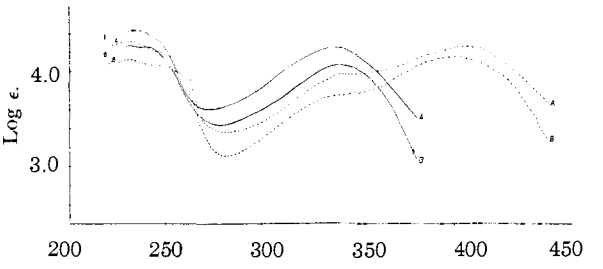


Fig. 2.—A, indolenine XVI, ———, in HCl, ---------; B, model compound XVIII, ———, in HCl, ---------.

vigorous decarboxylation on short warming in acid, affording the desired aldehyde in good yield, presumably *via* the interesting oxide-opening indicated on formula VIII (arrows). The preparation and reactions of oxytryptamine (III) have been discussed by Witkop[12]; only the salt has been isolated, for when an aqueous solution of the salt is made basic, no amine is isolable by extraction, the substance presumably remaining in solution as the zwitterionic hydrolysis product. Several other approaches to the synthesis of this material were unsuccessful in our hands. The Claisen condensation of oxindole with ethyl N-carbobenzoxyglycinate yielded IX but attempts to reduce this material catalytically to oxytryptamine afforded no authentic material. The longer synthesis later advanced by Harley-Mason[13] from isatin *via* X proved to be the most reliable. Equimolar quantities of the aldehyde and oxytryptamine hydrochloride were stirred 12 hr. in aqueous buffer in concentrations of 0.03–0.04 *M* at room temperature and the basic product isolated by acidifying the chilled solution, filtering and basifying the filtrate, a procedure which affords no oxytryptamine itself.[12] With this simulation of cell-medium conditions, the condensed base Vb was isolated in 38% yield at *p*H 6.0 and in 54% yield at *p*H 7.0. Preparation of Vb by boiling the components in ethanol afforded a 92% yield. The constitution of Vb is assured by the analyses of its salts and the tosyl derivative Vc, by the infrared spectra (oxindole: 2.93, 5.87 μ; tosyl derivative: no new NH band near 3 μ, SO_2 bands at 7.4 and 8.6 μ) and by the ultraviolet spectrum, which is essentially equal to the sum of its composite chromophores (oxindole and homoveratrole) as shown in Fig. 1. Hot permanganate oxidation of Vb yielded 3,4-dimethoxybenzoic acid. The entire series was also undertaken with 2,3-dimethoxyphenylacetaldehyde, made in the same way, with completely analogous results and yields throughout.

It is notable that in all cases only a single compound was isolated from the reaction of these components, so that a high degree of stereoselectivity must in fact be operative in the condensation, and it only remains to determine whether the single stereo-isomer of Vb which has been formed possesses the same stereochemistry as that of strychnine. This was achieved by converting the tosyl deriva-

(12) K. Freter, H. Weissbach, B. Redfield, S. Udenfriend and B. Witkop, *J. Am. Chem. Soc.*, **80**, 983 (1958).

(13) J. Harley-Mason and R. F. J. Ingleby, *J. Chem. Soc.*, 3639 (1958).

tive Vc by mild Bischler–Napieralski conditions (polyphosphoric acid in refluxing phosphorus oxychloride) to the cyclized indolenine XI, identified by its analysis and infrared spectrum (oxindole bands disappear, weak C=N at 6.2 μ, tosyl bands unchanged). The imine group is reducible with sodium borohydride, yielding the dihydro compound XII showing no carbonyl in the infrared and a simple ultraviolet spectrum equivalent to the indoline produced by reducing the oxindole Vc with lithium aluminum hydride (Fig. 3), which serves as an adequate model. The dihydro compound formed a mono-N-acetyl derivative with no NH band in the infrared. The conjugation introduced between the two aromatic rings in XI is clearly demonstrated by the ultraviolet spectrum (Fig. 2) which is very similar to that of the model compound XIII.[14] Since in the model compound the chromophoric system may achieve total coplanarity the same must be true of the chromophore in XI,[15] a fact which fixes its stereochemistry definitely. In the perspective drawing XIV, coplanarity is only attainable if ring B is fused to ring C by an equatorial bond at C_7 (strychnine numbering) as shown, the other possibility, XV, presenting aromatic π-electrons virtually perpendicular to those of the imine, with the effect of insulating the two chromophores so that the spectrum would show only a simple composite of the two separate aromatic rings, one conjugated to C=N. Since, in XIV, one bond of the pyrrolidine ring is thus forced into an axial orientation at C_7, the ring can only be *cis*-fused, as shown. *This relative stereochemistry, then, is that which is produced in the initial condensation, and it is identical with that in strychnine (VII).* The relative ease of the Bischler–Napieralski reaction as well as the stability of the indolenine XI to acid and base may also be taken as only acceptable with this stereochemistry, models of the other diastereomer of Vb implying a large measure of steric strain in the corresponding indolenine. The ultraviolet spectra of the indolenines XI and XIII in acid show identical shifts to longer wave lengths (Fig. 2) on formation of the quaternary salts (from which the respective indolenine spectra are regenerated unchanged on neutralization), thus confirming the identity of their chromophore geometries.

Reaction of the coupling product with formaldehyde, again under aqueous room temperature conditions, proceeded in good yield to the cyclized XVI, analogous to the formation of hexadehydroyohimbol (II) by Hahn. The skeleton of XVI is very similar to that of the *Mitragyna* alkaloids discussed below, and its stereochemistry from the foregoing must be represented as indicated (orientations shown relative to the pyrrolidine ring). When this material was refluxed in boiling pyridine, conditions used to effect the isomerization of these alkaloids (*vide infra*),[16] it was recovered unchanged in high

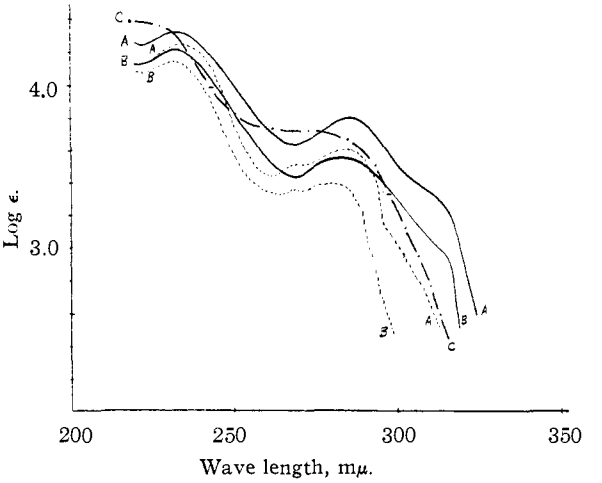
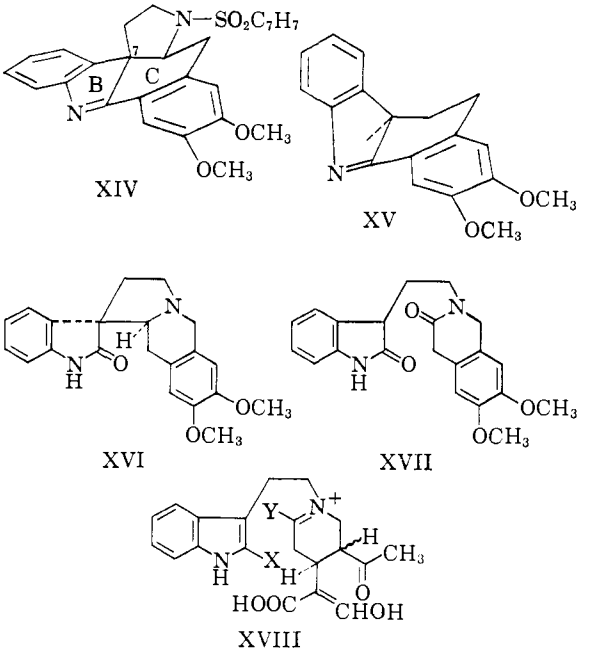


Fig. 3.—A, indoline XVII, ———, in HCl, ---------; B, indoline from $LiAlH_4$ reduction product of coupling product tosylate XIIc, ———, in HCl, ---------; C, coupling product tosylate XIIc, -·-·-.

yield, no other product being isolated. The compound XVI also reacted with mercuric acetate, precipitating mercurous acetate, by analogy with isorhyncophylline[16]; the neutral product showed the expected 6 μ band in the infrared arising from the lactam, XVII, formed by hydrolytic ring-cleavage of the initial immonium ion.



Discussion

Strychnos Alkaloids.—The experiments described may be taken as a demonstration that under conditions presumably extant in the living cell hydroxytryptamine condenses readily with a phenylacetaldehyde to produce one only of the two possible diastereomeric products V and that this product, presumably possessing the thermodynamically more stable configuration, has the configuration of natural

(14) R. B. Woodward, M. P. Cava, W. D. Ollis, A. Hunger, H. U. Daeniker and K. Schenker, *J. Am. Chem. Soc.*, **76**, 4749 (1954); we are grateful to Prof. Woodward for the gift of a sample of this material.

(15) See the discussion of E. A. Braude and F. Sondheimer, *J. Chem. Soc.*, 3754 (1955), and subsequent papers. The spectrum of XV has the same maxima but of slightly lowered intensity, so that it corresponds to steric hindrance of type (1), which implies only very small deviations from coplanarity.

(16) J. C. Seaton, M. D. Nair, O. E. Edwards and Leo Marion, *Can. J. Chem.*, **38**, 1035 (1960).

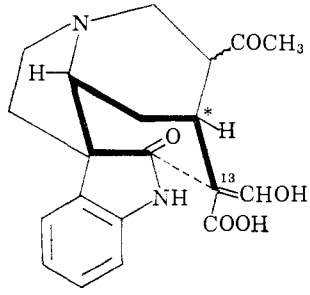
strychnine. In these terms it may be taken as a possible model for the central biogenetic condensation leading in the plant to the strychnoid alkaloids. Whether the components chosen here are the correct ones merits some discussion, however.

Since the commencement of the present work, Wenkert has made an important contribution to the biogenesis of indole alkaloids in his suggestion[17] that shikimic acid, the natural precursor of phenylalanine,[18] is itself the appropriate precursor of the aldehyde component in the biogenetic reaction; with a tryptamine the particular component suggested would form the anhydrosalt, XVIII,[19] which is poised for a spontaneous reaction either at the α-position to yield yohimbinoid skeletons (X = H) or in the β-position as in the present examples (oxindoles, X = OH) to yield strychnoid alkaloids.

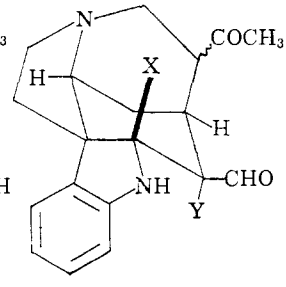
Knowledge of the coupling reaction stereochemistry from the present work permits construction of a model of the geometry of such a coupling reaction leading to strychnoid skeletons as pictorialized in XIX, the starred asymmetric center shown with the absolute configuration of the precursor XVIII.[19] Structure XIX provides the necessary proximity for the subsequent bond formation (. . . . .) closing the central ring; furthermore this model exhibits an essentially unstrained conformation (the ring being formed lies in a chair conformation) with virtually ideal π-electron overlap between the oxindole carbonyl and the aldehyde enol for this subsequent aldol-condensation ring closure to XXa; decarboxylation and dehydration then yields the full skeleton of C-fluorocurarine (XXI), one of the *Strychnos* curare alkaloids,[9] with its three asymmetric centers fully accounted for.[20]

In strychnine itself, however, the central ring has five asymmetric centers (VII and XXII), and while these would probably be correctly generated by reduction of this unsaturated aldehyde (corresponding to C-fluorocurarine) to XXc, a more attractive route is available.

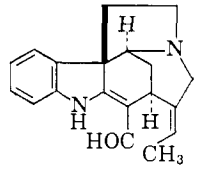
In a recent important note van Tamelen announced the successful condensation of the dialdehyde XXXIII in hot acetic acid–sodium acetate to yield XXIV,[21] an elegent reaction which provides another model for strychnine biogenesis in which tryptamine itself may be envisioned as condensing with the shikimic intermediate *via* XVIII. The stereochemistry of the main coupling reaction is almost certainly the same for the case of indole or oxindole, since the oxindole takes up, as its enol (X = OH in XVIII), the same geometry as indole in providing the necessary anionoid activity at the 3-position. Hence the present work may be taken in conjunction with van Tamelen's demonstration to provide the stereochemistry of this route to strychnine; in detail, β-condensation of the tryptamine indole in XVIII would yield an indolenine corresponding to XIX and ideally suited as discussed above for condensation to XXb and decarboxylation to XXc. This affords the correct absolute stereochemistry at all five sites for the carbon skeleton of the Wieland–Gumlich aldehyde VI and of strychnine itself (XXII). This analysis suggests that the biosynthesis of strychnine *in vivo* can proceed from tryptamine *via* the shikimic acid derivative XVIII by a series of spontaneous reactions taking the thermodynamically most favored steric course under conditions which will reasonably obtain in cell media.[24] Furthermore, these spontaneous reactions take a steric course of themselves which results in the stereochemistry of natural strychnine without any requirement of enzyme mediation. In this connection, also, it is of interest to note that in the broad complex of curare alkaloids[9] formed by dimerization of variants of the Wieland–



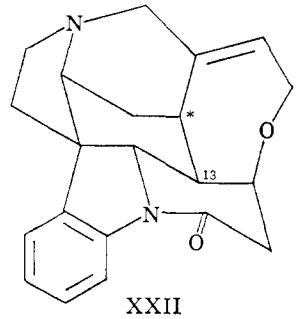
XIX



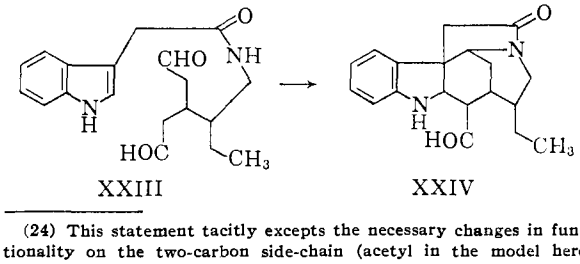
XXa, X=OH, Y=COOH
b, X=H, Y=COOH
c, X=Y=H



XXI



XXII



XXIII XXIV

(17) E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, **81**, 1474 (1959); E. Wenkert, *Experientia*, **15**, 165 (1959).

(18) B. D. David in W. D. McElroy and H. B. Glass, "A Symposium on Amino Acid Metabolism," The Johns Hopkins Press, Baltimore, Md., 1955, p. 799.

(19) The single non-epimerizable asymmetric center in XVIII is starred and shown in the absolute configuration demonstrated by Wenkert to be true of this center in a wide variety of indole alkaloids; that it is not universal, however, is demonstrated in the next section.

(20) Conversion of the acetyl group to ethylidene and N-methylation remain to complete the biosynthesis of C-fluorocurarine, but these are considered to be biologically unexceptional and are in any event not relevant to the stereochemical discussion.

(21) E. E. van Tamelen, L. J. Dolby and R. G. Lawton, *Tetrahedron Letters*, **19**, 30 (1960). The same basic conception was embodied in a proposal of Robinson[22] but was never successfully executed.[23]

(22) R. Robinson and J. E. Saxton, *J. Chem. Soc.*, 2598 (1953).

(23) A. R. Katritzky, *ibid.*, 2581, 2586 (1955).

(24) This statement tacitly excepts the necessary changes in functionality on the two-carbon side-chain (acetyl in the model here) which will form the seven-membered oxide ring; the stereochemistry of that ring is, however, fixed by the rest of the carbon skeleton. The formation of strychnine from the Wieland–Gumlich aldehyde (VI) is presumed to proceed by N-acetylation and aldol condensation and has been carried out in the laboratory by F. A. L. Anet and R. Robinson, *Chem. and Ind.* (*London*), 245 (1953).

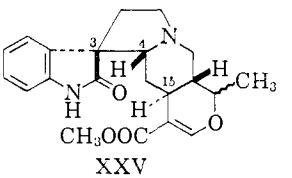
Gumlich aldehyde (VI) the dimerization reaction is simply one of Schiff base formation, which is a spontaneous reaction like the coupling condensation above. These stereochemical conclusions, therefore, combined with the demonstration of spontaneous coupling herein, lend support to the view that alkaloid production in the plant may be a fortuitous process with no intrinsic metabolic teleology.

Of the possible modes of biogenesis of the strychnoid skeleton, then, the coupling of tryptamine with an intermediate from shikimic acid provides a convincing route to strychnine itself as well as the strychnos curare alkaloids and such variants as strychnospermine and spermostrychnine,[25] all of which are presumably products of the Wieland–Gumlich aldehyde. Thus, whereas the involvement of the hydroxytryptamine used in these experiments would seem in many instances to provide a less facile route to most *Strychnos* alkaloids than tryptamine itself, it may well be implicated in the formation of more oxidized forms such as C-fluorocurarine as well as those alkaloids containing an intact oxindole ring.

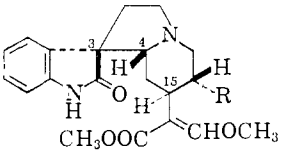
Mitragyna and Related Alkaloids.—The oxindole ring is a common feature of these alkaloids as seen in the structures of mitraphylline (XXV),[26] rhyncophylline (XXVIa),[7] uncarine A[27] (epimeric with mitraphylline at C_4 and C_{15}) and corynoxeine (XXVIb)[28]; the stereochemistry of these alkaloids recently has been deduced as shown.[29] Thus it is highly probable that the oxytryptamine used in the present experiments is in fact the precursor here; implication of its existence in nature is afforded by the natural occurrence of the corresponding oxytryptophan.[8,12] Its condensation with the shikimic acid precursor would yield the intermediate XVIII (X = OH) which is remarkably akin in structure to these alkaloids. In fact the simple biogenetic coupling reaction need only be followed by reduction of the pendent acetyl group and methylation to yield rhyncophylline directly! The stereochemistry, however, requires some comment.

In most of these cases the alkaloid is accompanied in the plant by an epimer with which it is interconvertible in boiling pyridine[16] and which, therefore, differs in configuration at C_4,[29] epimerizing by a reversal of the condensation examined herein. This experimental evidence suggests that the same course of equilibration may occur in the plant, although it may be expected to be as little as 1/500 as fast; based on the time of 12 hr. used to equilibrate the compounds in boiling pyridine,[16] this implies that equilibration may be achieved in the plant cell in 8 months or less. Since this represents an upper limit, it is more likely that in less than 1–2 months substantial equilibration may occur to produce each of the two epimers which are found,[30] so that this route to a second epimer must be considered as available and likely.[31]

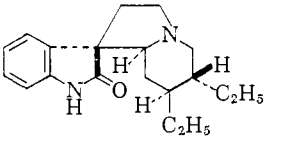
The implication of the oxytryptamine component in the biogenesis of the mitragyna skeleton is highly probable; the involvement of Wenkert's aldehyde component is favored by the intimate relation of its functionality with that of the natural alkaloids as well as by a sense of unity in the entire indole alkaloid field, but the compelling evidence, adduced by Wenkert for the other indole alkaloids, of the unique unepimerized asymmetric center (C_{15} *cf.* XVIII) is



XXV



XXVIa, R = C_2H_5
b, R = $CH{=}CH_2$



XXVII

surprisingly lacking. Thus, mitraphylline has been isolated as both dextro- and levorotatory enantiomers on more than one occasion[33] so that the absolute stereochemistry at C_{15} is necessarily not always the same as in the shikimic-derived precursors. Another case is apparently to be found in corynoxeine.

(25) F. A. L. Anet, G. K. Hughes and E. Ritchie, *Aust. J. Chem.*, **6**, 58 (1953).

(26) J. C. Seaton, R. Tondeur and Leo Marion, *Can. J. Chem.*, **36**, 1031 (1958).

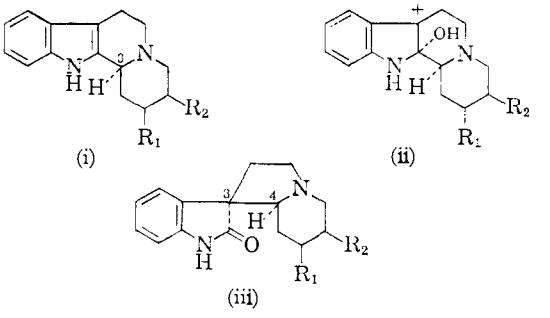
(27) T. Nozoye, *Chem. Pharm. Bull. (Japan)*, **6**, 300 (1958).

(28) N. An Cu, R. Goutarel and M.-M. Janot, *Bull. Soc. chim. France*, [5] **24**, 1292 (1957).

(29) J. B. Hendrickson, *J. Am. Chem. Soc.*, **83**, 650 (1961).

(30) The experimental work on the isolation of these alkaloids does not permit a judgment as to the extent of heating and hence, presumably, epimerization of the crude extracts. It seems unlikely, however, that such artifactual epimerization is the sole source of the less stable epimer in the extracts. It usually in fact has been the less stable epimer that was first isolated, but since this epimer is also always the less soluble of the two,[16] this is not surprising.

(31) Another mode of formation of the Mitragyna alkaloids must also be considered, *i.e.*, the oxidation-rearrangement of a yohimbinoid material (i) to an oxindole (iii). Laboratory precedent for the reaction exists[32] and, as it is analogous mechanistically to the conversion of tryptophan to oxytryptophan, its existence *in vivo* may be inferred. If attack of the oxidant is considered to be irreversible, it is reasonable that it should attack on the less hindered side, *i.e.*, that of C_3–H, producing (ii), which must rearrange to (iii), which then has the opposite relative stereochemistry at the newly-formed centers (C_3–C_4) to that of strychnine or that formed in the spontaneous biogenetic reaction.



(i) (ii) (iii)

In any event, however, β-oxidation of the indole (i) should be more facile than α- and would lead directly to an *indoxyl* skeleton by an analogous path.[32] The rarity of indoxyl alkaloids and their apparent absence from *Mitragyna* sources constitute a serious argument against the operation of this biogenetic pathway here. Also the stereochemical argument rules it out as a route to strychnine or the alkaloids from the Wieland–Gumlich aldehyde.

(32) B. Witkop and J. B. Patrick, *J. Am. Chem. Soc.*, **75**, 2572 (1953).

(33) T. Nozoye, *Chem. Pharm. Bull. (Japan)*, **6**, 306 (1958); see also reference 26 and previous references noted therein.

xine,[28] which must be epimeric with rhyncophylline at both C_{15} and C_{20} since the two are convertible to *enantiomers* of the same parent compound (XXVII).[34] Reinspection of Wenkert's biogenetic proposal,[17] however, indicates that, should the conversion of shikimic acid proceed normally to prephenic acid *before* reaction with formaldehyde, the plane of symmetry so created would then necessarily create equal amounts of the two epimers of XVIII at the starred carbon, and the observed optically inactive alkaloids become possible. At the same time this observed lack of optical specificity in these alkaloids weakens the force of that argument for these shikimic-derived precursors which is based on the unique configuration of C_{15} (*cf.*, the starred center in XVIII). On the other hand, the sense of biogenetic unity in the indole alkaloids is strengthened by the isolation of corynoxeine and corynoxine from the same plant (*Pseudocinchona africana*) which yields corynantheine and various stereoisomers of yohimbine. The virtually complete spectrum of possible epimers in both the yohimbinoid and mitragyna-type alkaloids lends some credence to the view advanced above that their biogenesis may proceed with no specific enzymatic mediation.

Acknowledgments.—The authors wish to express their sincere gratitude to Professors R. B. Woodward and R. C. Cookson for helpful and stimulating discussion, to the former for gifts of material, and finally to the National Science Foundation.[1]

Experimental[35]

3,4-Dimethoxyphenylacetaldehyde.—In a 500 ml. round bottom three-necked flask equipped with a stirrer, a dropping funnel and a dry nitrogen inlet, 35.0 g. (0.211 mole) of 3,4-dimethoxybenzaldehyde (purified by recrystallization from ether), 23.0 g. (0.212 mole) of freshly distilled methyl chloroacetate and 100 cc. of *t*-butanol were placed. The flask was immersed in a cold water bath and stirred vigorously while a solution of 8.0 g. (0.205 mole) of potassium in 200 cc. of *t*-butanol was added over 45 minutes *via* the dropping funnel. When the addition was complete, the dropping funnel was replaced with a reflux condenser fitted with a drying tube and the reaction slurry stirred for an additional 3 hr. under a slight positive pressure of nitrogen. At the end of this time, most of the *t*-butanol was removed *in vacuo* with slight warming and the yellowish residue stirred with about 300 cc. of ether and just enough water (50 cc.) to dissolve all the solids. The ether phase was separated and the aqueous residue washed with three 100 cc. portions of ether. The ether extracts were combined and dried over anhydrous Na_2SO_4. Concentration of the ether solution to about 50 cc. and addition of a cold solution of 8.8 g. of NaOH in 75 cc. of methanol, precipitated sodium β-(3,4-dimethoxyphenyl)-glycidate, as a white solid which was washed twice with ether and once with acetone; weight after drying, 42.2 g. (0.171 mole, 81%).

A portion of this salt, 13.7 g. (55.7 mmole) on neutralization and decarboxylation with 10% HCl followed by extraction with chloroform and high vacuum distillation yielded 2.86 g. (15.9 mmole; 34.4%) of the colorless liquid aldehyde, b.p. 112–118° (6–0.7 mm.), infrared (chloroform) 5.83, 8.67, 8.80μ. The last pair of bands was found in most compounds containing the 3,4-dimethoxybenzyl system.

The 2,4-dinitrophenylhydrazone was prepared, filtered through a short alumina column and recrystallized from methylene chloride/ethanol to an orange crystalline solid, m.p. 166–168°.

Anal. Calcd. for $C_{16}H_{16}O_6N_4$: C, 53.33; H, 4.48; N, 15.55. Found: C, 53,33; H, 4.43; N, 15.41.

3-(N-Carbobenzoxyglycyl)-oxindole (IX).—In the following procedure, all glassware was dried rigorously by flaming, and the ether used as solvent was distilled from metallic sodium. Into a 200 cc. three-necked flask equipped with stirrer, reflux condenser with $CaCl_2$ tube and a pressure equilibrated dropping funnel, was placed 3.16 g. (13.1 mmoles) of ethyl N-carbobenzoxyglycinate, previously dried *in vacuo* over P_2O_5 for 3 days, and then by 80 cc. of anhydrous ether and 1.04 g. (23.6 mmoles) of sodium hydride suspended in mineral oil. All additions were performed rapidly to exclude atmospheric moisture as much as possible. The reaction vessel was immersed in an ice bath, and after it had been thoroughly flushed with a dry nitrogen stream, 1.16 g. (8.70 mmoles) of oxindole were rapidly added. Vigorous evolution of hydrogen was noted and the reactants were stirred for a total of 3.5 hr. The ice bath was removed and the mixture stirred for an additional 3 hr. at room temperature. In order to decompose all remaining hydride, 8 cc. of absolute ethanol was added, and then 25 cc. of ice cold 10% HCl. The ether was removed *in vacuo* and the aqueous acid mixture extracted with 50 cc. of $CHCl_3$ in three portions. The chloroform fractions were combined and dried over anhydrous magnesium sulfate. Filtration and evaporation of the chloroform left a turgid red-brown residue. On triturating with 5 cc. of ice cold ether and standing for 10 minutes, a white crystalline solid separated out. This was filtered off and washed with ether to give 0.657 g. (2.01 mmoles, 23.3%) of a white crystalline solid, recrystallized from absolute ethanol to m.p. 168–169.5°, infrared (KBr) 5.83, 5.87, 5.94μ. The compound gave a deep blue color with ferric chloride reagent.

Anal. Calcd. for $C_{18}H_{16}O_4N_2$: C, 66.65; H, 4.97; N, 8.64. Found: C, 66.77; H, 5.25; N, 8.82.

Hydrogenation of 3-(N-Carbobenzoxyglycyl)-oxindole.—In a 50 ml. flask attached to a microhydrogenation apparatus, 0.130 g. (0.57 mmoles) of PtO_2, 10 cc. of glacial acetic acid and 0.04 cc. of concd. H_2SO_4 were placed. After flushing, hydrogenation of the PtO_2 was commenced and after 2 hr. and 43 minutes, no further uptake of hydrogen was observed. Into the flask, 0.242 g. (0.75 mmole) of 3-(N-carbobenzoxyglycyl)-oxindole was placed and hydrogenation commenced again. When 1.85 mmoles of hydrogen had been taken up, hydrogenation was stopped, the system evacuated and flushed with nitrogen through a previously weighed Ascarite tube, and the wt. of CO_2 evolved was found to be 0.037 g. (112%). To the reaction solution, 10 cc. of water was added and the catalyst filtered off. The filtrate was shaken up with 5.0 g. of hydroxide-charged Amberlite resin (IRA-410) and filtered. The filtrate was treated with 15 cc. of a 10% soln. of HCl and washed with three 10 cc. portions of ether. The aqueous phase was evaporated *in vacuo* to leave a yellow-brown solid which after washing with ether and drying in a vacuum desiccator over KOH for 24 hr., was found to weigh 0.118 g. (0.55 mmoles, 74%, if oxytryptamine hydrochloride). The solid did not melt sharply and charred at about 240°. It was soluble in methanol and ethanol and insoluble in ether, benzene, chloroform, tetrahydrofuran, ethyl acetate and *t*-butanol. It was highly hygroscopic and darkened and dissolved when exposed to air for 30–60 seconds. The infrared (Nujol) spectrum closely resembled that of authentic oxytryptamine hydrochloride, tail at 3.85 μ, broad band at 5.93 μ. Attempts to prepare the picrate salt and the benzoyl derivative were unsuccessful.

3-(β-Aminoethyl)-oxindole (III) was prepared by the method of Harley-Mason,[13] isolated and used as its hydrochloride.

A. Condensations in Cell-medium Conditions.—The 3,4-dimethoxyphenylacetaldehyde and the oxindole-3-ethylamine hydrochloride were carefully weighed into a 100 cc. round-bottom flask. The buffer solution (McIlvaine's citrate/phosphate solution) was added and the resulting mixture stirred at room temperature for 12 hr. During the first hour, an orange-yellow gum appeared at the walls of the reaction vessel and the buffer solution became impregnated with a fine cloudy material. At the end of the re-

(34) The direct comparison apparently has not been made but the melting points of corynoxinane and rhyncophyllane are both 70° while their rotations, $[\alpha]_D$, are −25° and +24°, respectively.

(35) We are indebted to Miss Heather King of this department for microanalyses. All ultraviolet spectra were run in 95% ethanol on a Cary model 11 recording spectrophotometer. Melting points are corrected.

action time, about 12 cc. of 10% H_2SO_4 were added and the mixture filtered through a thin Celite pad. The pale yellow filtrates were chilled in ice and made basic with concd. NH_4OH to precipitate a pale yellow solid. This was taken up in chloroform and the chloroform dried over anhydrous $MgSO_4$. Filtration of the chloroform and evaporation *in vacuo* left a pale yellow glass, infrared (chloroform) 5.87, 8.63, 8.75 μ. The yields were

	*p*H 6.0	*p*H 7.0
Amine hydrochloride	1.92 mmoles	1.62 mmoles
Aldehyde	1.99 mmoles	1.67 mmoles
Buffer solution	50.0 cc.	50.0 cc.
Product	0.74 mmole	0.87 mmole
Yield	38.3%	53.5%

The picrate was prepared and recrystallized with difficulty from methanol to give pale yellow crystals, m.p. 195–197°.

Anal. Calcd. for $C_{26}H_{25}O_{10}N_5$: C, 55.02; H, 4.44; N, 12.35. Found: C, 54.93; H, 4.63; N, 12.33.

The perchlorate salt was also prepared and recrystallized from absolute ethanol to m.p. 255–260°.

Anal. Calcd. for $C_{20}H_{23}O_7N_2Cl$: C, 54.73; H, 5.28; N, 6.38. Found: C, 54.58; H, 5.53; N, 6.48.

B. **Preparative Condensation to (Vb).**—To a solution of 2.58 g. (12.2 mmoles) of oxindole-3-ethylamine hydrochloride in 30 cc. of ethanol and 60 cc. of water, 4.1 g. (30.1 mmoles) of hydrated sodium acetate was added. The reaction flask was warmed on the steam bath and a solution of 2.92 g. (16.2 mmoles) of 3,4-dimethoxyphenylacetaldehyde in 30 cc. of ethanol was added over 10 minutes. The solution was refluxed for 48 hr. on the steam-bath. Most of the ethanol was removed *in vacuo* on the steam-bath, and the solution was acidified with 120 cc. of 5% sulfuric acid and filtered free of insoluble material. The filtrate was cooled in ice and neutralized with a saturated sodium bicarbonate solution to precipitate a faintly yellow solid. This was taken up in chloform and the organic phase dried over anhydrous magnesium sulfate. The drying agent was filtered off and the chloroform removed *in vacuo* to leave 3.80 g. (11.2 mmoles, 92%) of a pale yellow solid identical in all respects with the product obtained in the previous condensation.

N-Tosyl Derivative (Vc).—To a solution of 0.557 g. (1.65 mmoles) of the secondary amine condensation product, in 1.0 cc. of pyridine, an excess of *p*-toluenesulfonyl chloride was added and the whole left at room temperature for 4 days. Extraction of all basic materials with dilute HCl left 0.580 g. (1.18 mmoles, 71%) of a neutral red-brown material which was recrystallized from absolute ethanol to give pure white crystals, m.p. 233–234°.

Anal. Calcd. for $C_{27}H_{28}O_5N_2S$: C, 65.83; H, 5.73; N, 5.69; S, 6.51. Found: C, 65.99; H, 5.53; N, 5.52; S, 6.37.

Lithium Aluminum Hydride Reduction of Vc.—Into a 50 cc. round-bottom flask equipped with a reflux condenser, were placed 0.594 g. (1.75 mmoles) of the 3,4-dimethoxytosylate Vc, 1.168 g. (30.8 mmoles) of lithium aluminum hydride and 20 cc. of tetrahydrofuran (freshly distilled from $LiAlH_4$). The reaction mixture was refluxed on an oil bath for 14 hr. and then poured into 100 cc. of ice cold 5% H_2SO_4. The acid solution was allowed to stand at room temperature for 1 hr. and then neutralized with solid $NaHCO_3$. Large amounts of a flocculent precipitate were observed as the *p*H changed from acidic to basic. The mixture was filtered through a Celite pad and the solids on the pad well washed with water. The Celite pad and the material on it was transferred to a 100 ml. erlenmeyer flask and warmed with 60 cc. of $CHCl_3$. The slurry obtained was filtered through a fresh Celite pad and the pad well washed with more warm $CHCl_3$. The $CHCl_3$ filtrate was dried over anhydrous $MgSO_4$ and evaporated to a pale yellow residue. The residue was dissolved in 10 cc. of CH_2Cl_2 and 10 cc. of MeOH and evaporated on the steam-bath to a volume of about 3 cc. On cooling, 0.248 g. of an almost white crystalline material were obtained, m.p. 145–147°; infrared (chloroform), no carbonyl; peaks at 2.90 (N-H); 7.49 and 8.62 μ (tosylate).

Concentration of the filtrate to about 1 cc. followed by addition of 2 cc. of MeOH provided an additional 0.147 g. of the same material. The yield of the almost white crystalline solid was 0.395 g. (1.04 mmoles, 59.7%).

Anal. Calcd. for $C_{27}H_{30}O_4N_2S$: C, 67.76; H, 6.32; N, 5.85; S, 6.70. Found: C, 67.75; H, 6.18; N, 5.86.

Cyclization to the Indolenine (XI).—A mixture of 2.32 g. (4.71 mmoles) of the 3,4-dimethoxy tosylate (Vc) and 2.23 g. of polyphosphoric acid was heated to reflux with 20 cc. of phosphorus oxychloride. The reaction mixture attained a yellow color in a few minutes and at the end of 30 minutes was almost black. It was refluxed for a total of 1.5 hr. in an oil bath. About 10 cc. of benzene was added and boiled off. The resulting black tar was carefully stirred with a few chips of ice and about 50 cc. of $CHCl_3$ was added. About 50 g. of chipped ice was added slowly over 30 minutes. The slurry was made basic with concd. NH_4OH, cooled and 20 cc. of $CHCl_3$ added. The $CHCl_3$ phase was separated and the aqueous layer washed with three 20 cc. portions of $CHCl_3$. The $CHCl_3$ phases were combined, washed with water, dried and evaporated to a brown residue. The residue was warmed with 25 cc. of MeOH and on cooling, 1.21 g. of pale yellow crystals were obtained, m.p. 242–243°. The filtrate was charcoaled once and concentrated to about 8 cc. for an additional crop of 96 mg. The total yield of crystalline indolenine was 1.31 g. (2.76 mmoles, 58.7%); infrared (chloroform), no oxindole peak; triplet at 6.23, 6.34, 64.6 μ; peaks at 7.47 and 8.62 (tosylate); recrystallized from methanol to white needles, m.p. 243°.

Anal. Calcd. for $C_{27}H_{26}O_4N_2S$: C, 68.33; H, 5.52; N, 5.90; S, 6.76. Found: C, 67.72; H, 5.45; N, 5.56.

Sodium Borohydride Reduction of the Indolenine (XI).—Into a 50 cc. flask equipped with a reflux condenser, 0.633 g. (1.33 mmoles) of the indolenine (XVI) were mixed with 0.308 g. (81.5 mmoles) of sodium borohydride and 20.0 cc. of freshly distilled diglyme. The slurry was heated to reflux in an oil-bath and kept there for a total of 3 hr. The contents of the reaction vessel were poured into 100 cc. of ice cold 5% HCl. When the reaction had subsided, the mixture was filtered through a Celite pad. The white solid left on the pad was washed well with water and then redissolved in about 50 cc. of $CHCl_3$. The $CHCl_3$ was dried over $MgSO_4$ and evaporated to a pale yellow solid. The solid was crystallized from a mixture of CH_2Cl_2 and MeOH to yield 0.580 g. (1.21 mmoles; 91%) of off-white crystals, m.p. 195–197°. Infrared (chloroform) one peak at 6.20; peaks at 7.46 and 8.62 μ (tosylate).

Anal. Calcd. for $C_{27}H_{28}O_4N_2S$: C, 68.04; H, 5.92; N, 5.88. Found: C, 68.06; H, 5.75; N, 5.63.

Preparation of the Formaldehyde Condensation Product (XVI).—In a 500 cc. erlenmeyer flask, 5.371 g. (12.25 mmoles) of the perchlorate salt of the 3,4-dimethoxy two component base was dissolved in 200 cc. of hot water. To this solution, 10.0 cc. of 37% formaldehyde (123.3 mmoles) was added and the whole heated on a steam-bath for 1 hr. Solid sodium bicarbonate was added carefully to the hot solution until it was basic. A pale yellow gum precipitated on the sides of the flask. The mixture was heated an additional hour on the steam-bath and then made strongly basic with concd. NH_4OH. It was cooled and extracted with $CHCl_3$. The $CHCl_3$ phase was washed well with water, dried over $MgSO_4$ and evaporated to a pale yellow glassy residue which could not be induced to crystallize. Infrared (chloroform) 2.80, 5.84, 6.20, 9.04 μ.

The glass was redissolved in about 10 cc. of absolute methanol and dry HCl gas was bubbled through the solution for about 45 minutes. On cooling, 3.72 g. of a white crystalline hydrochloride separated out. Further concentration of the filtrate yielded an additional 0.63 g. of the same salt. Total yield of hydrochloride salt was 43.5 g. (11.25 mmoles; 91.6%), m.p. 199–201°.

The perchlorate salt was prepared by mixing the free base with perchloric acid in methanol; white crystals, m.p. 246–247.5°.

Anal. Calcd. for $C_{21}H_{23}O_4N_2Cl$: C, 55.93; H, 5.14; Cl, 7.86. Found: C, 55.83; H, 5.02; Cl, 7.69.

The pK_a of the perchlorate salt was obtained by titration with NaOH and found to be 5.6.

Formaldehyde Condensations in Cell-medium Conditions.—The free base was dissolved in a sodium acetate/acetic acid buffer by warming on a steam bath, 37% aqueous formaldehyde was added and the *p*H of the mixture was

measured. The reaction mixture was then stirred at room temperature for a length of time and after basification with concd. NH_4OH, the organic materials were extracted with $CHCl_3$. The $CHCl_3$ phase was washed well with water, dried and evaporated. The infrared spectrum of the residue then was compared with spectra of known mixtures of starting material and authentic product (XVI) and the yield ascertained by comparison of relevant peaks:

2-Component base, Vb (mmoles)	5.68	0.83
Formaldehyde (mmoles)	12.33	123.3
Time	1 day	8 days
Concentration $\times 10^{-3}$ M	28.40	4.14
pH	4.1	4.5
Yield of XVI	18.9%	60.9%

Mercuric Acetate Oxidation of (XVI).—In a 100 ml. erlenmyer flask, 180 mg. (0.51 mmole) of the 3,4-dimethoxy three component amine (XVI) was dissolved in 11 cc. of 20% acetic acid. To this 540 mg. (1.69 mmoles) of mercuric acetate was added and the solution heated on the steam-bath After 10 minutes colorless platelets began to crystallize out. After the crystals were filtered off and identified as mercurous acetate; wt. 143 mg. (0.275 mmole). The filtrate was heated on the steam-bath for an additional 12 hr. during which time no further precipitation was observed. The solution was cooled, made basic with $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ phase was dried over anhydrous $MgSO_4$ and evaporated to a yellow-brown glass which could not be induced to crystallize but contained a new infrared band at 6.0 μ.

Permanganate Oxidations of Vb.—In a 250-ml. erlenmyer flask, 1.20 g. of the 3,4-dimethoxy amine Vb was dissolved in 20 cc. of acetone. About 0.25 g. of KOH was added and then 10.0 g. of $KMnO_4$ in small portions over an hour. An immediate precipitate of MnO_2 was observed. After stirring for 3 hr., the flask was warmed on the steam-bath till almost all the acetone evaporated and 100 cc. of water added. The mixture was heated on the steam-bath for an additional 3 hr. Enough SO_2 gas was bubbled through to dissolve all the solids and the solution made basic with 40% KOH. The flocculent white precipitate was filtered off and washed four times with 20-cc. portions of 10% KOH. The filtrate was made acid and continuously extracted with ether for 24 hr. The ether was dried and evaporated to yield about 30 mg. of a yellowish material which was recrystallized from water to white needles m.p. 180–182°. The mixed m.p. with a sample of 3,4-dimethoxybenzoic acid prepared by oxidation of the aldehyde showed no depression.

The Analogous 2,3-Dimethoxyphenyl Series.—The other compounds prepared were entirely analogous in their methods of preparation, chemical behavior and spectra to the corresponding compounds in the 3,4-dimethoxyphenyl series.

2,3-Dimethoxyphenylacetaldehyde.—Prepared as above and distilled as a colorless oil, infrared (chloroform) 5.81, 7.87, 8.53, 9.22 μ; the last three peaks were typical of the 2,3-dimethoxyphenyl system in almost all of the compounds prepared; yield from the benzaldehyde, 20%.

Preparative Condensation with Oxytryptamine.—2,3-Dimethoxyphenylacetaldehyde (2.33 g., 12.9 mmoles) and 2.33 g. (10.9 mmoles) 3-(β-aminoethyl)-oxindole hydrochloride and 4 g. sodium acetate were condensed as above (procedure B) to afford 3.44 g. (10.1 mmoles) of a pale yellow solid, obtained crystalline from acetone only after extensive purification, white needles, m.p. 166–168°. The N-tosyl derivative (corresponding to Vc) was prepared and recrystallized from methanol, m.p. 199–200°.

Anal. Calcd. for $C_{27}H_{28}O_5N_2S$: C, 65.83; H, 5.73; S, 6.51. Found: C, 65.80; H, 5.74; S, 6.40.

The perchlorate of the free base crystallized from absolute ethanol, m.p. 224–225°.

Cyclization to the Indolenine (Corresponding to XI).—0.959 g. (1.95 mmoles) of N-tosyl derivative above, 0.926 g. polyphosphoric acid, in 7 cc. of phosphorus oxychloride, as before, yielded 0.627 g. (68%) of pale yellow crystals, m.p. 255–256.5°, from methanol.

Anal. Calcd. for $C_{27}H_{26}O_4N_2S$: C, 68.33; H, 5.52; N, 5.90; S, 6.76. Found: C, 68.14; H, 5.57; N, 6.15; S, 6.74.

Reduction with Sodium Borohydride.—Reduced as above, this indolenine afforded 89% yield of fine white crystals, m.p. 162.5–164.0° from methanol, corresponding to XII.

N-Acetyl Derivative of Indoline Corresponding to XII.—The sodium borohydride reduction product above was acetylated directly, 0.23 g. in 2 cc. of pyridine and 1 cc. of acetic anhydride, left overnight at room temperature. The solution was chilled in ice, acidified with cold 10% HCl and the pale yellow precipitate filtered and recrystallized from methylene chloride–methanol to a 75% yield of white crystals, m.p. 274–278°; infrared (chloroform), no N-H peak below 3.3 μ; amide at 6.06 μ.

Anal. Calcd. for $C_{29}H_{30}O_5N_2S$: C, 67.15; H, 5.83; N, 5.40; S, 6.18. Found: C, 67.38; H, 5.96; N, 5.17; S, 6.06.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, LOS ANGELES, CALIFORNIA]

Stereochemistry of the *Mitragyna* Alkaloids

BY JAMES B. HENDRICKSON

RECEIVED FEBRUARY 27, 1961

Application of conformational analysis to the *Mitragyna* alkaloid structures permits assignment of the stereochemistry of mitraphylline, rhyncophylline, uncarine, and corynoxine.

Structures for the *Mitragyna* alkaloids were first proposed by Loudon in 1955[1] and confirmed in recent years for uncarine A and B and mitraphylline, all represented by I, by the Japanese school of Kondo,[2] and for mitraphylline[3] and rhyncophylline[4] (IIa) by Marion and co-workers in Canada. Some tentative stereochemical assignments have been made[2] but these suffer both from a lack of adequate experimental evidence and insufficient attention to the potential stereochemical and conformational complexity of these structures. Evidence is now available, however, to make rational stereochemical assignments to the *Mitragyna* alkaloids.

The keystone in the evolution of this stereochemistry lies in the widespread existence among the alkaloids and their degradation products of pairs of interconvertible isomers; this was particularly evident in the early degradative studies on the isomers uncarine A and uncarine B.[5] However, it remained for the Canadian group to show that

(1) J. D. Loudon, *Spec. Pub. Chem. Soc. no. 3*, 12 (1955).

(2) H. Konda and T. Nozoye, *Ann. Rept. Itsuu Lab. (Tokyo)*, **7**, 44 (1956); T. Nozoye, *Chem. Pharm. Bull. (Japan)*, **6**, 300, 306, 309 (1958).

(3) J. C. Seaton, R. Tondeur and Leo Marion, *Can. J. Chem.*, **36**, 1031 (1958).

(4) J. C. Seaton and Leo Marion, *ibid.*, **35**, 1102 (1957).

(5) H. Kondo and T. Ikeda, *J. Pharm. Soc. Japan*, **61**, 416, 453 (1941); H. Kondo and T. Nozoye, *Ann. Rept. Itsuu Lab. (Tokyo)*, **1**, 71 (1950).