Metabolites of Mitragynine

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Abstract □ Microbial transformation of the alkaloid mitragynine by the fungus Helminthosporum sp. has elaborated two major metabolites. The compounds were isolated from the biological milieu and their structures were elucidated as mitragynine pseudoindoxyl and hydroxy mitragynine pseudoindoxyl. Tests to date have indicated quite high pharmacological activity by mitragynine pseudoindoxyl. Both metabolites were synthesized to verify structures.

Keyphrases □ Mitragynine—microbial transformation by Helminthosporum sp., isolation and identification of metabolites □ Microbial transformation—isolation and identification of two metabolites from Helminthosporum sp. transformation of mitragynine □ Indole alkaloids—microbial transformation of mitragynine by Helminthosporum sp., isolation and identification of metabolites

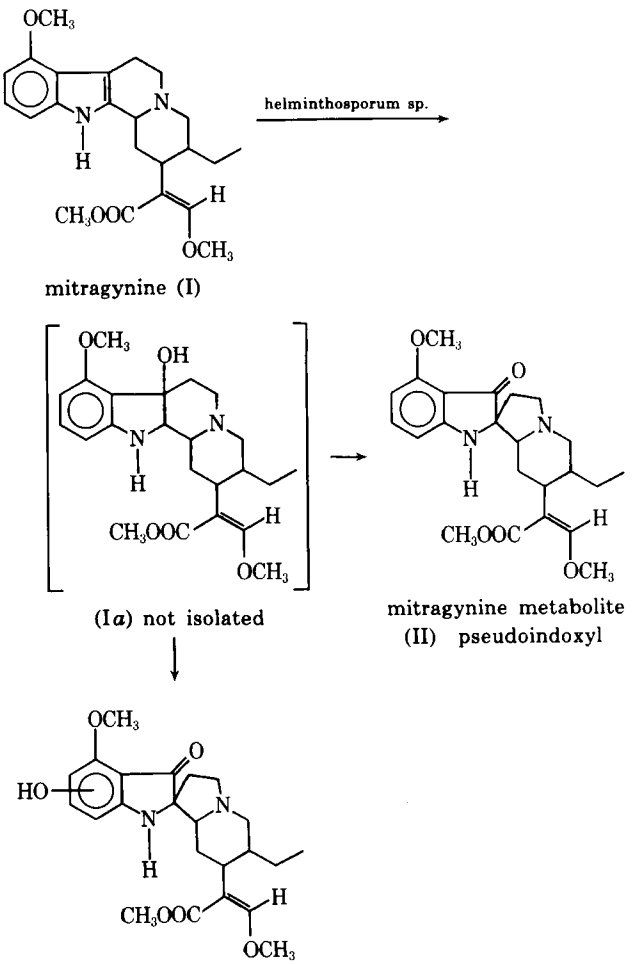
Mitragynine, an indole alkaloid isolated from the tree *Mitragynine speciosa*[1], indigenous to Borneo, Thailand, and New Guinea has been reported to exhibit analgesic and antitussive properties in experimental animals. The chemical structure of this material was defined in 1963 (1) and was confirmed by crystallographic studies (2). As part of continuing studies of the chemistry and biology of mitragynine, the pure material was subjected to biotransformation by the fungus Helminthosporum sp.[2]. A mixture including at least two major metabolites was found to be quite active pharmacologically; therefore, further work was undertaken to isolate each major metabolite and to test them biologically. The most active compound, II, $C_{23}H_{30}N_2O_5$, of this mixture was isolated in this laboratory and displayed analgesic activity in the D'Amour–Smith test (3) for analgesia almost 10-fold (9.4:1) over mitragynine, I, when administered by both oral and intraperitoneal routes to animals (4). A second active metabolite, III, $C_{23}H_{30}N_2O_6$, was also isolated in these laboratories in much smaller amounts but displayed lower activity (20–38%) compared to that of mitragynine. Considerable work has been reported (5, 6) describing many related alkaloids, but the two compounds isolated and identified in these studies have not been described.

The microbial transformation appears to proceed *via* oxidative and hydroxylative pathways (Scheme I). The primary metabolite, II, was isolated and identified as the pseudoindoxyl derivative of mitragynine [methyl ester of 6′-ethyl-2′,3′,6′,7′,8′,8′α-hexahydro-4-methoxy-α-(methoxymethylene)-3-oxospiro[indoline-2,1′(5*H*)indolizine]-7′-acetic acid]. The second metabolite, III, was identified as the ring A hydroxylated derivative of II.

Structure elucidation required extraction of the compounds from the fermentation mixture and chromatographic separation on a neutral alumina column followed by preparative TLC and spectral analysis. Selective chemical oxidation (7, 8) was carried out on mitragynine and resulted in the partial conversion of mitragynine into the major active metabolite, II. Spectral evidence obtained from both the synthesized and isolated compounds was identical. Because of the low level of biological activity associated with Compound III and the potential complexity of the synthetic problem, no attempt was made to prepare this compound chemically.

EXPERIMENTAL

Microbial Transformation—The pH of 9.5 liters of 0.5% glu-



Scheme I—Proposed microbial metabolic pathway for mitragynine

[1] The material used in this paper is *Mitragyna speciosa, KORTH*, a member of the Rubiacea. Specimens were collected by Dr. A. H. Beckett in November and December 1962 in the vicinity of Bangkok, Thailand. A voucher specimen of the plant has been deposited with Professor R. E. Schultes, Botanical Museum, Harvard University, Cambridge, Mass.

[2] ATCC 20154, available from the American Type Culture Collection, Rockville, Md.

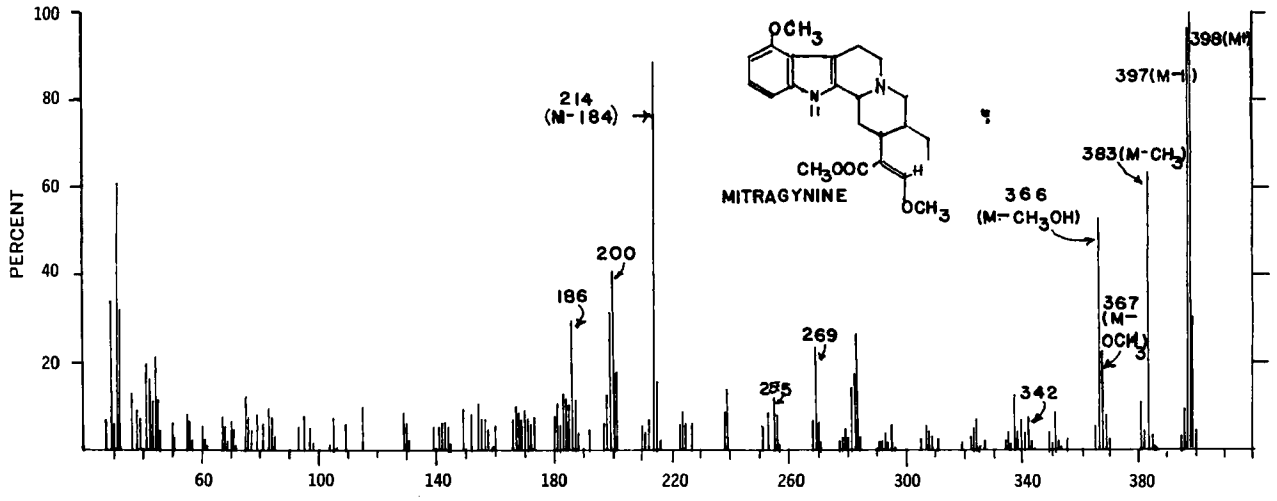


Figure 1—*Mass spectrum of mitragynine.*

cose in 2% corn steep liquor in a 10-liter fermentation flask was adjusted to pH 5.0 with dilute hydrochloric acid, and the solution was heated in an autoclave for 75 min at 17 psi and 121°. Helminthosporum sp. was grown in 500 ml of 0.5% glucose in 2% corn steep liquor for 48 hr. This culture was introduced into the prepared medium, and the system was incubated for 48 hr at 30° at 200 rpm with aeration at 0.5 v.v.m[3]. After this time the pH was adjusted to 8.0 with 1 *N* aqueous sodium hydroxide solution. Mitragynine hydrochloride (TLC pure, one spot) (10.88 g) in 100 ml of 95% ethanol and 100 ml of deionized water were added.

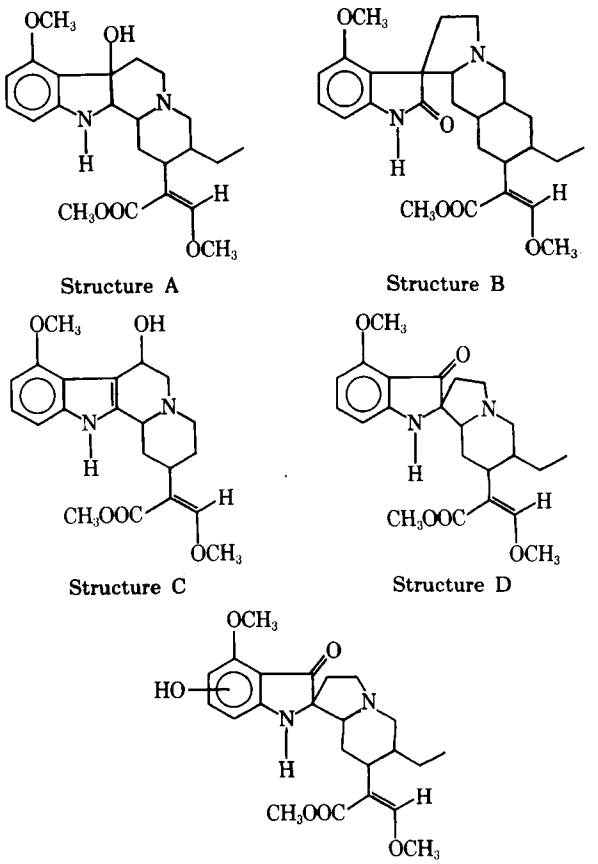
The fermentation was allowed to proceed at 30° with agitation at 250 rpm and aeration at 1 v.v.m. The pH was monitored with a pH meter and adjusted with dilute acid or alkali to maintain it at 8.0. Samples (4–5 ml) were taken at intervals and were adjusted to pH 9.2 and shaken with an equal volume of freshly opened peroxide-free ether. The ether phase was separated and concentrated *in vacuo* at 30°. The residue was taken up in chloroform–methanol (9:1) and spotted on neutral alumina chromatography plates (Fig. 1). The plates were developed for 10 cm in a paper-lined tank using chloroform–ethyl acetate (1:1), air dried, observed under UV light, and sprayed with modified Dragendorff reagent to determine the progress of the transformation.

After 50 hr the fermentation was terminated. The pH was adjusted to 9.2, the broth was centrifuged, and the supernate was decanted and extracted with half its volume of chloroform. The chloroform was backwashed with water and dried over anhydrous sodium sulfate. The chloroform extract was then concentrated to dryness *in vacuo*. Benzene was added to the residue, and the mixture was stirred and filtered. The benzene filtrate was evaporated to dryness *in vacuo*. Cyclohexane was added to the residue, and the mixture was filtered. The cyclohexane filtrate was then transferred onto a neutral alumina column and eluted with the following solvents: cyclohexane, toluene, ether, chloroform, and methanol.

The collected fractions were monitored by TLC. Fractions containing the active metabolites (R_f 0.13 and 0.23; Fig. 1) were combined and evaporated to dryness *in vacuo*. The residue was dissolved in cyclohexane with a small amount of toluene. This solution was placed on a second neutral alumina column and rechromatographed using the same eluting solvents. Examination of the fractions gave several cuts where single spots of each of the active metabolites were found. The overall yield was 73.5% of metabolized microbial product, 24.3% of metabolite II, and 1.4% of metabolite III. Two compounds were isolated and studied from both the structural and biological viewpoints. Biologically these were the most active metabolites when compared to mitragynine. Pharmacological work related to mitragynine was already reported (4).

Mass Spectral Analysis—Because many indole alkaloids are not readily distinguished from one another by their optical spectral properties and because mass spectral fermentation patterns offer convenient and very characteristic fingerprints for many alkaloid species (9–14), a mass spectral study was carried out to augment the UV, IR, and NMR spectral data. This consisted of: *(a)* 70-ev electron-impact spectra at medium resolution, *(b)* exact mass measurements of major peaks, and *(c)* mass spectral analysis of compounds after treatment with a silylating reagent, *N,O*-bis-(trimethylsilyl)trifluoroacetamide.

The mass spectra of mitragynine and its major metabolites are reproduced in Figs. 1 and 2. High-resolution mass measurements of the major fragmentation peaks were made and empirical formulas were established. Mitragynine was found to have a molecular weight of 398.2250 corresponding to an empirical formula of $C_{23}H_{30}N_2O_4$, while the major metabolite II had a molecular weight of 414.2160 corresponding to $C_{23}H_{30}N_2O_5$. Metabolite III, a hydroxylated mitragynine indoxyl, showed an increase of 16 amu corresponding to an empirical formula of $C_{23}H_{30}N_2O_6$. Exact mass



Structure A

Structure B

Structure C

Structure D

Structure E

Possible structures for metabolites

[3] v.v.m. = cubic liters of air per cubic liter of growth medium per minute.

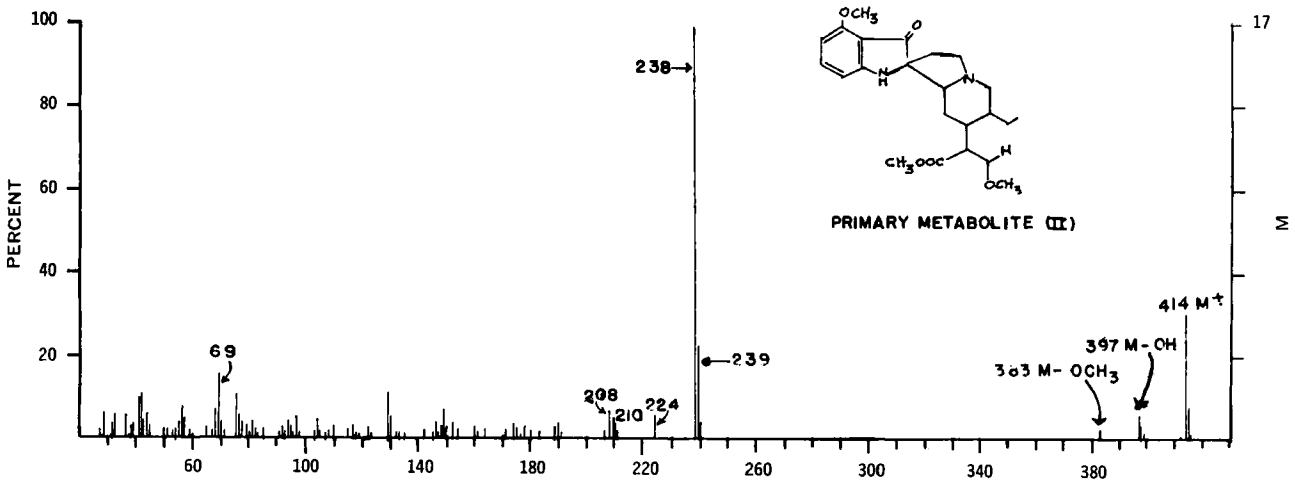


Figure 2—*Mass spectrum of mitragynine metabolite (II).*

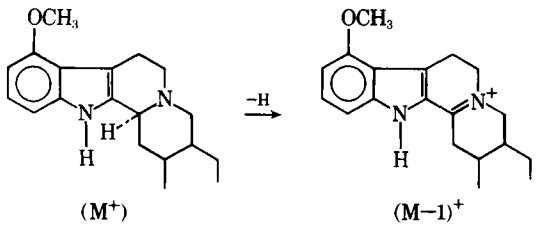
measurements were not made on III since the spectrum corresponded to that of the primary metabolite, the pseudoindoxyl II plus the additional 16 amu. Further evidence through derivatization as the silyl compound obviated the need for additional measurements. This increase of 16 amu for metabolite II and 32 amu for metabolite III over that of the parent mitragynine due to the addition of one and two atoms of oxygen, respectively, suggested four possible structures, A–D, for metabolite II and E (Fig. 1) for metabolite III.

Comparative examination of the mass spectral data of mitragynine and its major microbial metabolite II shows characteristic, unique fragmentation patterns (9). The $(M - 1)^+$ peak in mitragynine is a very large peak, whereas it is comparatively insignificant in the primary metabolite II. This $(M - 1)^+$ peak is large only in indole compounds having a tetrahydro-β-carboline moiety which can form an ammonium ion in conjugation with the indole system by losing one hydrogen. This is a very characteristic decomposition pattern which has been defined through extensive deuterium labeling (3) (Scheme II).

The mass spectra of indole alkaloids where an $(M - 1)^+$ peak is weak or absent provide an indication that the compound may be either an indole or pseudoindoxyl species (Scheme III) (9).

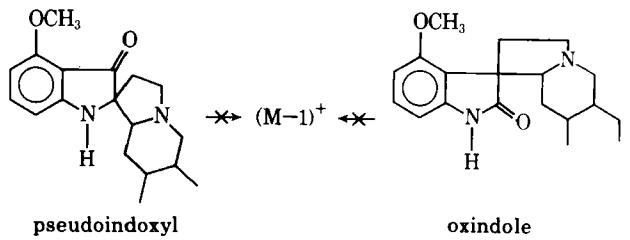
The base peak in the spectrum of mitragynine occurs at *m/e* 214, a loss of 184 amu, whereas the base peak in the major metabolite is at *m/e* 238. The difference of 24 amu between these two fragment ions does not correspond to a difference of 16 amu between the molecular weights of these two compounds. This suggests that fragmentation in both molecules occurs *via* different pathways (Schemes IV and V) and that the molecular ion shift technique (11) frequently used to establish similarities could not be applied because two basically different structures are present. The sequence of fragment formation is further substantiated by the presence of a metastable ion (*m** 115.4) for the transition and is verified by exact mass measurement of the peak at *m/e* 214 (214.111 = $C_{13}H_{14}N_2O$).

It is evident that since the mitragynine ion (214) has two nitrogens, the indole nucleus and the nitrogen in ring C contribute to its composition. In the major microbial metabolite, on the other hand, the most intense ion is at mass *m/e* 238 (238.1436 = $C_{13}H_{20}NO_3^+$), having only one nitrogen present. To obtain two such fragments from a compound, structural elements differing from mitragynine would have to be present. Such a moiety would occur from a pseudoindoxyl (*m/e* 238) or from an oxindole (*m/e* 239) (Scheme VI). Deuterium labeling (3) has shown that bonds 5–6 and 3–7 are broken by electron-impact mass spectrometry in oxindole compounds while bands 5–6 and 7–8 are broken in pseudoindoxyl compounds and that fragment ions (*m/e* 238 and 239) are derived from the alicyclic portion of the molecules. Since the most abundant peak in the mass spectrum of the mitragynine metabolite occurs at *m/e* 238 and not 239, the evidence is that the compound is a pseudoindoxyl and not an oxindole species. These conclusions are also in agreement with UV, NMR, and IR data provided. Other structural features are given in the fragmentation pathways shown in Schemes IV and V.



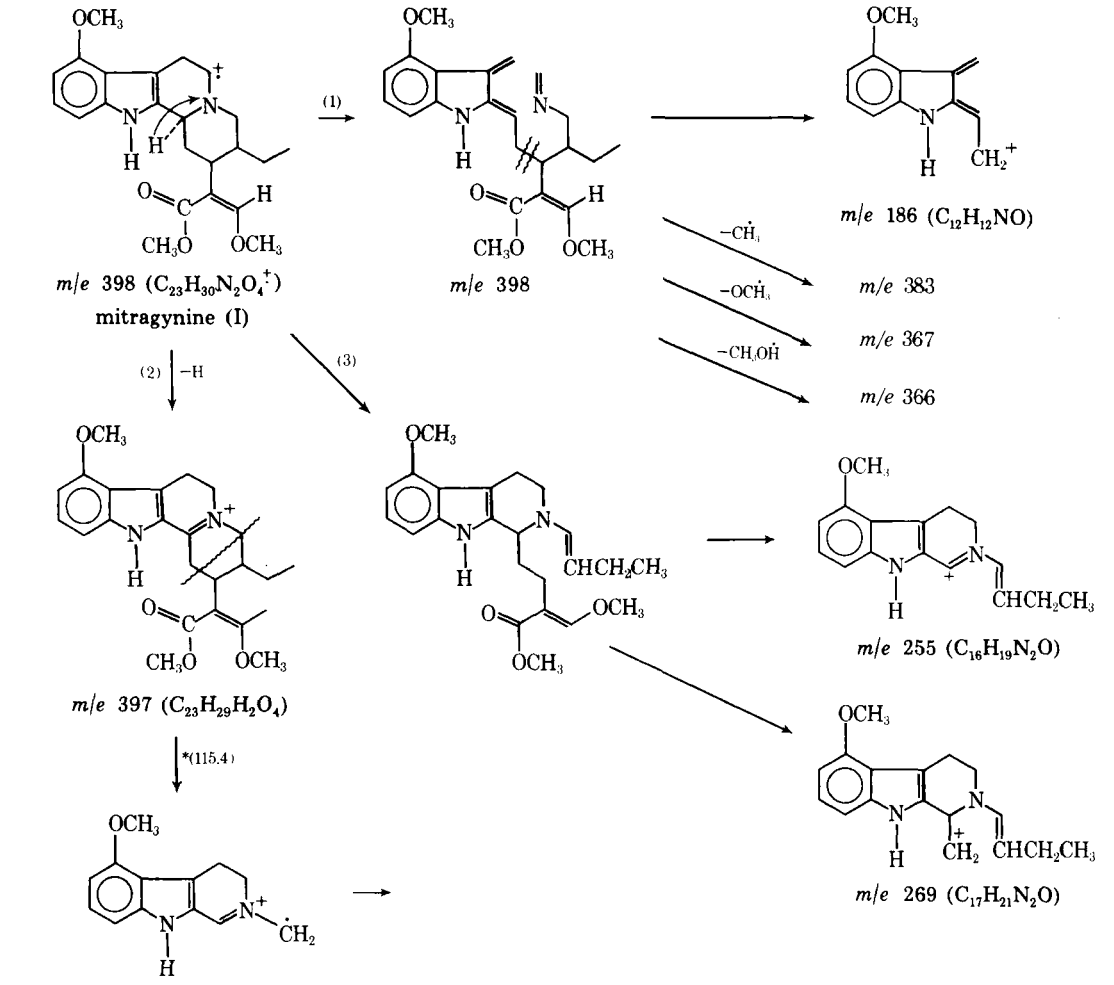
Scheme II

Additional diagnostic data were obtained from Compounds I, II, and III by treatment with diazomethane and a silylating reagent[4] prior to mass spectral fragmentation to establish the position and nature of the two added oxygens in metabolite III. The compounds (approximately 1–2 μg), dissolved in peroxide-free ether, were cooled in ice and treated with an excess of a freshly prepared diazomethane solution for approximately 30 min until the solution remained yellow. The reacted compounds were then isolated by evaporating the ether, and electron-impact mass spectra were obtained on them. The mass spectra of I and II were identical to the unreacted spectra, indicating that no reactive carboxylic acid, alkylatable phenolic, or reactive olefin groups were present. However, III showed an increase in mass of 14 amu (*m/e* 446) on the molecule, indicating alkylation had taken place on a phenolic hydroxyl in ring A. These same underivatized Compounds, I–III, were then dissolved in 5–6 μl of the silylating reagent[4] for 15 min and gently heated (~50°). The acetonitrile solvent was removed with a stream of nitrogen while heating at 50–60°. Upon rerunning the mass spectra of the compounds, the molecular ion of mitragynine had increased by 72 amu to *m/e* 470, equivalent to the addition of one trimethylsilyl group. This observation agrees with the structure of mitragynine, I, containing one replaceable or reactive hydrogen on the nitrogen of the indole nucleus. Metabolite II also increased by 72 amu to *m/e* 486. That only a single silyl derivative was produced agrees with earlier mass spectral assignments for II having no hydroxyl groups on the molecule. Metabolite III showed an increase in mass from *m/e* 430 to 574 (M^+ + two trimethylchlorosilane groups). The mass fragmentation pattern of each derivatized sample showed a loss of $M^+ - 15$, which was not diagnostic but sug-

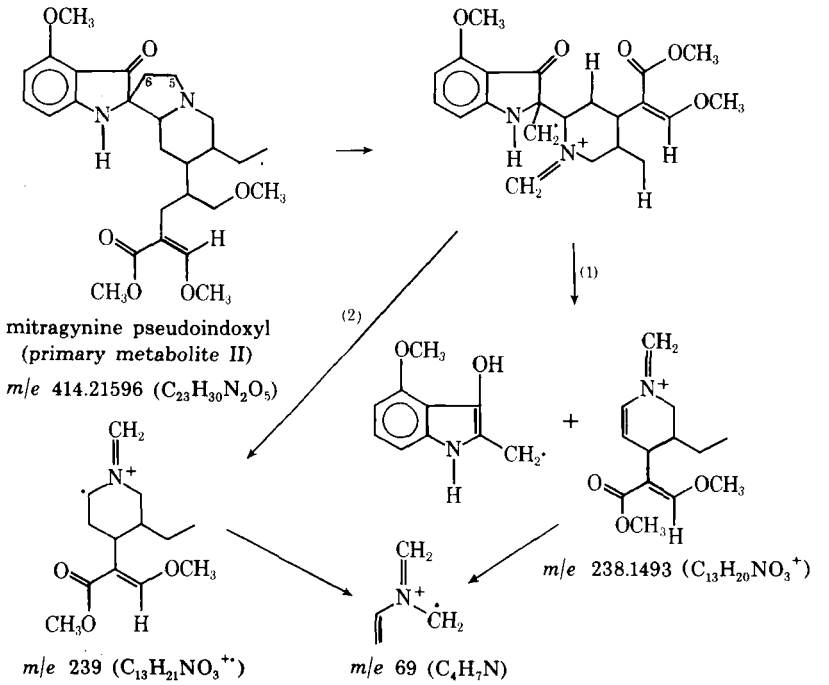


Scheme III

[4] *N,O*-Bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane, Pierce Chemical Co.



Scheme IV—Fragmentation of mitragynine



Scheme V—Fragmentation of mitragynine pseudoindoxyl

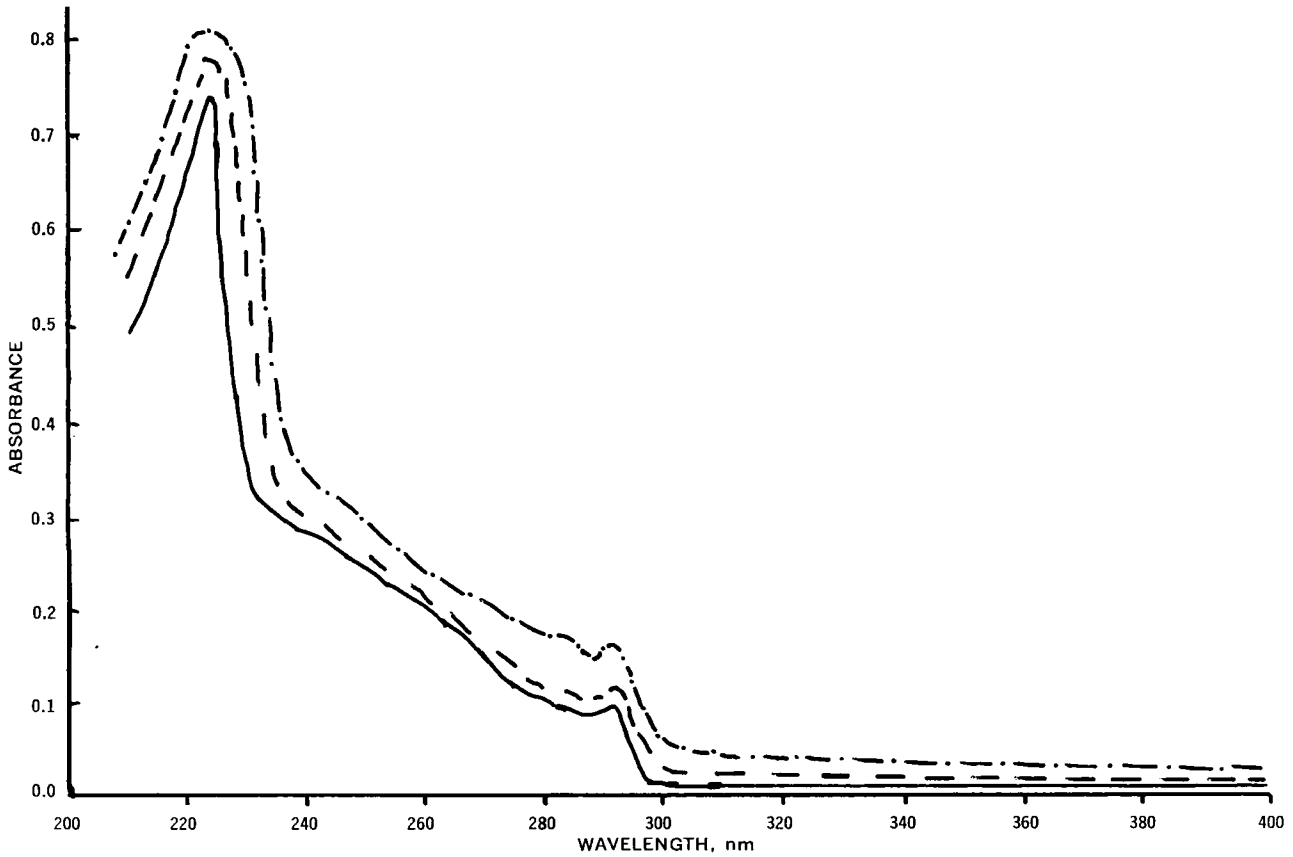


Figure 3—*UV absorption spectrum of mitragynine (I). Key: -·-, in neutral ethanol; ——, in acidified ethanol; and ---, in alkaline ethanol.*

gested the loss of a methyl from the trimethylsilyl compounds[5]. Metabolite III also showed the presence of an intense ion at *m/e* 238 plus a less intense one at *m/e* 239. Since this latter fragment ion had not increased in mass (mass shift), it was reasonable to assume that the hydroxyl is on the aromatic ring (A) of the indole and not on the alicyclic portion of the molecule. UV spectral data obtained in acid and alkaline solutions of these metabolites provided additional evidence that the hydroxyl is phenolic and not alicyclic, as discussed later.

These deductions and findings agree with those of Beckett *et al.* (15) and Lee *et al.* (16) who described the mass spectral data of several mitragynine-type indoles and oxindoles. These compounds have corresponding *m/e* 239 ions but no *m/e* 238 ions as do the pseudoindoxyl compounds isolated and identified here. To date, there is no evidence in the products obtained and isolated from the microbial transformation with Helminthosporum sp. that any oxindole compound was formed. This evidence shows that there has only been selective conversion to the pseudoindoxyl species.

On the basis of the mass spectral data, Structures A, B, and C were eliminated. Additional spectral evidence described here helps verify these deductions.

UV Data—Table I presents the UV spectral absorbance data of mitragynine (I), mitragynine metabolite II, and mitragynine metabolite III. Comparison of the UV absorption spectral curves (Figs. 3–5) of mitragynine metabolites II and III with that of mitragynine shows that the metabolites II and III have the characteristic indole absorption peaks at 285–290 nm and approximately 220–225 nm, which indicate the presence of an intact indole nucleus; however, metabolites II and III have two additional peaks: II at 239 and 296 nm and III at 242 and 320 nm. None of the absorption peaks in metabolite II is shifted with a change in pH of the solvent. When determined at pH 1.0 and 10.0, there were no detectable shifts of the absorption maxima or changes in their intensity. These data suggested that the additional oxygen present in metabolite II probably was located in a position other than ring A [*e.g.*, test for hindered phenol was negative (17)]. When this test was carried out on metabolite III, a positive (reddish-orange) color was produced.

Since the original sample of purified mitragynine showed the absence of an absorption peak above 290 nm, the presence of a peak above 360 nm indicated that the C ring—if metabolite II still had one intact—might have unsaturation present in conjugation with the indole portion of the molecule. Metabolite III did, however, show a shift in the position of the absorption maxima from 320 to 343 nm and from 242 to 262 nm, indicating that the oxygen

Table I—UV Absorption Spectral Data

Medium[a]	Mitragynine (I) λ_{max}	Mitragynine (I) Log ϵ	Metabolite II λ_{max}	Metabolite II Log ϵ	Metabolite III λ_{max}	Metabolite III Log ϵ
Ethanol (neutral)	225	4.67	216	4.40	222	4.45
			239	4.44	242	4.41
	290	3.88	288	3.89	285	4.22
			396	3.60	320	3.04
1 *N* HCl–ethanol[b]	225	4.73	216	4.32	222	4.46
			234	4.36	242	4.42
	290	3.77	290	3.83	285	4.21
			395	3.54	320	3.06
1 *N* NH_4OH–ethanol[c]	225	4.68	219	4.36	221	4.64
			237	4.43	285	4.24
	291	3.86	288	3.80	285	4.24
			395	3.53	343	3.43

[a] Alcohol USP. [b] Concentrated hydrochloric acid added to ethanol to produce a 1 *N* solution. [c] Concentrated ammonium hydroxide added to ethanol to produce a 1 *N* solution.

[5] See S. P. Markey, H. A. Thobhani, and K. B. Hammond, "Identification of Endogenous Urinary Metabolites by Gas Chromatography–Mass Spectrometry; A Collection of Mass Spectral Data," B. F. Stolinsky Research Laboratory, Department of Pediatrics, University of Colorado Medical Center, Denver, Colo., 1972.

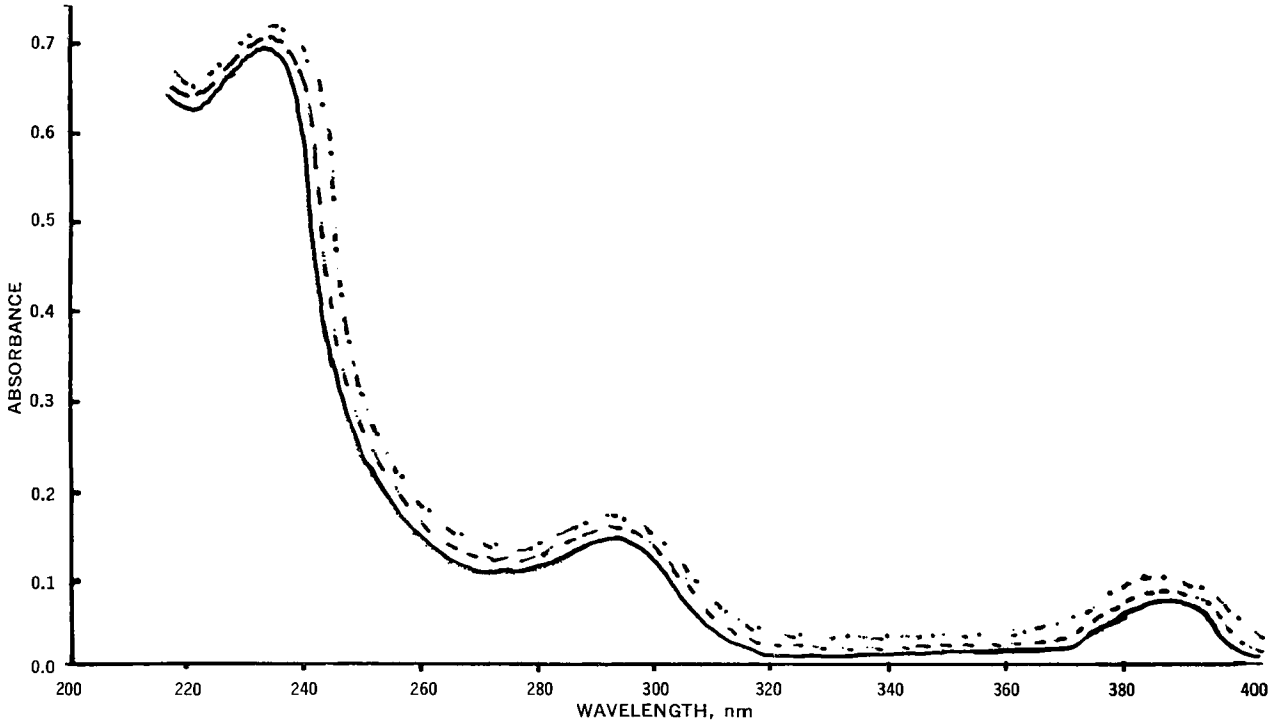


Figure 4—*UV absorption spectrum of mitragynine metabolite II. Key: –·–, in neutral ethanol; ——, in acidic ethanol; and ---, in alkaline ethanol.*

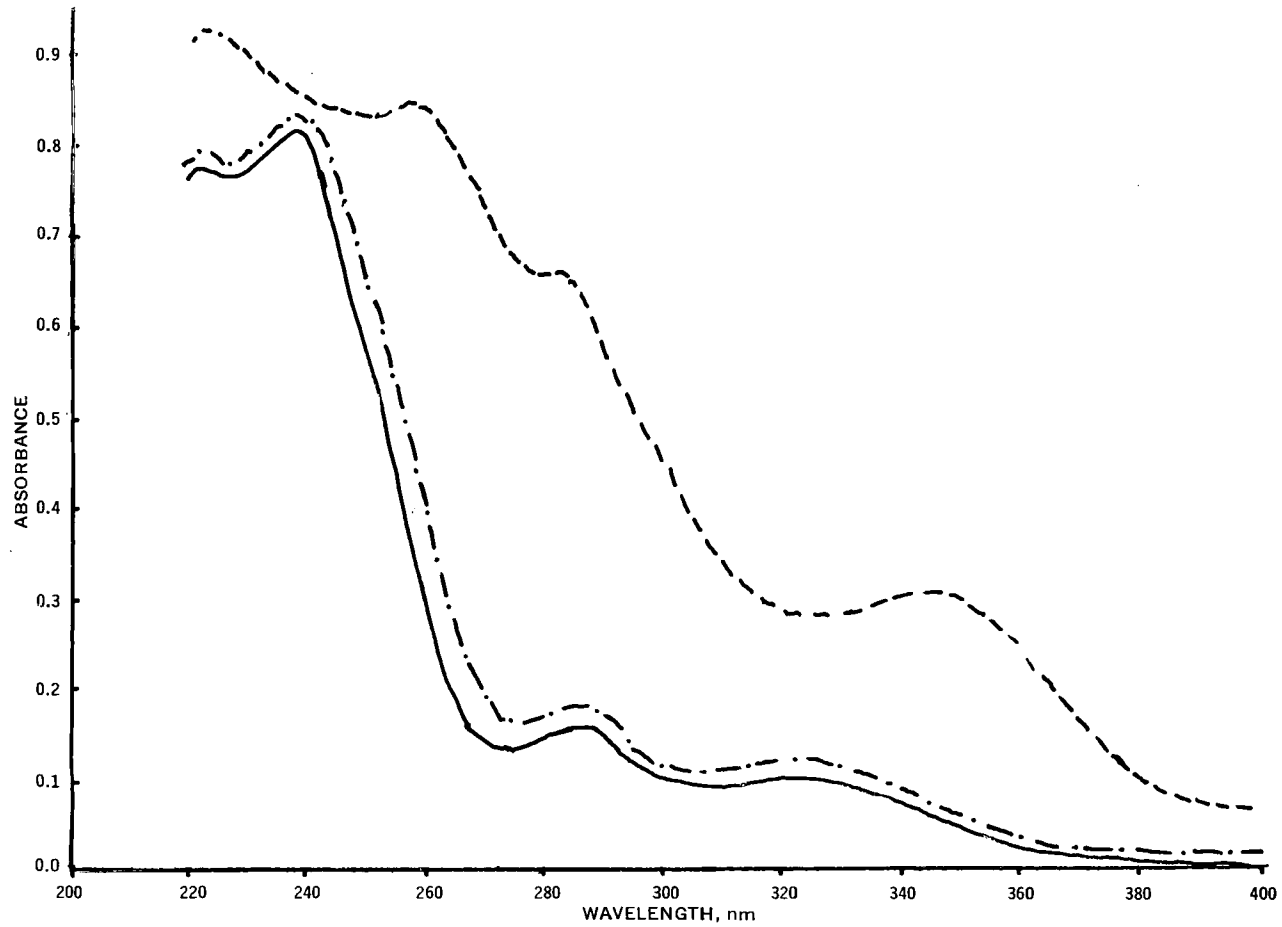


Figure 5—*UV absorption spectrum of mitragynine metabolite III. Key: –·–, in neutral ethanol; ——, in acidic ethanol; and ---, in alkaline ethanol.*

Table II—NMR Spectral Data[a]

Protons	Mitragynine (I)[b]			Metabolite II[b]		
	$CDCl_3$		D_2O	$CDCl_3$		D_2O
CH_3	0.88	Triplet	No change	0.88	Triplet	No change
CH_2 (ring)	2.0 –2.5	Multiplet	No change	2.0 –2.5	Multiplet	No change
OCH_3 (aromatic)	3.83	Singlet	No change	3.83	Singlet	No change
$OCOCH_3$	3.67	Singlet	No change	3.63	Singlet	No change
OCH_3 (vinyl)	3.64	Singlet	No change	3.58	Singlet	No change
CH_3 of ethanol	1.20	Triplet	No change	1.20	Triplet	No change
CH_2 of ethanol	3.45	Quartet	No change	3.45	Quartet	No change
One aromatic proton	6.42	Quartet	No change	6.25	Quartet	No change
Two aromatic protons	6.82–7.20	(Multiplet)	No change	7.10–7.40	(Multiplet)	No change
Olefinic protons	7.40	Singlet	No change	7.34	Singlet	No change
Proton on indole N—H	7.98	Singlet	Disappears	5.20	Singlet	Disappears

[a] Data values in parts per million from tetramethylsilane. [b] Compounds recrystallized and isolated as alcoholates.

of metabolite III was phenolic and was located on the aromatic A ring. The inference that metabolite II might have unsaturation in the C ring was not substantiated. A careful examination of the indole alkaloids listed in Ref. 18 failed to reveal a model structure with an absorption band above 360 nm. Reference to Sangster and Stuart's (19) review showed that pseudoindoxyl compounds and no other indole alkaloids reviewed had four absorption bands in their UV spectra in the approximate vicinity of metabolite II: 215–225, 225–235, 285–295, and 390–405 nm. Witkop and Patrick (20) reported these same absorption maxima for pseudoindoxyl compounds in their studies. Furthermore, they reported that these peaks were also insensitive to alkali and acid pH. A study of the UV spectral data in this review showed that oxindole alkaloid spectra were similar to indole alkaloids containing a tetrahydrocarboline moiety and that their UV spectra did not resemble metabolite II. These findings helped to substantiate the mass spectral findings and to exclude oxindoles from further consideration, thus adding support to the conclusion that Structure D fits all data for Compound II.

The limited investigation of metabolite III also suggests that Structure E is in accord with the spectral observations.

IR Spectral Data—The IR spectra of mitragynine and metabolites II and III show an N—H stretching vibration at approximately 3365 cm^{-1} (mineral oil mull). Carbonyl peaks attributable to the carboxymethoxy ether group for all three compounds are found at approximately 1690 and 1640 cm^{-1}. In addition, there is an OH stretching vibration at 3558 cm^{-1} in all three compounds due to the OH contributed by the ethanolate (see *NMR Data*). Structural changes attributed to changes in the indole ring are seen by the appearance of a moderately strong band at 1590 cm^{-1}. These findings are in agreement with the bands of the carboxyl region reported for other similar alkaloids having the pseudoindoxyl structure (7, 21–24) with similar functional groups on the indole nucleus. No evidence from the IR data could be found for oxindole formation.

NMR Data—The NMR spectra of mitragynine and the major metabolite II were run in deuterated chloroform (60 MHz) and were then shaken in deuterium oxide to exchange active hydrogens in these compounds (Table II). An insufficient amount of metabolite III was isolated to obtain a useful NMR spectrum. The observed chemical shifts for mitragynine very closely resembled those obtained by Trager *et al.* (25) and for indole pseudoindoxyls by Wenkert *et al.* (22) and Hendrickson and Sims (23). The NMR data agree with the structure assigned to Compound II. Examination of the NMR data shows that the indole nuclei, the proton on the indole nitrogen, and components of rings C and D are intact and that the methoxyl groups are present on mitragynine as well as the major metabolite II.

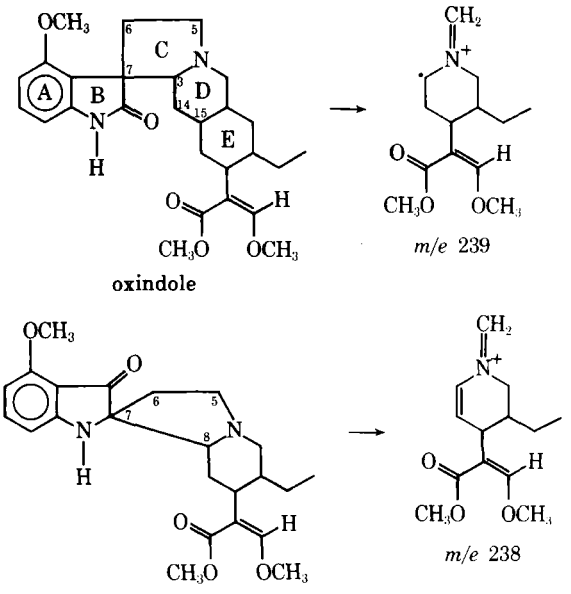
Table III—Microelemental Analysis Results

Element	Mitragynine I, $C_{23}H_{30}N_2O_4 \cdot C_2H_5OH$		Metabolite II, $C_{23}H_{30}N_2O_6 \cdot C_2H_5OH$		Metabolite III, $C_{23}H_{30}N_2O_6 \cdot C_2H_5OH$	
	Calc.	Found	Calc.	Found	Calc.	Found
C	67.54	67.34	65.20	65.48	63.01	63.21
H	8.16	7.92	7.88	7.66	7.61	7.49
N	6.30	6.18	6.08	6.12	5.88	5.88

Elemental Analysis Data—Both the major metabolites and mitragynine samples used in this study were isolated as alcoholates and run as such for elemental composition (Table III), NMR, and IR studies. Differential thermal analysis (DTA) examination of the samples showed that the alcohol was lost between 80 and 90° and that decomposition of the free base occurred near the melting points (mitragynine mp 102–104°, metabolite II mp 151–153°, and metabolite III mp dec.)[6].

TLC—TLC was employed throughout this study to check and verify the integrity of the samples and to prepare highly purified materials for analytical purposes. Trace impurities were removed from the compounds by preparative TLC; the separated spots were scraped from the plates and the highly purified samples were isolated and rechecked by additional TLC work. Only when a single TLC spot was found after this treatment was a sample used for structural characterizations. While this procedure might have been overly cautious, artifacts that frequently arise were avoided, thus making structural characterizations more definitive. Two TLC systems giving good separation were developed and employed throughout this work (Table IV).

Spot tests on TLC plates containing the metabolites as well as mitragynine gave positive reaction with Dragendorff reagent, modified Ehrlich reagent, iodoplatinate, and diazotized *p*-nitroaniline (weak red). When tested with Emerson reagent and 2,4-dinitrophenylhydrazine, the results were inconclusive because of the orig-



Scheme VI

[6] Melting-point determination on Mettler hot stage microscope.

Table IV—TLC Data for Mitragynine and Its Microbial Metabolites

Band	R_f	Color	Compound
		System 1[a]	
8	0.60	Yellow	?
7	0.49	Yellow	Mitragynine (I)
6	0.41	Yellow	?
5[b]	0.32	Yellow	?
4[c]	0.23	Yellow	Metabolite II
3[d]	0.13	Yellow	Metabolite III
2	0.05	Orange	?
1	0.00	Orange	?
		System 2[e]	
9	0.40	Yellow	Metabolite II
8	0.36	Yellow	Metabolite III
7	0.27	Yellow	?
6	0.25	Yellow	Mitragynine (I)
5	0.19	Yellow	?
4	0.13	Orange yellow	?
3	0.09	Orange yellow	?
2	0.05	Pink	?
1	0.00	Orange red	?

[a] Silica gel GF on 5.1 × 20.3-cm (2 × 8-in.) plates run for 10 cm; solvent of chloroform–ethyl acetate (1:1 v/v); equilibration time of 5 min. Compounds 1, 2, 6, and 8 were obtained in insufficient amounts for biological testing. [b] Slightly active. [c] Most active. [d] Active. [e] Silica gel GF on 5.1 × 20.3-cm (2 × 8-in.) plates run for 10 cm; solvent of benzene–methanol (95:5 v/v); equilibration time of 5 min.

inal color of the spots. All spots on the thin-layer plates were visible in ordinary white light as well as under short wavelength (254 nm) UV light. Test for hindered phenol employing ferric chloride in pyridine (17) was negative for Compounds I and II but showed a slight increase in color for Compound III. Since a positive test with 2,4-dinitrophenylhydrazine produces an orange-yellow reaction and the compounds had a yellow color, it was difficult to determine whether the test was weakly positive or negative. Since other spectral data (mass, IR, UV, and NMR) were more diagnostic and definitive, the spot test data were only used for semiqualitative purposes during the separations. The migration characteristics are described and the spots which indicate biological activity are listed in Table IV.

Synthesis of Metabolite II (Mitragynine Pseudoindoxyl)—Mitragynine and other indole alkaloids been reported to be converted into their corresponding indole and pseudooxindole analogs by reaction with oxidizing agents such as lead tetraacetate (7) and *tert*-butyl hypochlorite which convert the indoles to the β-hydroxyindolenines. Treatment of these compounds with mild alkali (sodium methoxide) converts the compounds into their corresponding pseudoindoxyl compounds. If the same reaction sequence is carried out and the β-hydroxyindolenine is treated with acid, the corresponding oxindole compounds are prepared (26).

Preparation of Mitragynine Pseudoindoxyl (II)—The procedure followed was essentially that of Finch *et al.* (7). In a 150-ml erlenmeyer flask, 0.25 mmole (111 mg) of mitragynine (as the alcoholate) was dissolved in 15 ml of methylene chloride and the solution was cooled in an ice–water bath. A freshly prepared 0.07 *M* solution of lead tetraacetate (50 ml), which was previously cooled in ice, was added dropwise over 10 min. Stirring and reaction were continued for an additional 60 min (cold), after which time the reaction mixture was poured over ice and stirred. The ice–water–organic mixture was then separated, washed several times with water, and dried (sodium sulfate). The volume was reduced to 10–15 ml in a stream of nitrogen. The resulting light-yellow reaction product was transferred to a neutral alumina[7] chromatographic column packed with 60 g of adsorbent.

Elution was carried out with pure methylene chloride followed by increasing amounts of methanol in methylene chloride. The fractions were collected and evaporated to dryness. Fractions containing the solvents from methylene chloride and 1% methanol in methylene chloride were combined. The dry fractions were then treated with 0.05 *N* sodium methoxide (prepared from sodium and methanol) and refluxed for 1 hr. The reaction product was treated with water, and methylene chloride solutions were dried with sodium sulfate, concentrated with a stream of nitrogen, and then prepared by TLC (System 1). The spots containing the desired compounds were scraped from the plates and eluted with chloroform–methanol (9:1). When a single spot was obtained on subsequent TLC, the compounds were recrystallized from a very small amount of ethanol and dried in a stream of nitrogen and spectral data were obtained. These data compared identically with spectral data obtained from Compound II isolated from the microbial transformation product and constituted final proof of structure.

One may speculate as to the action of the fungus at this time. It is possible that the products formed may simply have been the result of air oxidation of mitragynine during the fermentation. It is, however, unlikely that ring hydroxylation (Compound III) is the result of air oxidation since there was no evidence of this compound in the synthesis of Compound II from mitragynine. In the synthesis, however, it was possible to isolate some of Compound Ia. This aspect of the work requires further investigation and will be reported later.

[7] Woelm-Brockmann grade III activity.

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Browning of Dextrates in Solid–Solid Mixtures Containing Dextroamphetamine Sulfate

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Abstract □ By using diffuse reflectance spectroscopy, the ethanol-mediated interaction between dextroamphetamine sulfate and dextrates in solid–solid mixtures was studied. Discoloration of the powder mixtures was accelerated by the presence of the amine and by storage at elevated temperatures. Heated samples showed two new absorption maxima at 330 and 300 nm in their reflectance spectra. The former was attributed to the chemisorption of the amine molecules on the surface of the dextrates, while the latter was attributed to the interaction between amine molecules and the dextrates. The rate of browning was determined by plotting remission function *versus* time at 300 nm for mixtures of dextroamphetamine sulfate and dextrates at three temperatures and two dextroamphetamine sulfate concentrations. Arrhenius-type plots were used to approximate the browning rate at 25°. The browning rate was considerably slower than that reported for solid–solid mixtures containing dextroamphetamine sulfate and spray-dried lactose USP.

Keyphrases □ Dextrates—browning in solid–solid mixtures containing dextroamphetamine sulfate, rate and variables □ Dextroamphetamine sulfate–dextrates—solid–solid mixtures, rate of browning, effects of temperature and concentration □ Discoloration—*dextrates–dextroamphetamine sulfate solid–solid mixtures*, rate of browning, effects of temperature and concentration □ Diffuse reflectance spectroscopy—monitoring rate of browning of dextrates–dextroamphetamine sulfate mixtures

Dextrates is dextrose prepared by the controlled hydrolysis of starch in combination with a special manufacturing process that yields spherical, porous beadlets[1]. It is composed of approximately 92% dextrose and 8% higher saccharides. The material is white, free flowing, and odorless and consists of aggregates of dextrose microcrystals intermixed and cohered with a small proportion of higher saccharides. The structure and dissolution characteristics of dextrates are unique when compared to those of dextrose USP and sucrose. Its absolute solubility is greater than that of dextrose USP but less than that of sucrose, yet its rate of solution is low when compared to dextrose USP and sucrose.

Henderson and Bruno (1) evaluated dextrates as an excipient in direct compression tablet production. It was concluded that dextrates and lactose[2] USP (beadlets) were superior to spray-dried lactose and lactose USP (anhydrous) for use as filler in direct compression. Both exhibited excellent flow characteristics, and dextrates showed improved compression and disintegration properties while lactose USP (beadlets) possessed greater physical stability.

A number of workers (2–4) reported that tablets prepared using lactose as a filler tend to discolor on storage. This phenomenon was accelerated by the presence of amines and/or certain lubricants and was dependent on the temperature, humidity, and light exposure at which the tablets were stored. With regard to color stability, tablets prepared with dextrates and stored at different temperatures and

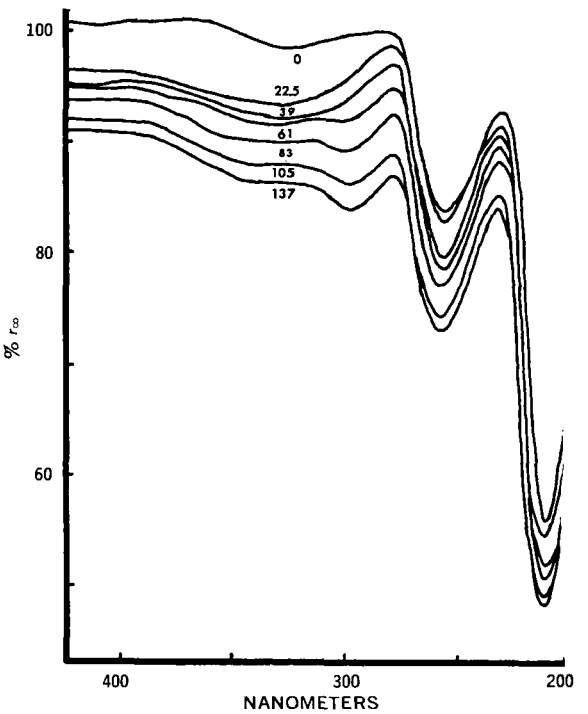

Figure 1—*Diffuse reflectance spectra of an equilibrated sample containing 10 mg of dextroamphetamine sulfate/g of dextrates after heating at 55° for various time periods (shown in hours).*

[1] Celutab, Penick and Ford Ltd., Cedar Rapids, Iowa.

[2] Foremost Dairies Inc., San Francisco, Calif.