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Preface.

The author wishes to express his sincere thanks to Dr. J. D. Loudon for his constant advice and encouragement throughout the period of these researches, also to Professor J. W. Cook for the interest he has shown in the work.

Thanks are due to Sir John Simonsen, F. R. S., and the Colonial Products Research Council for arranging the supply of botanical material and to Messrs. T. and H. Smith, Ltd., for extracting some of it.

The author acknowledges his indebtedness to the Department of Scientific and Industrial Research for a Maintenance Allowance, and to Mr. J. Cameron and Miss Christie for carrying out microanalyses.

Summary.

Mitraphylline is isolated from the bark of Mitragyna rubrostipulaceae and is separated from β-sitosterol, quinovic acid and nitrogenous bases comprising alkaloidal congeners. The molecular formula, $C_{21}H_{26}O_4N_2$, the presence of one methoxyl group - probably incorporated in a methoxycarbonyl group, and the absence of methylimino groups are confirmed; but the alleged presence of an olefinic linkage is refuted and, contrary to previous statements, it is found that the alkaloid may be induced to yield a mono-acetyl derivative. The ultra-violet and infra-red absorption spectra of mitraphylline are measured and are shown to be consistent with an oxindole structure.

Distillation of mitraphylline from zinc dust in vacuo is found to yield degradation products of considerable significance. In particular, isoquinoline is isolated from the basic fraction while a neutral product, $C_{10}H_9ON$, is identified as either 3-ethylidene- or 3-vinyl-oxindole since it affords 3-ethyloxindole on hydrogenation. Mitraphylline may therefore be regarded as a derivative of N-(β-3-oxindolylethyl)perhydroisoquinoline, in which only the suspected methoxycarbonyl group

and one unidentified oxygen atom remain to be located. Moreover, the neutral compound, $C_{10}H_9ON$, is now shown to be identical with the "methylcarbostyril" which Barger, Dyer and Sargent had obtained from the related alkaloid, rhynchophylline. In the light of these facts the evidence reviewed shows that in the Mitragyna bases an oxindole group of alkaloids co-exists with the β-carboline type indicated by previous investigations.

Synthetic work described includes the syntheses of 1:7-dimethyltryptophan and of 9-methyl-, 1:9-dimethyl-, 2:9-dimethyl- and 1:2:9-trimethyl-β-carbolines.

The apparatus used for the extraction of the bark and a device for the chromatography of colourless substances are described.

CONTENTS.

The Mitragyna Alkaloids.

Introduction.

The natural order of Rubiaceae comprises among others the genera Cinchona, Remijia, Psychotrea, Corynanthe and Mitragyna, and is rich in alkaloids. Interest in the alkaloids of the Mitragyna genus dates from the researches of Miss Field[1] in 1921, but despite the attentions of a number of workers their structures have remained largely unknown.

Considerable attention has been given to the pharmacology of these bases. Under the name "Kratom", the leaves of M. speciosa are chewed as a narcotic in Siam. The view that "Kratom"is a cure for the opium habit is not generally accepted although the drug is used for this purpose by the natives of Perak[2]. Mitragynine has been reported to have a general depressant effect on plain muscle, to facilitate the passage of autonomic impulses and in some respects to resemble cocaine and quinine[3]. It has no action on pathogenic organisms. According to Raymond-Hamet,[4,5,6,7,8,9,10], rhynchophylline (mitrinermine) lowers blood pressure, paralyses sympathetic nerve endings and unlike yohimbine but like

echitamine, does not reverse the action of adrenaline. Rhynchophylline has been shown to be markedly more toxic to frogs than mitraphylline and Massion[11] has stated that the latter resembles mitragynine in action but is weaker.

A number of the Mitragyna alkaloids have been isolated and characterised and some degradative work carried out upon them. The known alkaloids of this genus are mitragynine, mitragynol, mitraphylline, mitraspecine, mitraversine, mitrinermine, and rotundifoline and these have been shown to be very similar to the alkaloids of the Ourouparia genus which are formosanine, gambirine, hanadamine, rhynchophylline and isorhynchophylline. Mitrinermine has indeed been found to be identical with rhynchophylline[12,13,14,15], and the latter name is preferred by most workers for chronological reasons. The locations of these alkaloids in the plants are shown in Table 1.

Table 1.

Name of Plant	Location	Alkaloid	References
M. ciliata	Leaves, bark	Rotundifoline Rhynchophylline	15 10,15
M. diversifolia	Leaves	Mitraversine	1
M. inermis	Bark	Rhynchophylline	16
M. macrophylla	Bark	Mitraphylline	17,18
M. rotundifolia	Leaves	Mitragynol Rhynchophylline Rotundifoline	13,15
M. rubrostipulacea	Leaves, bark fruit wood	Mitraphylline	19,20,21,26

Table 1 -Contd.

Name of Plant	Location	Alkaloid	References
M. speciosa	Leaves, bark fruit, wood	Mitraspecine Mitragynine	22 23
M. stipulosa	Bark	Mitraphylline Rhynchophylline	10

In most cases molecular formula have been established and some similarity can be seen in them. The molecules of the alkaloids all contain 21 or 22 carbon atoms and 2 nitrogen atoms. All possess 4 oxygen atoms with the exception of mitragynol and rotundifoline which contain 5. The formulae are tabulated below (Table 2).

Table 2

Alkaloid	Formula	References
Mitragynine	$C_{22}H_{30-2}O_4N_2$	1,23
Mitragynol	$C_{21}H_{28}O_5N_2$	15
Mitraphylline	$C_{21}H_{26}O_4N_2$	26
Mitraspecine	?	
Mitraversine	$C_{22}H_{26}O_4N_2$	1
Rhynchophylline	$C_{22}H_{24-8}O_4N_2$	10,13,24,25
Rotundifoline	$C_{22}H_{26}O_5N_2$	13

The bases are all monoacidic and the non-basic nitrogen atom has always been considered to be indolic. Herzig-Meyer determinations on mitragynine, rhynchophylline mitragynol and rotundifoline failed to show any trace of methylimino groups[27].

Methoxyl determinations by the Zeisel method show that mitraphylline and mitraversine each contains one methoxyl group, rotundifoline, rhynchophylline and mitragynol two each and mitragynine three[27]. Alkaline hydrolysis of mitraphylline[15], rotundifoline[13], rhynchophylline[13] and mitragynine[1,23] gives rise in each case to acidic products and on this basis it has been stated that there is a methoxycarbonyl in each of these alkaloids[27]. When heated with methanolic potassium hydroxide, mitragynine first appears to combine with a molecule of alcohol to form a product, $C_{23}H_{34}O_5N_2$, containing four methoxyl groups and this, on further heating is changed to a monocarboxylic acid $C_{21}H_{28}O_4N_2$, containing two methoxyl groups. On remethylation with diazomethane this product does not regenerate mitragynine[23]. Rotundifoline yields rotundifolic acid, $C_{21}H_{24}O_5N_2$, which is decarboxylated on heating with quicklime to a crystalline base, $C_{20}H_{24}O_3N_2$[13]. Rhynchophylline provides rhynchophyllic acid which can be methylated to <u>iso</u>rhynchophylline

and this can be converted to a mixture of rhynchophylline and its acetyl derivative by the action of boiling acetic anhydride[24]. Mitraphylline is hydrolysed to mitraphyllic acid and this does not yield mitraphylline when it is methylated with diazomethane[27]. Unfortunately the above products are amorphous and consequently the analytical figures obtained are not trustworthy.

Information about the other oxygen atoms in the alkaloids is scanty. On the basis of colour reactions Ongley[27] suspects the presence of enolic or phenolic groups in rotundifoline and mitragynol and he has pointed out that the formation of an acetyl derivative of rhynchophylline might mean that this alkaloid possesses a hydroxyl group. Carbonyl activity has not been reported by any workers in this field nor has there been any indication of methylene dioxy groups There are uncharacterised oxygen atoms in every alkaloid of this genus.

Specific rotations, melting points and a variety of salts have been described for most of the known alkaloids.

In general the alkaloids have one double bond as shown by hydrogenation over Adam's catalyst and none over palladium. Exceptions to this rule are mitragynol which could not be hydrogenated, and mitraphylline which absorbed one mole of hydrogen over palladium and three

over Adam's catalyst.

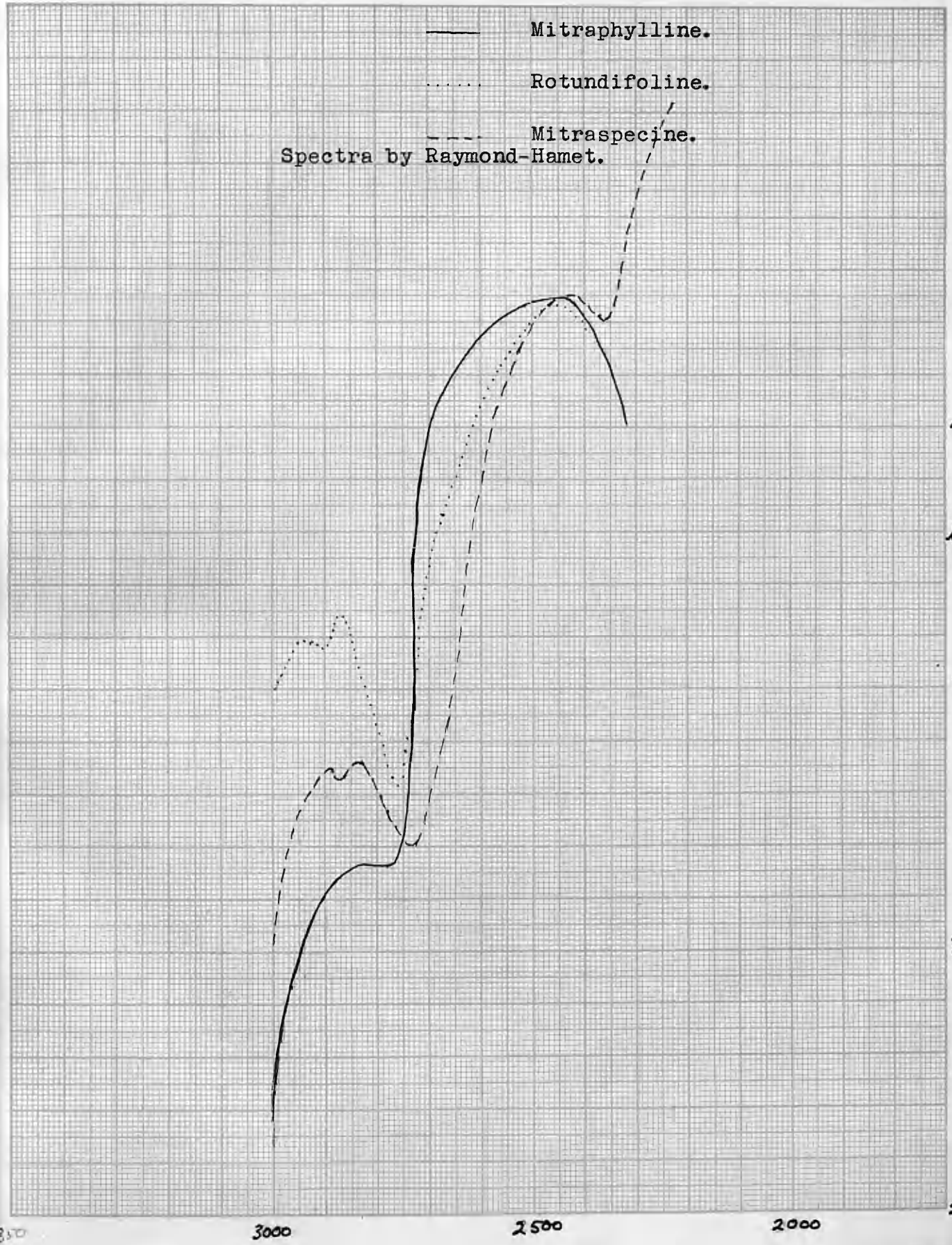
Active hydrogen atoms were estimated by Ongley[27] by the Zerewitinoff method and he obtained results that were higher than those of earlier workers.

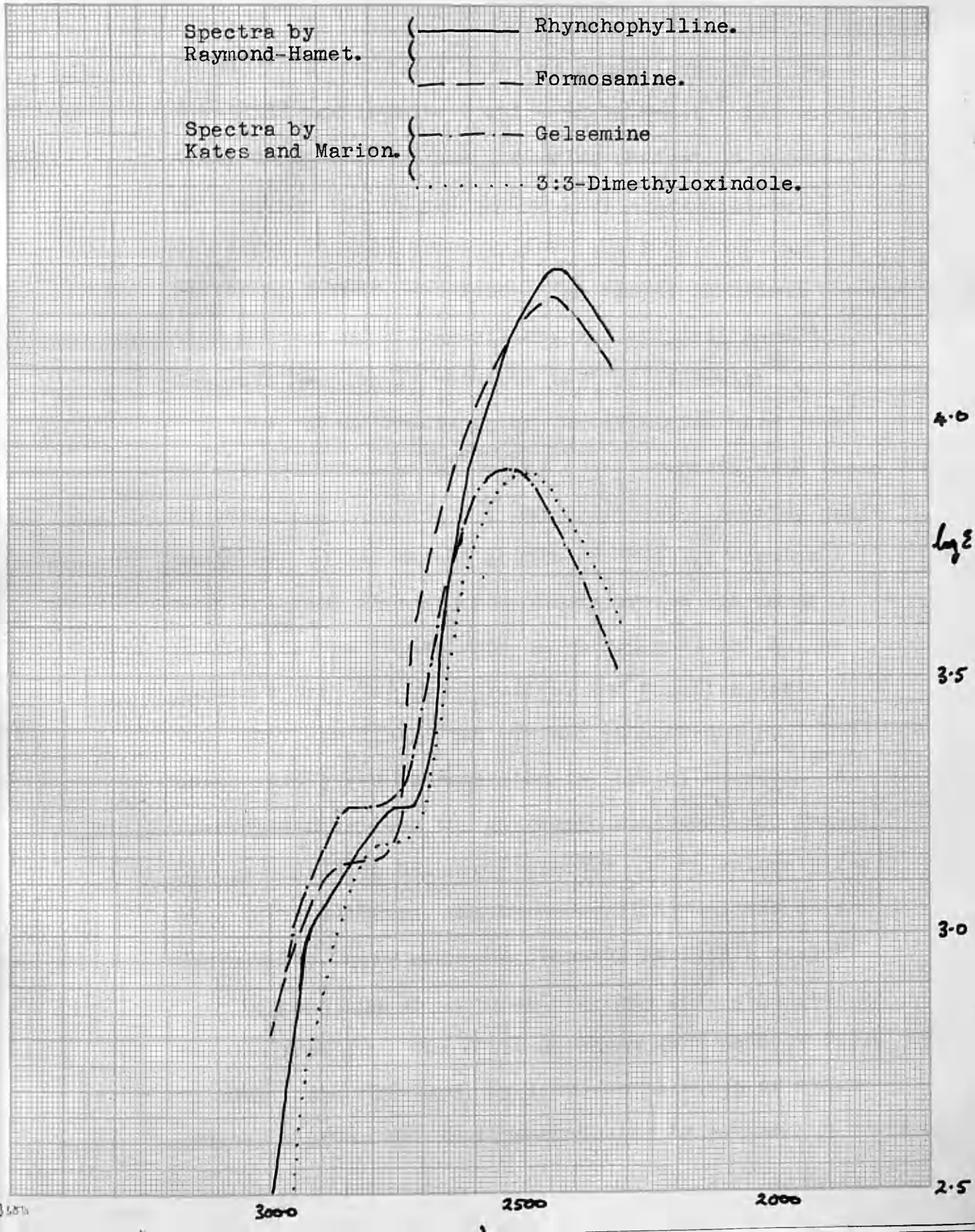
The experimental results are shown in Table 3.

Table 3

Alkaloid	m.p.	OMe	NMe	Act. H	Pot. titn.	H_2/PtO_2	H_2/Pd
Mitragynine	106-15°	3	0	3	monoacid	nil	nil
Mitragynol	130°	2	0	4	monoacid	nil	nil
Mitraphylline	263°	1	0	4	monoacid	3	1
Mitraversine	237°	1	0	-	-	-	-
Rhynchophylline	215-6°	2	0	3	-	1	nil
Rotundifoline	233-4°	2	0	3	-	1	nil

There has been much speculation on the results obtained from ultra-violet spectroscopic studies of the alkaloids. With the exception of mitragynine the spectra are extremely similar to one another. They show maxima at 2700-2900Å and 2300-2500Å, and minima at 2800Å and 2300Å. The exception, mitragynine, has two additional peaks at 3550Å and 2910Å. These curves are said to be characteristic of indolic alkaloids and the spectra of the Mitragyna alkaloids have been compared with those of harmine, harmaline, peganine, sempervirine, aspidospermine, gelsemine, yohimbine and strychnine and with those of various indolic degradation products of alkaloids such as yobyrine and lysergic acid[27].


Mitraphylline.
Rotundifoline.
Mitraspecine.
Spectra by Raymond-Hamet.
4·0
log ε
3·5
3·0
2·5
3000
2500
2000

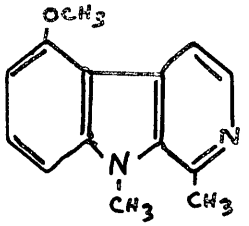

Spectra by Raymond-Hamet.
Rhynchophylline.
Formosanine.
Spectra by Kates and Marion.
Gelsemine
3:3-Dimethyloxindole.
4·0
log ε
3·5
3·0
2·5
3000
2500
2000

It has been concluded from the comparisons that the alkaloids are indolic[27] and Raymond-Hamet[28] has suggested that, on account of the differing absorption spectra, mitragynine may be segregated from mitraphylline, mitraspecine and rotundifoline. This is confirmed by differing colour reactions. The almost identical absorption spectra of mitraphylline, rhynchophylline and formosanine closely resemble that of gelsemine[29].

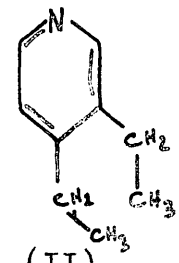
The colour reactions of the alkaloids have been extensively studied by Ongley[27] and compared with those of carbazole, indole, 2-, and 8-hydroxyquinoline, calycanthine, yohimbine and quinine. Positive results were obtained in tests for indole groups and imino groups (except in the case of mitragynol) and in the Ipatieff test for unsaturation, and negative tests for methylene dioxy and =CO and $-CH_2.CO-$groups. Enolic tests were given by rotundifoline and mitragynol. A resemblance between the Mitragyna alkaloids and the Curare alkaloids has been reported in the colour given with ceric sulphate, and between mitragynol and calycanthine in their behaviour towards Ehrlich's reagent.

Degradation of the alkaloids has been attempted by various workers. The first attempts were made by Field in 1921[1], who obtained, by lime distillation of mitragynine, a green oil that behaved like an indole. A base,

$C_{14}H_{14}ON_2$, isolated from the products of zinc dust distillation of mitragynine by Ing and Raison[23], has recently been shown to be 1:2-dimethyl-6-methoxy-β-carboline[30] (I). Barger, Dyer and Sargent[13] obtained



a crystalline solid from rhynchophyllic acid by heating it with lime. Its formula was established as $C_{10}H_9ON$. This product was not identified but they suggested it was a methylcarbostyril. Examination of its ultra-violet spectrum led Ongley[27] to believe that it was an indole, probably a 2:3-dimethylhydroxyindole. Degradation of rhynchophyllic acid by heating with soda lime gave rise to a mixture of ammonia, oxygenated indoles, a pyridine base, a negligble acidic fraction, a neutral skatole-like substance and a basic fraction which was analysed as its picrate and is apparently of formula C_8H_9ON or $C_8H_{11}ON$[13]. Dehydrogenation of rotundi-foline by distillation with selenium produced a mixture of indoles, ammonia and pyridine-like bases from which a crystalline picrate[13] was isolated. This was identified by Ongley[15] as 3:4-diethylpyridine (II).



(II)

The degradative work described in the present thesis was carried out on mitraphylline. This alkaloid occurs in the bark of M. macrophylla and M. stipulosa and in the bark leaves and fruit of M. rubrostipulaceae (Adina rubrostipulata). Although the alkaloid from M. rubrostipulaceae was thought by Denis[21] to be a distinct alkaloid and called "rubradinine" by him, it was shown to be identical with mitraphylline by Raymond-Hamet[26]. For the present researches mitraphylline was extracted from M. rubrostipulaceae in a specially constructed Soxhlet apparatus. Chromatographic technique was employed for the separation and purification of the alkaloid.

From evidence accumulated during the present researches and from the above data it is shown that mitraphylline is a derivative of N-(β-3-oxindolylethyl)-perhydroisoquinoline. The facts reviewed show that an oxindole group of alkaloids co-exists with the β-carboline type in the Mitragyna genus.

The Mitragyna Alkaloids.

Extraction, Separation and Purification of Mitraphylline.

One hundredweight of the bark of M. rubrostipulaceae was ground to a fine powder and preliminary extraction experiments were carried out on this powder. Batch extraction by cold chloroform with agitation and by boiling chloroform, and continuous extraction in a Soxhlet apparatus yielded acidic and neutral material but no basic product. Alkaloids were obtained by treating the powdered bark with quicklime to liberate the bases and extracting by the above methods. The continuous Soxhlet extractor (which gave best results in these experiments) was constructed from a five-litre aspirator bottle and a five-litre flat-bottomed flask. By this procedure, basic and neutral substances were obtained from the bark. Acidic material was recovered by extraction of the residual bark with dilute sodium carbonate. Acidification of the carbonate solution precipitated the acidic material.

Large scale extraction of the bark was effected in a Soxhlet-type extractor, capable of holding 5Kg. of the bark powder. Extraction was continued until a sample of extract liquour withdrawn by means of the sampling tap gave a negative result in a test for

alkaloid by Mayer's reagent (potassium mercuric iodide). It was normally necessary to carry out extraction for about sixty hours before this degree of completion was reached.

The extract thus obtained was contaminated considerably with ferric salts, presumably arising from the action of the chloroform on the iron drums of the extractor.

The chloroform solution was evaporated to dryness and the pasty brown residue was triturated repeatedly with hydrochloric acid. The alkaloids were precipitated with ammonium hydroxide and filtered. They were found to contain some ferric hydroxide. The precipitated bases were therefore dissolved in methyl alcohol in which ferric hydroxide is insoluble, and the solution was filtered from undissolved material and evaporated to dryness affording the crude alkaloids.

Separation.

It had been reported that mitraphylline, a crystalline base, occurred as the sole alkaloidal constituent of the bark of M. rubrostipulaceae. It has been found, however, that there is a complex mixture of alkaloids present and that mitraphylline is the main constituent.

Attempts to purify the alkaloids by crystallisation

showed that there was a non-crystalline portion which, unlike mitraphylline, was ether-soluble. Fractional precipitation of the ether-soluble part showed no more than that it consisted of a complex mixture of alkaloids.

The chromatographic method of separation was resorted to, and tests were carried out using alumina, heavy and light calcium carbonate, and heavy and light magnesium carbonate. Alumina proved to be too strong an adsorbent, and there was loss of material on the column. The various varieties of calcium and magnesium carbonates were satisfactory adsorbents, but the flow of eluant through the columns was too slow for extensive use, except in the case of heavy magnesium carbonate. Accordingly, heavy magnesium carbonate was adopted as the adsorbent for the separation of the alkaloids.

The alkaloid was shaken with cold ether and the ether-soluble fraction yielded no mitraphylline by chromatography. There was separation into six distinct bands on the magnesium carbonate column, and these bands were shown to be easily separable. The ether-insoluble residue was dissolved in chloroform and passed into a column of magnesium carbonate. Elution with chloroform yielded mitraphylline, crystalline and almost pure. Further elution with acetone, then with methyl alcohol,

gave the other constituents of the ether-insoluble fraction. These have not been obtained crystalline.

The extraction of one hundredweight of the bark yielded 44g. of mixed ether-soluble alkaloids, 13.6g. of mitraphylline and 4.2g. of other ether-insoluble alkaloids.

Characterisation and Degradation of Mitraphylline.

The accepted formula for mitraphylline, $C_{21}H_{26}O_4N_2$, has been confirmed by us, by analysis of mitraphylline itself, of its picrate, and of the hitherto unknown sulphate and acetyl derivative. These analyses certainly conform closely to the required percentages for $C_{21}H_{26}O_4N_2$, but it must be noted that the figures obtained do not entirely disprove the formula $C_{21}H_{24}O_4N_2$. It is doubtful if analytical results could ever give an answer to this problem. In mitraphylline with its molecular weight of 370, the variation in the percentage hydrogen for a difference of two atoms of hydrogen is only 0.5, and in its derivatives the difference is even less. The analytical results are shown in the accompanying Table 4.

Table 4.

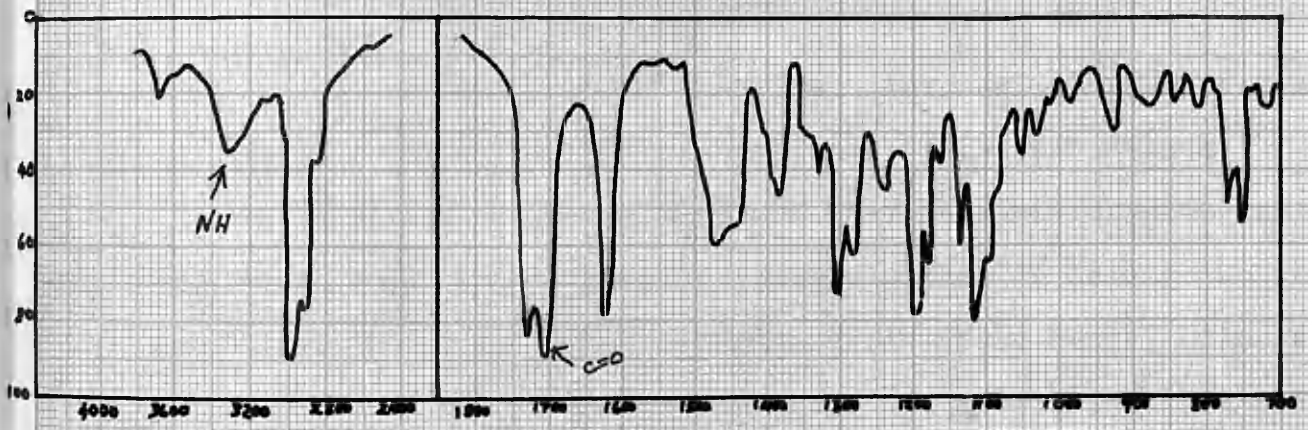
		Mitraphylline	Picrate	Sulphate	Acetyl-mitraphylline
Found	C	68.4 67.9	53.8	48.7	67.2
	H	6.9 7.4	4.8	6.4	6.7
	N	7.4 7.7	11.5	5.5	7.0
$C_{21}H_{26}O_4N_2$ requires	C	68.1	54.1	48.4	67.0
	H	7.1	4.85	6.5	6.8
	N	7.4	11.7	5.4	6.8
$C_{21}H_{24}O_4N_2$ requires	C	68.4	54.1	48.5	67.3
	H	6.6	4.5	6.15	6.35
	N	7.6	11.7	5.4	6.85

The figures appear to support the formula $C_{21}H_{26}O_4N_2$.

As stated above we have been able to prepare the

sulphate and the acetyl derivative of mitraphylline, neither of which has hitherto been obtained. Mitraphylline sulphate crystallises out when mitraphylline is dissolved in 50% sulphuric acid. Its analysis shows that it is the acid salt of mitraphylline and contains three molecules of water of crystallisation. Previous attempts to acetylate proved unsuccessful and in the present researches it has in fact been found that by the normal acetylation techniques mitraphylline is unaffected. This led to the conclusion that the basic nitrogen of mitraphylline was tertiary and that it contained no hydroxyl groups. Infra-red spectral measurements have now been made and these show that there is indeed no hydroxyl group in mitraphylline. In spite of these observations the acetyl derivative of mitraphylline has now been prepared m.p. 164-5° by boiling the alkaloid with acetic anhydride containing a grain of ferric chloride. Undoubtedly the acetylation has taken place on the neutral nitrogen atom as happened when the alkaloid gelsemine was similarly treated by Janot[31]. It is interesting to note that, although Moore[32] acetylated gelsemine in 1910, for many years the result could not be repeated until the above conditions were discovered by Janot in 1951. Gelsemine is an oxindole alkaloid, and contains a basic tertiary nitrogen atom and no hydroxyl

Infra-red Spectrum of Mitraphylline in Nujol.



group.

Zeisel determination indicated the presence of one methoxyl group in mitraphylline and repeated Herzig-Meyer determinations showed no evidence of methylimino. These results have been confirmed by the infra-red spectrum of the alkaloid which shows a typical =NH band at 3300 $cm.^{-1}$ and no hydroxyl band.

Alkaline hydrolysis of other alkaloids of the Mitragyna genus has led to inconclusive results due to the amorphous nature of the products obtained; we can claim no greater success. The most exhaustive study of alkaline hydrolysis was made by Ing and Raison[23] on mitragynine. They found that when mitragynine was boiled with methanolic caustic potash an acid group was produced presumably by hydrolysis of an ester, affording an amphoteric acid which could not be crystallised and which did not yield mitragynine on re-esterification. The interesting observation was made that an intermediate base was formed in the course of this reaction; ethanol or propanol gave different intermediate bases. Analyses of the amorphous picrates of these bases suggested that a molecule of solvent had combined with the alkaloid in each case to form theintermediate base. Although we did not consider that analysis of amorphous products

was justified, it can be said that a parallel exists between the products from mitraphylline and those from mitragynine. Hydrolysis of mitraphylline produced an amphoteric substance which was amorphous (and gave an amorphous hydrochloride) and an intermediate base. This base on further treatment yielded the amphoteric product. Unfortunately the amorphous nature of the products makes it difficult to base any conclusions on this work.

It had been reported that mitraphylline absorbed one molecule of hydrogen over palladium black catalyst[27]. This has now been investigated further, and the conclusion has been reached that there is no isolated double bond in the molecule of mitraphylline. The first hydrogenation determination showed an uptake of 0.9 mol. hydrogen but the product when isolated had a melting point identical with that of mitraphylline and undepressed on admixture with it. The supposed "dihydro"-mitraphylline afforded an acetyl derivative of melting point identical with that of acetylmitraphylline, and again there was no observed depression in the melting point when the samples were mixed. A specimen of the hydrogenated mitraphylline absorbed no more hydrogen in acetic acid over palladium black. This evidence suggested that mitraphylline did not have an isolated double bond in its molecule and that the anomalous

results were due to a highly unsaturated substance, occurring with mitraphylline and not separable from it by crystallisation. This theory was confirmed by hydrogenation of a specimen of mitraphylline which had previously been boiled with potassium dichromate in acetic acid. This sample absorbed no hydrogen whatsoever. When a sample of mitraphylline was chromatographed carefully on alumina it was found that in addition to the band of mitraphylline there were two small yellow bands on the column. (It may be noted that the analyses recorded for mitraphylline were obtained from samples (a) boiled with dichromate/acetic acid, and (b) hydrogenated over Pd.) The nature of the unsaturated material is unknown but it is of interest to note that gelsemine, which bears many similarities to mitraphylline, occurs with sempervirine[33], a highly unsaturated alkaloid of yellow colour. This phenomenon of "apparent unsaturation" is not unknown; it has been reported in the case of nor-melinonine-A[34]. Rotundifoline has been reported to absorb one molecule of hydrogen and give a product whose melting point is the same as that of rotundifoline itself and which is not depressed when the sample is mixed with a sample of rotundifoline[27]. It seems that this might be another case of "apparent unsaturation".

Hydrolysis by reagents other than alkali has

been studied. After boiling for several days with water mitraphylline was recovered unchanged. Likewise, boiling with N sulphuric acid for several hours had no effect on the alkaloid. Results of a surprising and interesting nature were obtained with more concentrated acid. Carbon dioxide (containing no detectable trace of carbon monoxide) was liberated when mitraphylline reacted with cold concentrated sulphuric acid. The other product of this reaction was an amorphous base which has not been characterised. Boiling 30% sulphuric acid also liberated carbon dioxide from mitraphylline but the basic product was more complex and contained a highly insoluble substance. Acetic acid containing as little as 5% sulphuric acid produced an amorphous base from mitraphylline when they were heated together at 100° for several hours. The evolution of carbon dioxide at once suggested carbonyl activity in the position β to the ester but mitraphylline exhibited no carbonyl activity and certainly did not behave as a β-keto ester. This reaction would appear to have some structural significance and is probably related to the oxygen atom which is still uncharacterised in mitraphylline. Tests on micro-quantities of rhynchophylline and rotundifoline showed that each of them liberates carbon dioxide on gentle warming with concentrated sulphuric

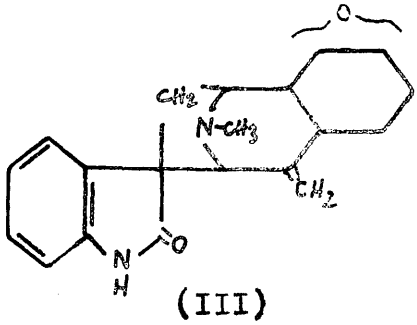
acid.

Mitraphylline is unchanged by boiling for several hours with potassium dichromate and acetic acid. As has been pointed out above, this procedure has been adopted for the purification of the alkaloid. Towards stronger oxidising reagents, however, mitraphylline is not so stable. Oxidation with concentrated nitric acid occurs on gentle warming and the products of this reaction were intensively studied. The effect of various concentrations of nitric acid were investigated and products obtained of which one was crystalline and proved to be oxalic acid. An amorphous product appeared to be a nitrophenol containing an acidic grouping other than phenolic.

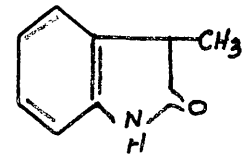
Various dehydrogenation techniques were employed in the study of mitraphylline. In order to aquire a method for the small scale dehydrogenation of mitraphylline with selenium, trial dehydrogenations were carried out on yohimbic acid. It was found possible to isolate the three products, yobyrine, tetrahydroyobyrine and ketoyobyrine[35] from dehydrogenations involving as little as 0.5g. of yohimbic acid. Chromatographic methods were used for this separation. Selenium dehydrogenation of mitraphylline afforded a mixture of unidentified bases and neutral products containing indoles. Zinc distillation at atmospheric pressure in a glass tube

yielded similar products.

Comparison of the colour reactions and of the ultra-violet absorption spectra suggested the segregation of mitragynine from mitraphylline, mitraspecine and rotundifoline[28]. The almost identical absorption spectra of mitraphylline, rhynchophylline and formosanine closely resemble that of gelsemine[29] (III). In turn the absorption spectra of gelsemine and 3:3-dimethyloxindole are almost superposable[36] and the oxindole nature of gelsemine has been proved by degradation to 3-methyloxindole[31](IV).



(III)



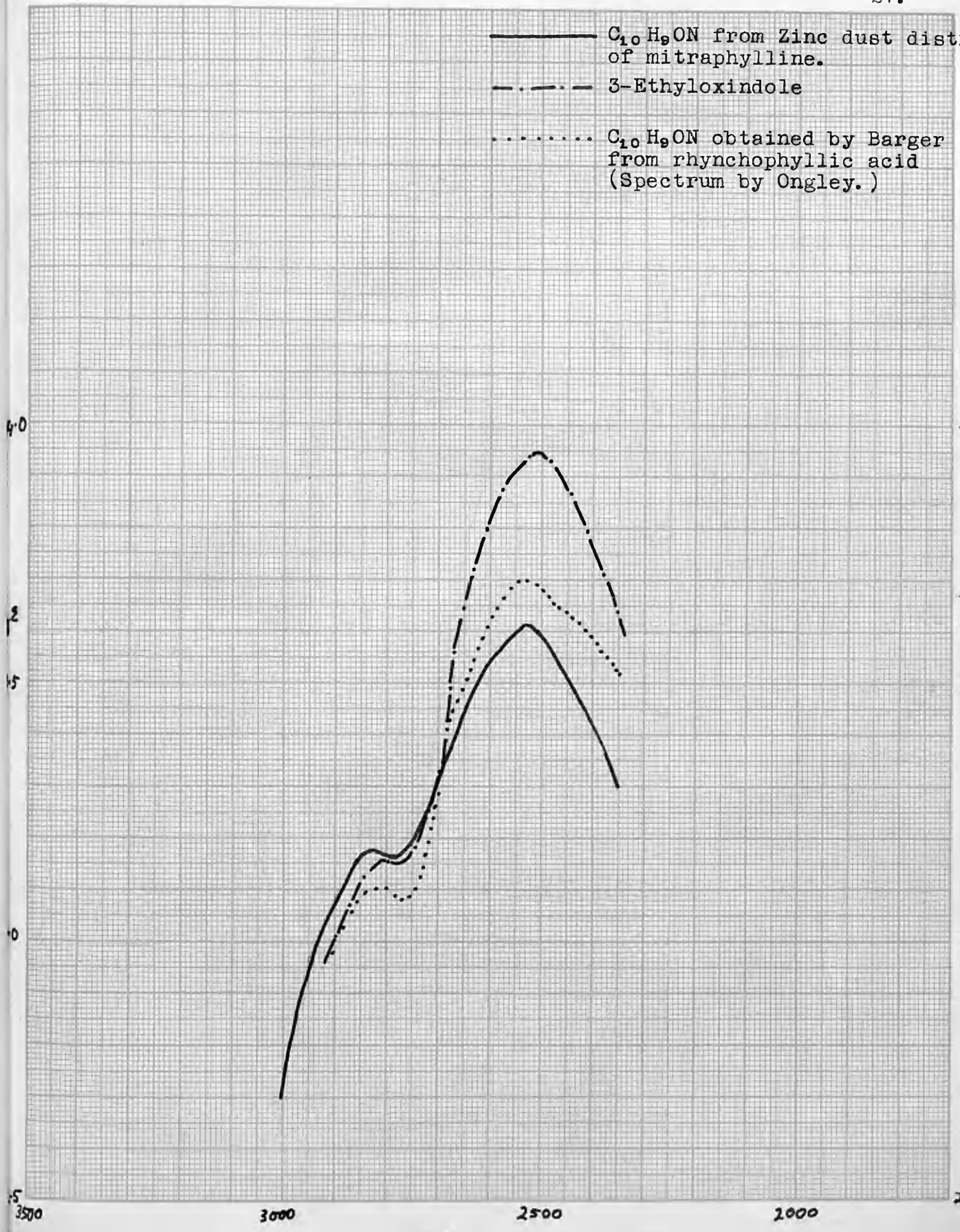
(IV)

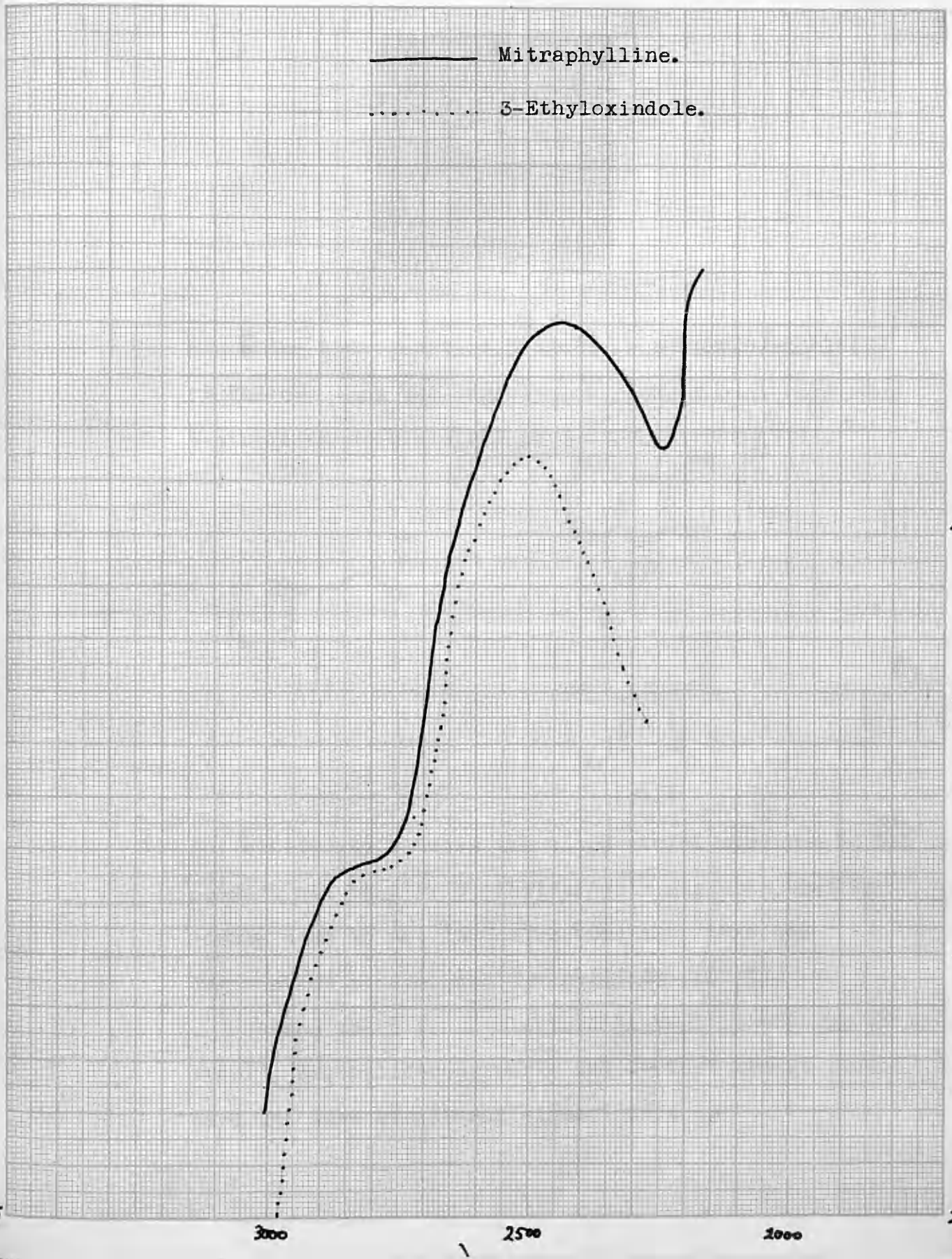
Other similarities between gelsemine and mitraphylline are (1) the reaction of each with acetic anhydride in the presence of ferric chloride to form an acetyl derivative[31]; and (2) the failure of each to couple with diazotised sulphanilic acid. The infra-red spectrum of mitraphylline is consistent with the presence of an oxindole grouping with a free imino hydrogen. These considerations suggested the working hypothesis that mitraphylline is a derivative of oxindole. This view has been confirmed by degradation.

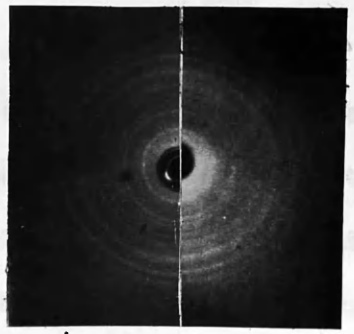
Oxindoles are reduced by zinc dust distillation to indoles but Janot[31] had managed to recover 3-methyl-oxindole from the zinc dust distillation of gelsemine by carrying out the reaction in a vessel evacuated to 10^{-4} mm. pressure. This procedure was adopted in the case of mitraphylline. With mitraphylline itself there was considerable recovery of unchanged base from the distillate, but more complete reaction was effected when the zinc was mixed with mitraphylline hydrochloride, an amorphous material obtained by bubbling dry hydrogen chloride into a solution of mitraphylline in chloroform. This degradation afforded a distillate of bases and neutral compounds, which were separated by extraction of their ether solution with dilute sulphuric acid. The bases were fractionated by distillation *in vacuo* and the lowest boiling fraction (micro-b.p. 239-45°) was converted into its picrate and fractionally crystallised. The main fraction from this crystallisation was a picrate m.p. 222° whose analysis indicated that it might be *iso*quinoline picrate and mixed m.p. of this picrate with authentic *iso*quinoline picrate showed no depression. The identity of the base with *iso*quinoline was confirmed by comparison of their styphnates. Another crystalline picrate m.p. 127-9° was isolated in quantity too small for identification. The neutral

products from the zinc dust distillation contained indoles and a crystalline, non-indolic material m.p. 179-81° which was separated from the indoles by chromatography. This compound which proved on analysis to have formula $C_{10}H_9ON$ was insoluble in dilute acid or alkali in the cold but dissolved in dilute alkali on boiling and was precipitated unchanged by acid. Barger, Dyer and Sargent[13] had obtained a product, $C_{10}H_9ON$, m.p. 182-4°, from rhynchophyllic acid which had similar properties and whose ultra-violet spectrum had been determined by Ongley[27]. The similarity in properties and in ultra-violet spectrum between the two products indicated that they were in fact identical.

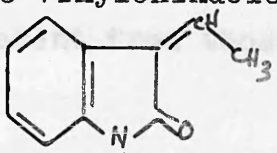
The structure of this neutral compound was now eludidated. On micro-hydrogenation, one mole of hydrogen was absorbed showing the presence of a double bond in the molecule. A larger sample was hydrogenated and the product obtained as a crystalline solid micro-m.p. 93-7°. On admixture with pure 3-ethyloxindole, micro-m.p. 96°, there was no depression in melting point. The identity of the two substances was confirmed by comparison of their X-ray diffraction photographs. Prints of these photographs are shown below and are identical in every detail


$C_{10}H_9ON$ from Zinc dust distn. of mitraphylline.
3-Ethyloxindole
$C_{10}H_9ON$ obtained by Barger from rhynchophyllic acid (Spectrum by Ongley.)
4·0
3·5
3·0
2·5
log ε
3500
3000
2500
2000

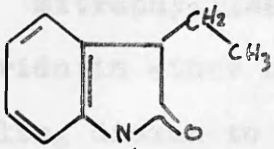

Mitraphylline.
3-Ethyloxindole.
4·0
3·5
3·0
2·5
log ε
3000
2500
2000



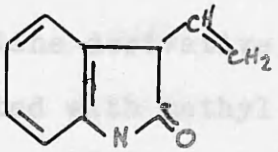
Since the hydrogenation product is 3-ethyloxindole(V) it follows that the degradation product must have been 3-ethylideneoxindole(VI) or 3-vinyloxindole(VII).



(VI)



(V)



(VII)

The similarity between the ultra-violet spectrum of the product and that of 3-ethyloxindole suggests that the double bond is not in conjugation i.e. that the degradation product was 3-vinyloxindole(VII).

Since there is no olefinic linkage in mitraphylline itself, 3-ethylideneoxindole and 3-vinyloxindole would have the same structural significance.

The active hydrogens in mitraphylline were estimated by the Zerewitinoff method using lithium aluminium hydride in pyridine and also using methyl magnesium iodide in boiling phenetole. These experiments showed that mitraphylline contained two active hydrogens. Using lithium aluminium hydride in pyridine it was found that rotundifoline contained three active hydrogens, and rhynchophylline two. The results for mitraphylline and rhynchophylline are different from those obtained by Ongley[27].

Mitraphylline was unaffected by lithium aluminium hydride in ether but was reduced by this reagent in boiling dioxan to an amorphous base which could not be induced to yield any crystalline derivative whatsoever.

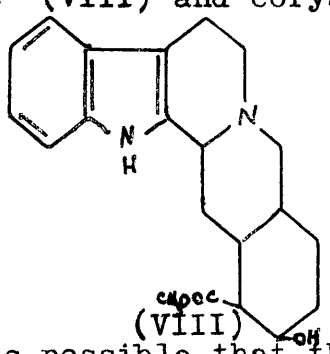
When mitraphylline reacted with methyl magnesium iodide in boiling phenetole a crystalline base was formed whose analysis conformed to the formula $C_{22}H_{28}O_2N_2$ and which contained no methoxyl group. This product was probably the isopropylidene derivative formed by the action of methyl magnesium iodide on the methoxycarbonyl of mitraphylline. Lack of time prevented further study of this interesting compound.

Structural Conclusions.

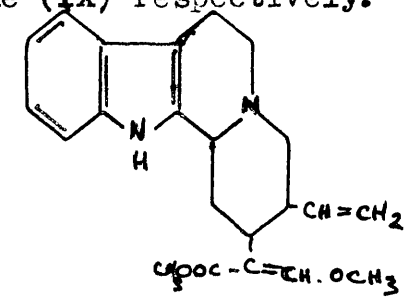
The results described afford considerable insight into the molecular structure of mitraphylline and hence of the related alkaloids of the Mitragyna genus. The oxindole theory based on an accumulation of evidence pointing to a close resemblance between mitraphylline and gelsemine, has been irrefutably established by isolation and identification of an oxindole in the degradation products. Undoubtedly the chromophore of the molecule of mitraphylline is contained in the oxindole and the almost identical absorption spectra of mitraphylline, rhynchophylline, rotundifoline and mitraspecine imply similarities in the chromophores. This is substantiated by the fact that the oxindole, $C_{10}H_9ON$, is identified with that earlier obtained from rhynchophylline. As has already been stated, one of the degradation products from mitragynine is known to be 6-methoxy-1:2-dimethyl-β-carboline, and this supports the hypothesis, based on spectroscopic evidence, that there are two chemical families in the alkaloids of this genus. It would appear that β-carbolines and oxindoles occur in the same botanical environment, and indeed mitragynine and mitraspecine are to be found in the same plant, namely M. speciosa. This is a point of some interest since it is already known that sempervirine, a β-carboline

derivative[41,42,43] is a congener of gelsemine, an oxindole derivative. Considerable biogenetical importance may be attached to this relationship.

The basic fractions so far isolated in the degradation of the oxindole branch of the Mitragyna genus are isoquinoline from mitraphylline and 3:4-diethylpyridine from rotundifoline. It is reasonable to suppose that these products represent similar molecular fragments of their respective alkaloids. Isoquinoline and 3:4-diethylpyridine are produced by degradation of yohimbine[38] (VIII) and coryantheine[39] (IX) respectively.



(VIII)

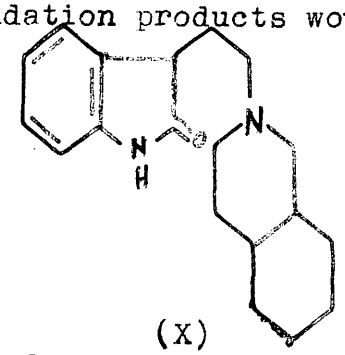


(IX)

It is possible that the alkaloids of Mitragyna genus have structures analogous to those of the various families of β-carboline alkaloids whose structures are already have received so much attention in recent years. Speculation on this relationship between the families must obviously await more extensively knowledge of the alkaloids of the Mitragyna genus.

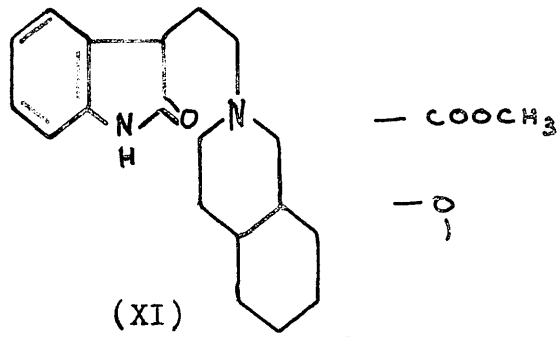
Much of the skeleton of mitraphylline is elucidated by the degradation products. The simplest and

most plausible formulation of the skeleton of the alkaloid from the two degradation products would be (X).



(X)

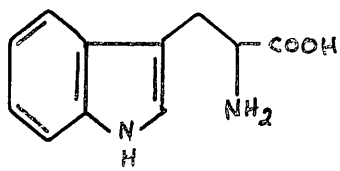
This accounts for 19 of the 21 carbon atoms of the molecule and with the suspected methoxycarbonyl group and one unidentified oxygen atom, the $C_{21}H_{26}O_4N_2$ required for mitraphylline is attained (XI).



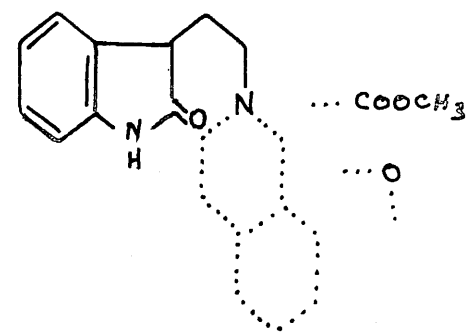
(XI)

The above formula can be confirmed to some extent by facts already known. The fragments obviously represent distinct moieties of mitraphylline since the nitrogen is directly attached to the benzene ring in the one and not in the other, and this renders it unlikely that they have arisen from a common source in the alkaloid molecule by ring enlargement or contraction. The double bond in the side chain of the oxindole (especially if it is in the vinyl position) suggests that the nitrogen of the other fragment is attached to the ω -position

of the oxindole. Confirmation is also found in Robinson's theory of biogenesis which postulates that indole alkaloids arise from tryptophan[44] (XII). The structure proposed for mitraphylline shows an obvious relationship to tryptophan



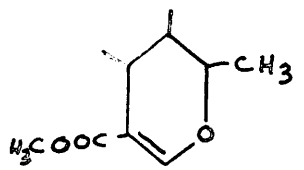
(XII)



Oxindoles that are monosubstituted in the β-position possess two active hydrogen atoms[54] and this is consistent with the experimental result obtained for mitraphylline.

There is no direct evidence on the nature of the unidentified oxygen atom of mitraphylline but some deduction is permissible from the data described. There is no hydroxyl group in the molecule as is shown by the infra-red spectrum of the alkaloid, and consequently the oxygen must be linked twice to carbon. This limits the number of possibilities to two. The oxygen must be present in an ether linkage or in a carbonyl group. The infra-red spectrum shows a carbonyl band at 1705 $cm.^{-1}$ and another at 1720 $cm.^{-1}$ but these are possibly accounted for by the methoxycarbonyl group and by the oxindole carbonyl. There has been no chemical

evidence of carbonyl activity. The evolution of carbon dioxide when mitraphylline is treated with concentrated sulphuric acid suggests that mitraphylline is a β-keto ester, but no indication of enolisation has been observed. Moreover, the free acid is presumably formed when mitraphylline is hydrolysed in alkaline solution yet the product does not lose carbon dioxide until concentrated sulphuric acid is added to it. Possibly the oxygen is present as an enol-ether as in alstonine (XIII) but this is unlikely if it be accepted that pure mitraphylline does not absorb hydrogen over Pd catalyst.



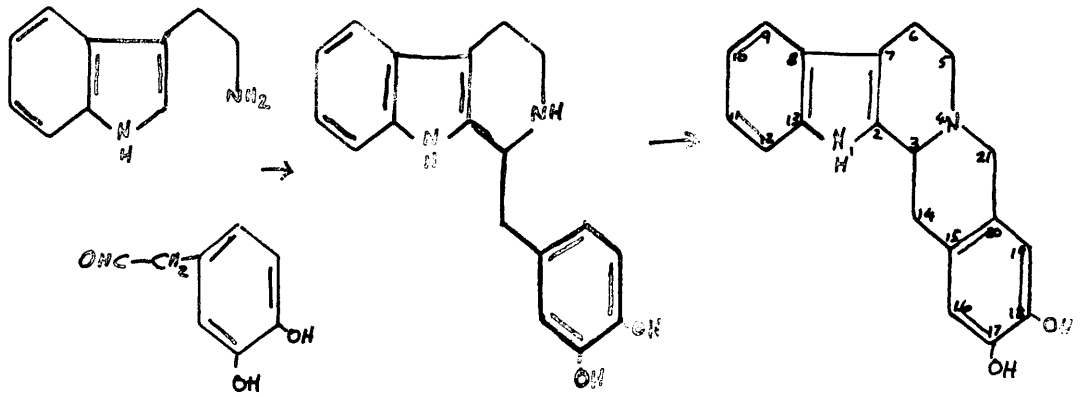
(XIII)

Moreover, a structure such as (XIII) does not provide a simple explanation of <u>iso</u>quinoline as a degradation product. An inert ether oxygen could not account for the evolution of carbon dioxide, but a suitably placed epoxide ring might accommodate the known facts.

<u>Biogenesis</u>:

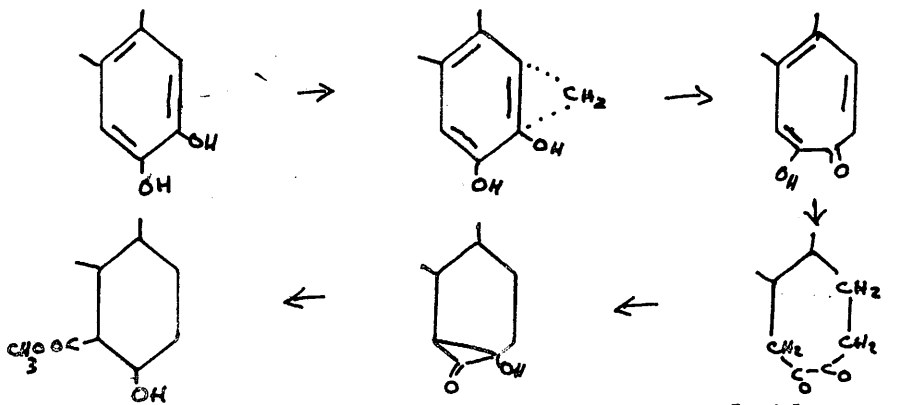
There is a close similarity between the molecular skeleton of yohimbine and that now postulated for mitraphylline. This and the fact that oxindole and β-carboline derivatives are known to co-exist in plants

leads to the conclusion that β-carboline alkaloids and oxindole alkaloids have a common scheme of biogenesis. The mode of biogenesis ascribed to yohimbine is the condensation of tryptophan with 3:4-dihydroxyphenylacetaldehyde to give (XIV), followed by condensation with formaldehyde to give (XV).

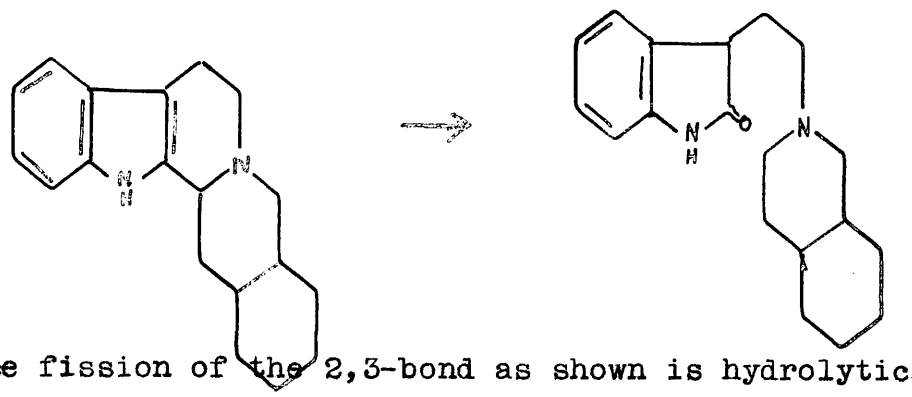


(XIV) (XV)

It has been suggested that the methoxycarbonyl group in the 16-position is formed through the intermediate formation of a tropolone ring[55].

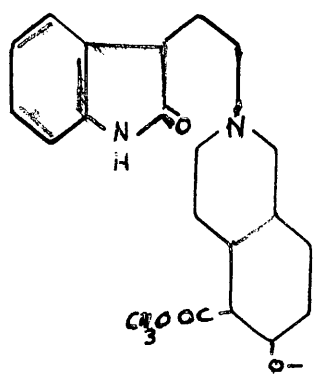


It is now suggested that oxindole alkaloids are formed by the fission of the 2,3 bond.



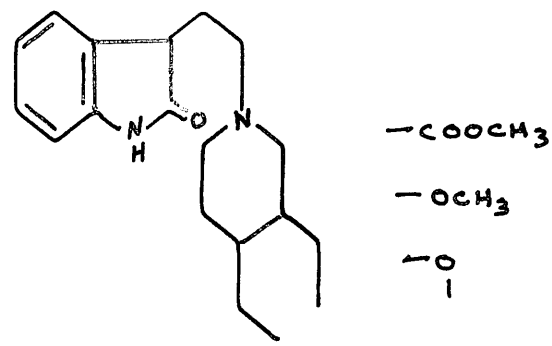
The fission of the 2,3-bond as shown is hydrolytic. If on the same general basis the fission is oxidative, carbon atom 3 would be oxygenated in the fission product. Carbonyl at position 3 is excluded by the basic properties of mitraphylline and hydroxyl at position 3 would make the alkaloid a carbinolamine which it is not; neither is it likely to be the internal O-ether of a carbinolamine since it is acid stable. On the other hand hydrolytic fission gives a direct explanation of the formation of the alkaloid.

On this basis the methoxycarbonyl group of mitraphylline would be expected to be in the 16-poaition and the unidentified oxygen to be attached to the 17-position.

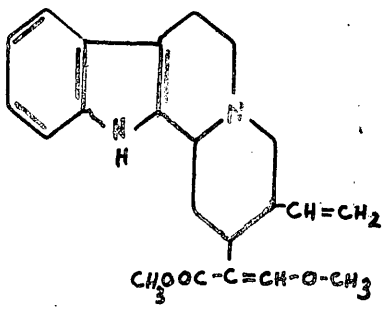


Rotundifoline.

It is noteworthy that with the establishment of the oxindole nature of these alkaloids, it is possible to speculate upon the structure of rotundifoline. As noted above, 3:4-diethylpyridine has been obtained by degradation of rotundifoline and together with the oxindole nucleus this accounts for 19 of the 22 carbon atoms of this alkaloid. Moreover, the methoxycarbonyl group of rotundifoline has been characterised by isolation of a crystalline decarboxylation product, and Zeisel determinations have shown that there is still another methoxyl group in the molecule. Accordingly, there is only one unidentified oxygen atom in the structure. On the simplest possible formulation the structure below can be written for rotundifoline.



This structure bears a striking resemblance to corynantheine (IX),



(IX)

but further data will be required before the structure of rotundifoline can be established with any certainty.

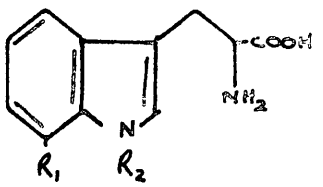
SYNTHESES OF SOME β-CARBOLINES.

Syntheses of Some β-Carbolines.

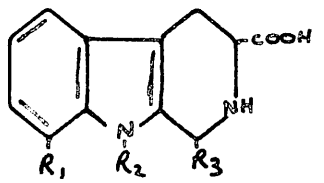
There has been increasing interest in β-carboline because of the frequency with which its derivatives are found among the degradation products of indole alkaloids. Among the degradation products as yet unidentified is a base, $C_{12}H_{10}N_2$, obtained by Barger[46] by treatment of the alkaloid with lime in a sealed tube at 300°. The reactions and characteristics of this compound indicate that it is a methyl-β-carboline isomeric with harman. This product melts at 183°. Another of the unidentified degradation products is "Base F", $C_{13}H_{12}N_2$, obtained by thermal decomposition of alstonine by Elderfield[47]. Ultra-violet spectroscopy suggests that it is a β-carboline and on this basis it would be an ethyl- or a dimethyl-β-carboline. Its melting point is 78-81°.

In the present series four β-carbolines were synthesised by condensing the corresponding tryptophan (XVI) with an aldehyde to form a tetrahydro-β-carboline carboxylic acid (XVII) which was dehydrogenated and decarboxylated to the carboline (XVIII) by boiling with potassium dichromate[53] in dilute acetic acid. Thus formaldehyde and acetaldehyde were condensed with 7-methyltryptophan (XVI; $R_1=CH_3$; $R_2=H$) to form 9-methyl-β-carboline (XVIII; $R_1=CH_3$; $R_2=R_3=H$) and

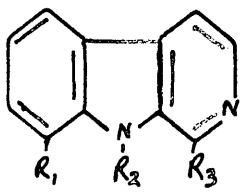
2:9-dimethyl-β-carboline (XVIII; $R_1=R_3=CH_3$; $R_2=H$) respectively. Likewise 1:7-dimethyltryptophan with formaldehyde gave 1:9-dimethyl-β-carboline (XVIII; $R_1=R_2=CH_3$; $R_3=H$), and with acetaldehyde gave 1:2:9-trimethyl-β-carboline (XVIII; $R_1=R_2=R_3=CH_3$)



(XVI)

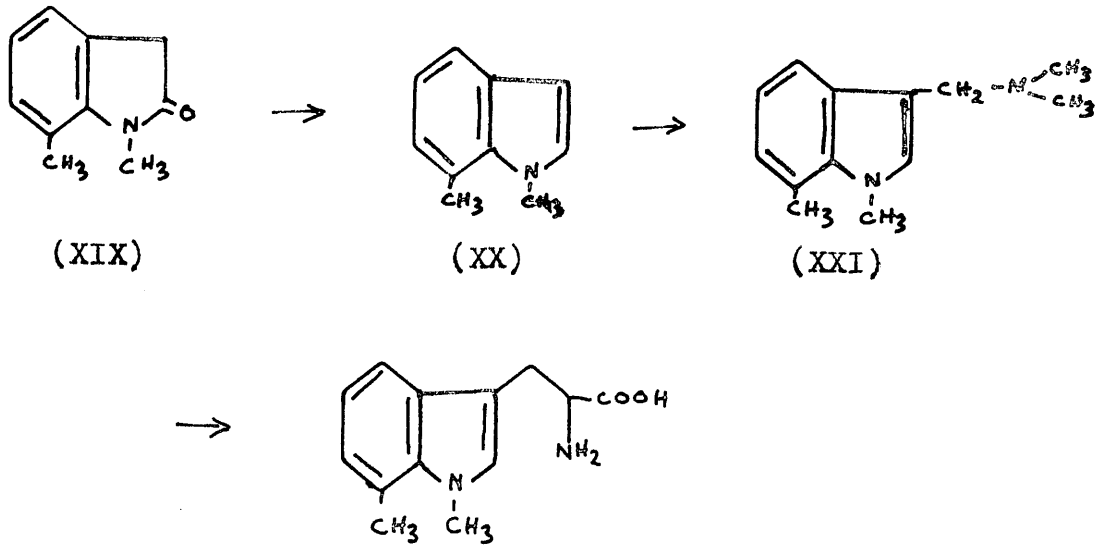


(XVII)



(XVIII)

7-methyltryptophan is already known and was prepared by the method of Rydon[45] from 7-methylindole. 1:7-dimethyltryptophan was synthesised by the method of Snyder, Smith[50] and Albertson[51] from 1:7-dimethylindole (XX) through 1:7-dimethylgramine (XXI). The 1:7-dimethylindole was itself formed by reduction of 1:7-dimethyloxindole (XIX) with lithium aluminium hydride.



(XIX) (XX) (XXI)

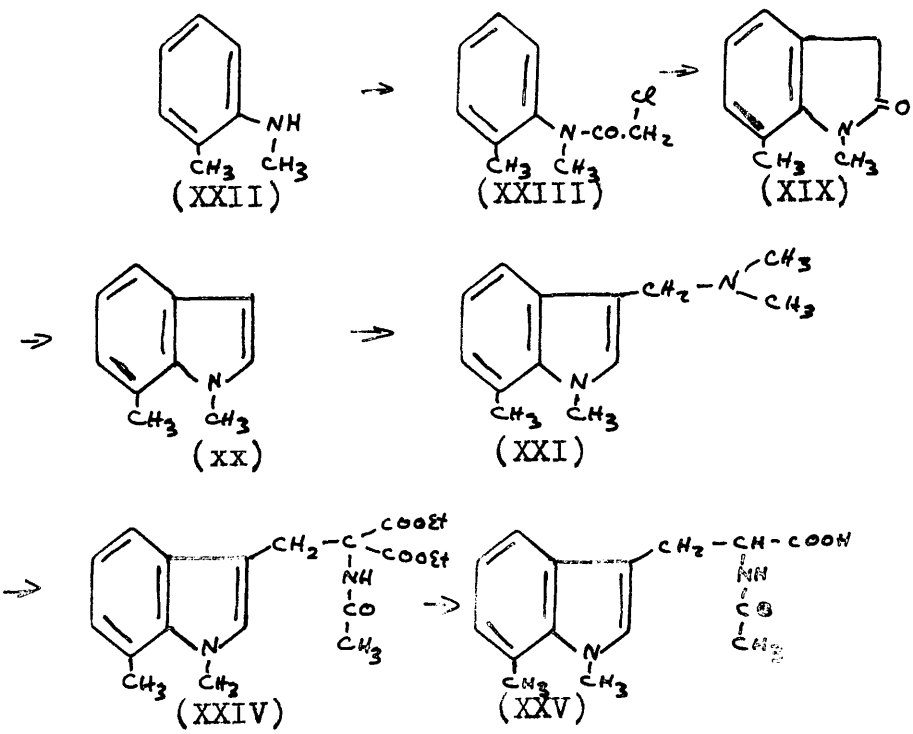
1:7-Dimethyltryptophan.

N-methyl-o-toluidine (XXII) was obtained by methylating the benzenesulphonyl derivative of o-toluidine with dimethyl sulphate in caustic soda solution and hydrolysing the methylated product with strong sulphuric acid. Chloroacetyl chloride reacted vigorously with N-methyl-o-toluidine to form N-methyl-chloroacetyl-o-toluidide (XXIII) which condensed intramolecularly under the influence of aluminium chloride affording 1:7-dimethyloxindole[48] (XIX).

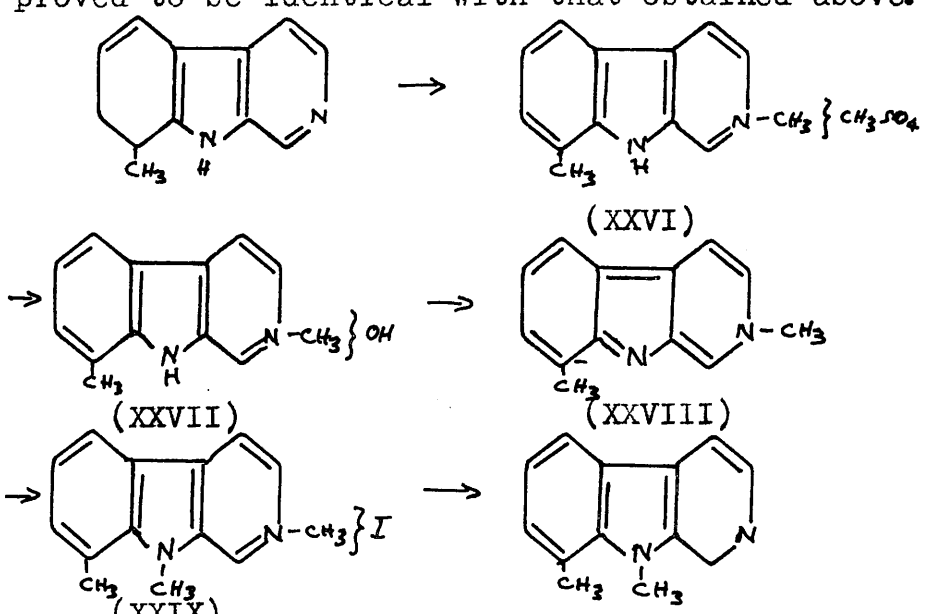
Julian[49] has reported that although oxindoles are normally unaffected by lithium aluminium hydride, it is possible to reduce N-methyl-oxindoles to indoles and indolines. Accordingly 1:7-dimethyloxindole (XIX) was reduced by lithium aluminium hydride and yielded 1:7-dimethylindole (XX).

Condensation of dimethylamine and formaldehyde

with 1:7-dimethylindole proceeded smoothly, and part of the resulting 1:7-dimethylgramine (XXI) was analysed as its picrate. The remainder of the gramine was converted to its methiodide which reacted with acetamidomalonic ester in boiling alcohol to give ethyl α-acetamido-α-ethoxycarbonyl-β-(1:7-dimethyl-3-indolyl)propionate[50] (XXIV). (XXIV) was hydrolysed and decarboxylated to 1:7-dimethylacetyltryptophan (XXV) by boiling with sodium carbonate solution. 1:7-dimethyltryptophan was obtained by hydrolysing its acetyl derivative with water in a sealed tube at 200°[52] and the β-carbolines were prepared from it as described above.

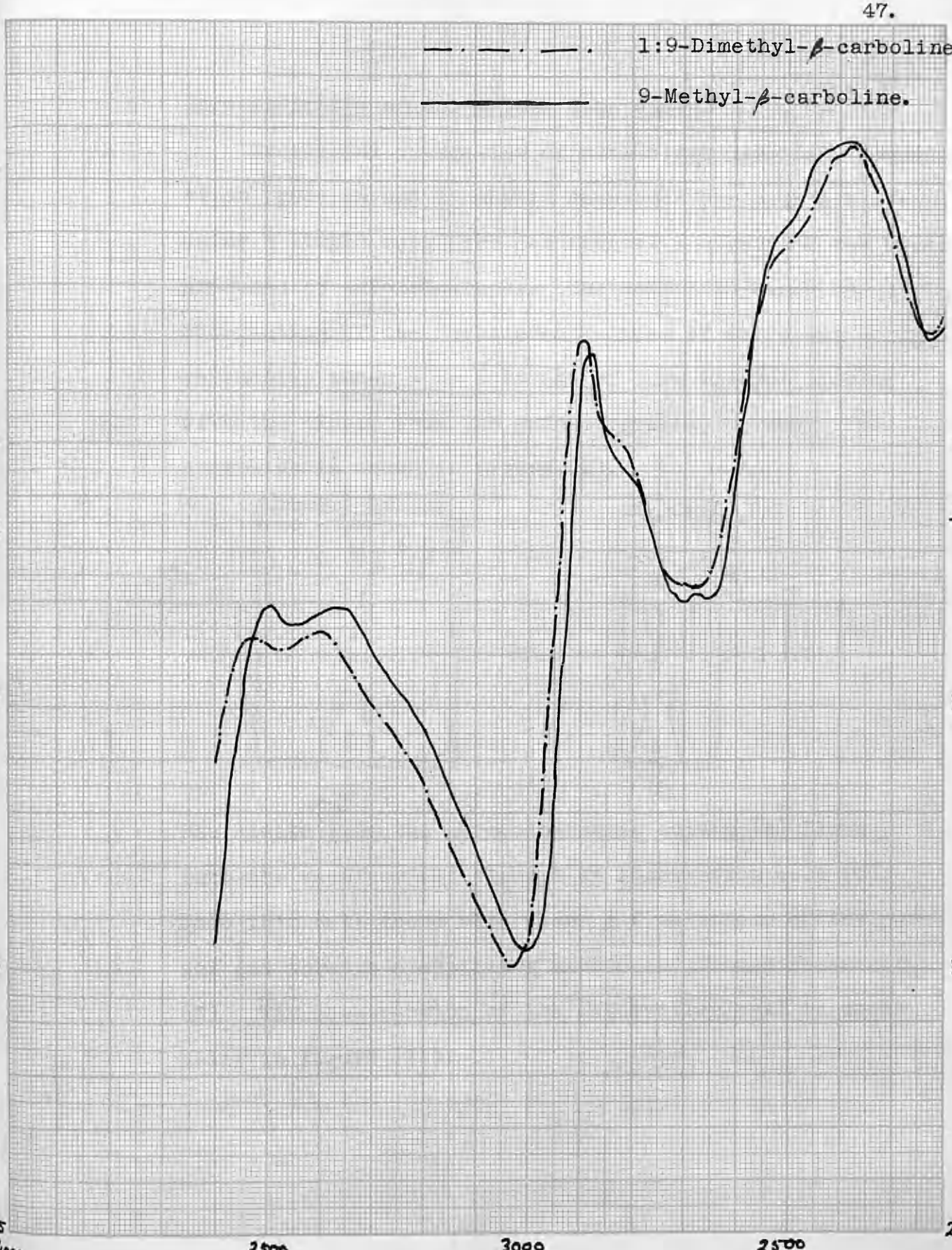


Confirmation of the structures of the products was obtained by methylating 9-methyl- and 2:9-dimethyl-β-carboline on the indole nitrogen atom by the method of Iyer and Robinson[56]. 9-Methyl-β-carboline was converted via the methosulphate (XXVI) to the methohydroxide (XXVII) whence by heating to form the anhydronium base (XXVIII) and subsequent addition of methyl iodide there was obtained 1:3:9-trimethyl-β-carbolinium iodide (XXIX). When this salt was heated to 300° methyl iodide was eliminated and 1:9-dimethyl-β-carboline was produced which was identical with that obtained above. The same method produced 1:2:9-trimethyl-β-carboline from 2:9-dimethyl-β-carboline. This trimethyl-β-carboline proved to be identical with that obtained above.



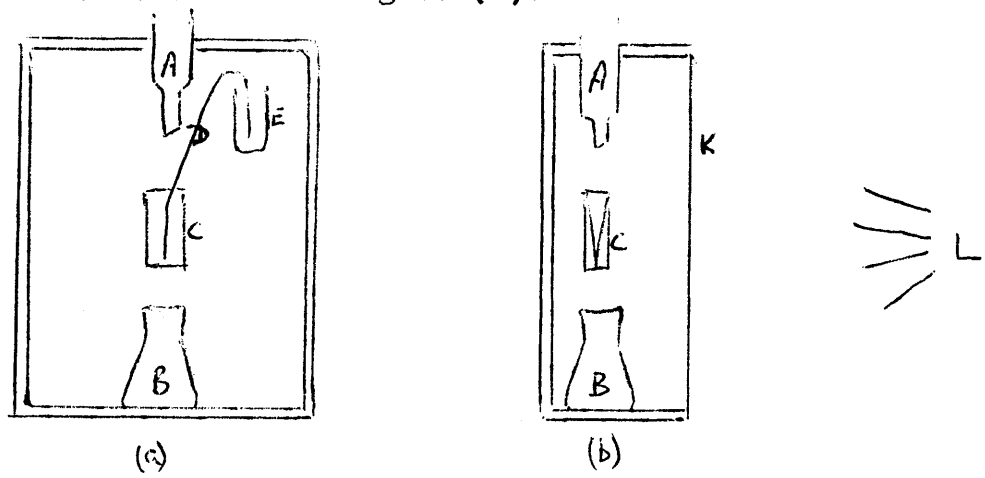
From a comparison of their melting points it appears that the base obtained by Barger from calycanthine does not correspond with 9-methyl-β-carboline; nor is Base F from alstonine identical with 2:9-dimethyl-β-carboline. The is some similarity in the melting points of Base F and 1:9-dimethyl-β-carboline.

In this department there has been collected a series of ultra-violet spectra of β-carbolines. The spectra of 9-methyl- and 2:9-dimethyl-β-carboline were measured for addition to this collection. The spectra are shown overleaf.

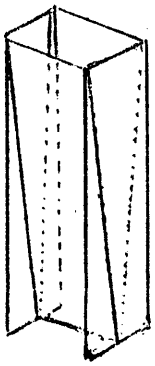

1:9-Dimethyl-β-carboline.
9-Methyl-β-carboline.
4·5
4·0
log ε
3·5
3·0
2·5
4000
3500
3000
2500

Chromatography of Colourless Substances.

Successful separation of alkaloidal material, degradation products and synthetic compounds has been achieved using a simple apparatus for frontal analysis of the bands produced by chromatography. The method is based on observation of the "schlieren" -lines of different refractive index - as a stream of pure solvent mingles with the eluate from the chromatographic column. The apparatus is shown in figure (I).



The eluate from the chromatographic column (A) flows into the receiver (B) through an observation cell (C). Into this cell there also flows a fine stream of the pure solvent through a capillary siphon (D) from a reservoir (E). The construction of the observation cell is shown below in figure (II).



The cell, which is made entirely from microscope slides cemented together with a silicate cement, has a slit at the narrow end of the V-shape. The capillary siphon extends to within 2mm. of the bottom of the cell and as the eluate drips through the cell, a constant level of eluate of about 1.5cm. is maintained by the capillary attraction of the walls. The mingling of the eluate and pure solvent can be observed in this region and as an aid to viewing, an adjustable background of black and white pattern is fitted behind the cell. The whole system is enclosed in a wooden box and observations are made through a glass window (K). Silica gel is used to maintain a dry atmosphere in the box. The position of the illuminating lamp (L) is shown in figure (Ib).

Procedure:-

The reservoir is filled with solvent and the siphon

started. The chromatogram is developed in the usual manner, while the cell is observed for any sign of "schlieren". The appearance of "schlieren" indicates the beginning of a band, and their disappearance, the end of a band.

Application:-

The apparatus has been used successfully for alkaloids, and indole/oxindole systems. Petroleum ether, benzene, ethyl acetate and methyl alcohol have given satisfactory results as eluanta, but ether could not be used because of evaporation and consequent deposition of material on the walls of the cell.

Sensitivity:-

It has been reported that "schlieren" can be seen when refractive indices vary by as little as 0.0001. We have frequently observed the beginning of a band when evaporation of a sample showed that only a minute quantity of eluted material was present.

Continuous Extractor.

An apparatus was built capable of effecting the large scale extraction of natural products from plant material. The moderate solubilities of the products rendered continuous extraction necessary, and the obvious choice for such a lengthy process was a modified Soxhlet apparatus. The photograph shows the form that this extractor finally assumed. The solvent reservoir was a 22l. oil drum with a small opening of 4"diameter. The solvent in this drum was heated by a gas ring and distilled, through an asbestos-lagged tube of 0.5" diameter, into a 40l. drum which held the bark to be extracted. The solvent filtered through the bark and returned to the reservoir through a siphon of 0.25" diameter. There was an outlet from the 40l. drum carrying a reflux condenser and on the siphon was a sampling tap. To avoid "channelling" the bark was packed in small linen bags and the even distribution of the solvent was ensured by the insertion of a perforated plate above the bags. The linen bags acted as filters and prevented clogging of the siphon and also facilitated the charging and discharging of the extractor.

This extractor ran almost continuously for five months and only rarely was any difficulty experienced in its operation.


B
GLASS

Experimental.

Extraction.

1. Extraction of Powdered Bark with Chloroform by Shaking.

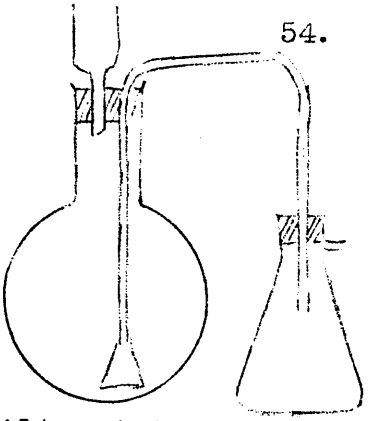
Ground bark (200g.) was shaken in a Winchester bottle with chloroform (1 l.) for 9 hours. The bark-chloroform mixture, which did not separate on standing overnight, was filtered and pressed. The chloroform solution, distilled to small bulk, yielded a pasty brown extract. The paste was extracted repeatedly with dilute hydrochloric acid and dilute ammonium hydroxide added to the acid solution to precipitate the alkaloid. There was a negligible yield of alkaloid.

This bark was extracted for another 6 hours by shaking with chloroform. More pasty brown material was obtained which again contained no alkaloid. The brown residue was triturated with dilute sodium carbonate solution. Addition of hydrochloric acid to the alkaline extract precipitated an acidic substance (0.3g.).

2. Extraction of Powdered Bark with Boiling Chloroform.

Ground bark (200g.) was boiled with chloroform (1 l.)

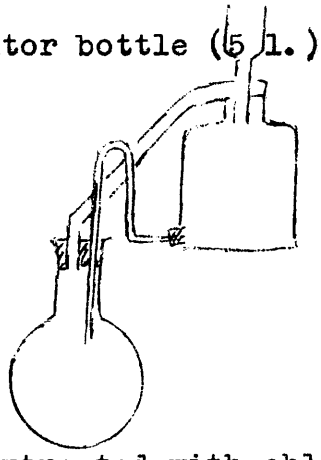
in a flask fitted as shown in sketch.



The solution was sucked through the filter into the filter-flask. Evaporation of the chloroform gave a brown paste. When treated as above this extract again gave no alkaloid. Acidic material (1.0g.) was obtained.

3. Extraction of Powdered Bark with chloroform in a Soxhlet Extractor.

A Soxhlet-type extractor was constructed as shown in the sketch from an aspirator bottle (5 l.) and a flask (5 l.).



Ground bark (200g.) was extracted with chloroform for 20 hours in this apparatus. The chloroform extract was worked up as above.

Yield: Alkaloid nil

Acidic substances 1.0g.

Neutral substances 2.7g.

Recrystallisation of the acidic material from ethanol-

water afforded white prisms m.p. 159° (micro.). Attempted crystallisation from dilute acetic acid gave a high melting acid m.p. 302-4° not depressed on admixture with quinovic acid. This suggests that the acidic material present in the bark is a precursor of quinovic acid and that it readily changes into quinovic acid on acid treatment.

4. Extraction of Bark/Quicklime with Chloroform by shaking.

Ground bark (200g.) was thoroughly mixed with powdered quicklime (40g.). The bark/quicklime mixture was moistened with water then spread out to dry at room temperature. When dry the mixture was shaken with chloroform (1 l.) for eight hours. The suspension thus formed was filtered and pressed and the chloroform distilled off. The brown residue was exhaustively extracted as above with dilute hydrochloric acid and then with sodium carbonate solution.

Yield:	Basic substances	0.05g.
	Acidic substances	nil.
	Neutral substances	1.0g.

5. Extraction of Bark/Quicklime with Boiling Chloroform.

Ground bark (200g.) was prepared as in (4) above then extracted for six hours with boiling chloroform in the apparatus described in (2) above.

Yield:	Alkaloidal material	0.8g.
	Acidic material	nil.
	Neutral material	1.0g.

6. Extraction of Bark/Quicklime with Chloroform in a Soxhlet Extractor.

Ground bark (200g.) was prepared as in (4) above and extracted for 6 hours with chloroform in the apparatus described in (3) above.

Yield:	Basic substances	0.75g.
	Neutral substances	1.1g.

7. Large Scale Extraction of Bark/Quicklime in a Soxhlet-type Extractor.

The extractor was constructed as shown in the section on extraction. (Photograph.)

The procedure for extraction was as follows:

1) The bark was cut, ground to a fine powder.

2) The powder was thoroughly mixed with powdered quicklime (20% of the weight of the bark).

3) The bark/quicklime mixture was moistened with water then spread out to dry at room temperature.

4) When dry the mixture was extracted with chloroform until a sample withdrawn from the sampling tap gave

a negative result in a test for alkaloid with Mayer's Reagent (K_2HgI_4).

The test was carried out as follows. Chloroform solution (25ml.) was withdrawn from the sampling tap and shaken with dilute hydrochloric acid (5ml.) in a separating funnel. The lower layer (chloroform layer) was run off and discarded. To the upper layer a sample of Mayer's Reagent was added. The appearance of a white cloudiness or precipitate indicated the presence of alkaloid.

The chloroform extract was evaporated, affording a brown paste. This paste was extracted repeatedly with dilute hydrochloric acid. Ammonium hydroxide was added to the acid solution precipitating the alkaloids together with a considerable amount of ferric hydroxide. This precipitate was extracted with methanol in a small Soxhlet extractor. Evaporation of the methanol solution gave the mixed alkaloids.

Separation.

1. Crystallisation.

Repeated crystallisation of the alkaloid mixture from methanol gave mitraphylline as white needles, m.p. 263°. The residual fraction did not yield further crystals.

2. Fractional Solution in Acetic Acid.

Mixed ether-soluble alkaloids (1g.) were dissolved in ether (10ml.) and extracted with successive quantities (2ml.) of 1% acetic acid. After ten such extractions the remaining alkaloid was extracted with excess dilute hydrochloric acid. To each acid solution dilute ammonium hydroxide was added and the precipitated alkaloid extracted with ether. Evaporation of the ether after drying over sodium sulphate gave the following fractions, none of which could be induced to crystallise. Melting pointsof the fractions suggest that a mixture is present in each case.

Fraction:	1	2	3	4	5	6	7	8	9	10	HCl
m.p.°C.	128	126	120	100	100	100	100	102	120	120	124
	-44	-40	-36	-10	-8	-8	-10	-15	-4	-6	-8

Chromatographic Separation of Ether-soluble Alkaloids.

Ether-soluble alkaloids (0.2g.) were dissolved in chloroform, passed through a column of alumina and eluted with methanol. Evaporation of the solvent showed that there was a considerable loss of material (0.06g.) on the column.

The experiment was repeated using magnesium carbonate, light and heavy, and calcium carbonate, light and heavy. Each of these adsorbents gave a satisfactory recovery of material but only the heavy magnesium carbonate allowed a rate of flow sufficiently fast

to permit extensive use.

Ether-soluble alkaloid (3.35g.) was dissolved in dry benzene and passed on to a column of heavy magnesium carbonate (200g.). Development with benzene caused the separation of five bands, visible due to their weak fluorescence in ultraviolet light. Bands (1) and (2) (0.62g.) were eluted together by benzene . Band (3) (0.54g.) was obtained by elution with benzene-chloroform (1:1). Chloroform eluted band (4) (0.70g.) and band (5) (0.96g.) wasobtained by elution with methanol. Total recovery = 2.82g. =85%.

The residue from evaporation of solvent from bands (1) and(2) was dissolved in benzene and adsorbed on a column of alumina. Separation of the two bands was achieved by development with benzene. None of the fractions was obtained crystalline.

Chromatographic Separation of Ether-Insoluble Alkaloid.

The separation of the ether-insoluble alkaloids was carried out as for the ether-soluble alkaloids, with magnesium carbonate (heavy) as adsorbent and chloroform as solvent. Bands were formed on the column which were eluted by chloroform (fraction 1), by acetone (fraction 2) and by methanol (fraction 3).

Yield from 1g. ether extracted alkaloid.

Chloroform insoluble residue		0.216g.
Fraction 1.		0.435g.
Fraction 2.		0.036g.
Fraction 3.		0.111g.
	Total	0.798g.

Fraction 1 was recrystallised from methanol in white needles m.p.263°. This fraction was mitraphylline. The residue, fraction 2 and fraction 3 could not be crystallised.

Attempted Acetylation of Mitraphylline.

Mitraphylline (10mg.) and sodium acetate (10 mg.) were dissolved in acetic anhydride (1ml.) and boiled for 30 minutes under reflux. The acetic anhydride was distilled off under reduced pressure. After a few drops of dilute ammonium hydroxide had been added the residue was dissolved in chloroform, dried over sodium sulphate, filtered and the filtrate evaporated to dryness. The residue was crystallised from methanol in white needles. m.p. 261° undepressed on admixture with mitraphylline.

Attempted benzoylation of Mitraphylline.

Mitraphylline (10mg.) was added to dilute caustic soda (1ml.) and a few drops of benzoyl chloride added. The mixture was shaken for 2 days, filtered and the gummy residue crystallised from methanol m.p. 263°, no depression on admixture with mitraphylline.

Action of Alkalis on Mitraphylline in presence of Alcohols.

The effect of alkali on mitraphylline in the presence of alcohols was studied as follows:

a) In ethanol + trace of potassium ethoxide.

b) In n-propanol + trace of potassium n-propoxide.

c) In methanol + trace of potassium methoxide.

d) In 4N methanolic potassium hydroxide.

---

a) Mitraphylline (10mg.) was dissolved in 10-20 times its volume of ethyl alcohol and a catalytic trace of potassium ethoxide added and the solution boiled under reflux for 6 hours. The ethanol was evaporated off and the residue crystallised from methanol. The first crop of crystals (5mg.) had m.p. 263° no depression on admixture with authentic mitraphylline. The remainder of the product was recovered from solvent and extracted with ether. The ether-insoluble part (1mg.) proved to be mitraphylline m.p. 261°. The ether solution was evaporated and yielded a small quantity of amorphous basic material m.p. 153°.

b) The experiment was repeated as above using n-propyl alcohol. A small quantity of mitraphylline was recovered (1-2mg.) m.p. 259° undepressed on admixture with authentic mitraphylline. The ether-soluble fraction could not be crystallised. m.p. pf amorphous base 115°

c) Mitraphylline (200mg.) was boiled under reflux for three hours with a trace of potassium methoxide. When the solution was evaporated to dryness, an ether-soluble product was obtained which did not crystallise even after many months in benzene/pet. ether. M.p. of amorphous material 205°. A small quantity of mitraphylline was also recovered m.p. 260°, no depression on admixture with authentic mitraphylline.

d) Mitraphylline (200mg.) and 4N methanolic potassium hydroxide (3 mol.) was boiled under reflux for one hour and left at room temperature overnight. An exact equivalence of methanol/hydrogen chloride was added and the precipitated potassium chloride filtered off. The filtrate was evaporated to dryness, ammonium hydroxide added; the precipitated base was extracted with ether and the ether solution dried over sodium sulphate then filtered. The filtrate was evaporated to dryness and yielded an amorphous base m.p. 203°. The ammonia solution was evaporated to dryness and afforded an amphoteric substance m.p. 173°, which could not be crystallised. The hydrochloride of this substance was prepared by passing dry hydrogen chloride into its chloroform solution. When dissolved in chloroform/methanol and left for several months the hydrochloride formed a glass.

Selenium Dehydrogenation of Yohimbic Acid.

Yohimbic acid (6.0g.) and selenium powder (4.5g.) were intimately mixed, and heated at 300° for 30 minutes in a flask, immersed in a metal bath and fitted with a long distillation tube. The flask was cooled and ground with its contents to a fine powder. An equal volume of sand, previously cleaned, was introduced and the whole mixture extracted with benzene in a Soxhlet extractor for 8 hours. The extraction was completed with methanol after removal of the benzene solution. The benzene solution was evaporated to dryness and crystals of yobyrine were collected at various concentrations. The residue from this crystallisation was dissolved in hydrochloric acid, precipitated with ammonium hydroxide and extracted with ether. The ether extract was dried over sodium sulphate, filtered and evaporated to dryness. The ether-insoluble precipitate was dissolved in benzene and chromatographed on alumina. The first band yielded tetrahydroyobyrine and thereafter yobyrine was eluted contaminated with a little keto-yobyrine.

The alcoholic extract afforded ketoyobyrine.

Yobyrine crystallised from benzene m.p. 217°.

Tetrahydroyobyrine crystallised from methanol m.p. 167°.

Ketoyobyrine crystallised from methanol m.p. 300°.

Note: It was possible to carry out this dehydrogenation with 0.5g. if the products were separated chromatographically.

Selenium dehydrogenation of Mitraphylline.

Small scale dehydrogenations in capillary tubes showed that hydrogenation selenide was evolved at temperatures above 205°. Evolution of hydrogen selenide became fairly rapid at about 230°.

Mitraphylline (0.5g.) was ground with selenium powder (0.35g.) and placed in a small flask with a long glass distilling tube attached. The flask was heated at 230° in a silicone bath. In one hour's time the evolution of hydrogen selenide had almost ceased and heating was discontinued. The flask and its contents were ground to a powder, mixed with cleaned sand and extracted first with chloroform then with methanol. Each fraction contained basic and neutral portions but no single compound could be isolated by chromatography on alumina or on magnesium carbonate, or by high vacuum distillation. Ammonia-like vapours were observed from the basic fractions and indole-like odours from the neutral fractions. The neutral fractions also gave positive tests with Ehrlich's reagent.

Attempted Oxidation of Mitraphylline with Potassium Dichromate.

Mitraphylline (370mg.) in acetic acid (5ml.) was shaken with 0.1N potassium dichromate (2 equivs.) for 3 days, then boiled for 4 hours. There was no change in the colour of the solution. Ammonia was added, and the precipitated base extracted with chloroform. The chloroform solution was dried over sodium sulphate then chromatographed on alumina. Chloroform elution gave mitraphylline (325mg.) crystallised from methanol, m.p. 263° not depressed on admixture with authentic mitraphylline. Methanol elution of the column yielded a very small amount of an amorphous substance.

Attempted Water Hydrolysis of Mitraphylline.

Mitraphylline (250mg.) was boiled in water (250ml.) for 36 hours. Part remained undissolved and was filtered off and crystallised from methanol, m.p. 261°, giving no depression in melting point when mixed with mitraphylline. The water solution was evaporated to dryness, and it yielded a basic residue which crystallised in needles m.p. 263° from methanol. This proved to be mitraphylline unchanged. The total recovery of mitraphylline was 215mg. A trace of amphoteric product was observed but could not be separated.

Attempted Acid Hydrolysis of Mitraphylline.

Mitraphylline (50mg.) was dissolved in N sulphuric acid (5ml.) and boiled under reflux. After 7 hours there was still basic material present. The pH of the solution was adjusted to 7 and the precipitate dissolved in chloroform. The chloroform solution was dried over sodium sulphate, filtered and evaporated to dryness. Recrystallisation from methanol afforded mitraphylline m.p. 259-61° (no depression with authentic specimen). The total recovery of mitraphylline was 30mg. There was no sign of amphoteric material.

Action of Nitric Acid on Mitraphylline.

a) Mitraphylline (100mg.) was heated with conc. nitric acid (10ml.) until red fumes were evolved. Intermittent heating was continued until the red fumes were replaced by the white fumes of nitric acid and the red solution became yellow in colour. One drop of the solution was smeared on a watch-glass and left overnight whereupon there developed evidence of crystallinity. The solution was evaporated to dryness. Part was found to be either soluble.

b) Mitraphylline was boiled with 20% nitric acid for 3 days and the solution evaporated to dryness and the residue treated with conc. nitric acid as above. There was no evidence of crystals in the evaporated solution.

c) Mitraphylline (100mg.) was treated as in (a) with fuming nitric acid (S.G. 1.50). A quantity of crystals similar to (a) was obtained.

d) In an attempt to nitrate before oxidation, excess nitrogen peroxide dissolved in chloroform was added to mitraphylline (100mg.) dissolved in chloroform. There was an immediate precipitate of brown oil. After 2 hours the chloroform was evaporated off and concentrated nitric acid was added to the oil. The procedure of (a) was then followed. Asmall quantity of crystalline product was again observed.

e) Experiment (d) was repeated using 1g. mitraphylline. To the final nitric acid solution, water was added, precipitating a yellow amorphous substance, which was filtered off. The filtrate was evaporated to dryness, and the residue was triturated with ether in which the crystals dissolved. After crystallising from ether/benzene several times the crystals were obtained as stout rods subliming then melting at 143-5° on the micro-melting point apparatus. Yield: 12mg. The product formed a compound with urea micro-m.p. 163° showing no depression with urea oxalate.

The amorphous ether-insoluble substance dissolved in caustic soda with the formation of a deep red colour.

Benzoyl chloride was added to this solution and the suspension shaken for two days. The red colour had by this time been replaced by a yellow and the red was not re-formed on addition of more caustic soda. The benzoyl product was still soluble in caustic soda. This shows that the nitration product was a nitrophenol containing an acidic group which could not be benzoylated.

Acetylation of Mitraphylline.

Mitraphylline (100mg.) and a small particle of ferric chloride were dissolved in acetic anhydride (2ml.) and boiled under reflux for 4 hours. The acetic anhydride was removed by heating under vacuum, and the residue obtained thereby was dissolved in water. Sodium carbonate solution was added and the precipitate extracted with chloroform. The chloroform solution was dried over sodium sulphate, filtered and evaporated to dryness, affording acetylmitraphylline. This product was crystallised from methanol in clusters of stout needles m.p. 164-5°. (Found: C, 67.2; H, 6.7; N, 7.0. $C_{23}H_{28}O_5N_2$ requires C, 67.0; H, 6.8; N, 6.8%)

No hydrogen was absorbed by this compound, dissolved in acetic acid in the presence of palladium black catalyst.

Hydrogenation of Mitraphylline.

Mitraphylline (13mg.) dissolved in acetic acid was hydrogenated over palladium black catalyst. There wasa an absorption of 0.9 mol. of hydrogen. The solution was filtered and concentrated by heating in vacuo. A little water was added and the base was precipitated with ammonium hydroxide then extracted with chloroform. The chloroform solution was dried over sodium sulphate, filtered and evaporated to dryness. The product crystallised in needles from methanol m.p. 263° giving no depression on admixture with mitraphylline.

The experiment was repeated on 1g. mitraphylline. There was again evidence of absorption of hydrogen but the exact volume could not be measured on the apparatus used. The product crystallised as follows:

Fraction 1 m.p. 263° - no depression with mitraphylline.

Fraction 2 m.p. 263° - ditto.

Fraction 3 m.p. 249-56° darkening on melting, no depression with mitraphylline

Fractions 1 and 2 were recrystallised in needles from methanol m.p. 263°. (Found: C, 67.9; H, 7.4; N, 7.7. $C_{21}H_{26}O_4N_2$ requires C, 68.1; H, 7.1 ; N,7.4. $C_{21}H_{28}O_4N_2$ requires C, 67.7; H, 7.5; N,7.5%.)

The hydrogenation product (100mg.) was boiled with acetic anhydride containing a grain of ferric chloride for 4 hours and the acetylation product isolated as previously described for acetylmitraphylline. The product, m.p. 163°, showed no depression in its melting point when admixed with authentic acetylmitraphylline.

In an attempt to acetylate "dihydro"mitraphylline no absorption of hydrogen was observed.

Mitraphylline (70mg.) was dissolved in acetic acid and N potassium dichromate (5 ml.) added. The solution was boiled under reflux for 4 hours, ammonia was added and the resulting precipitate extracted with chloroform. The chloroform solution, after drying over sodium sulphate and filtering was evaporated to dryness and the product was crystallised twice from methanol m.p. 263°. This sample absorbed no hydrogen when dissolved in acetic acid and over palladium black catalyst.

(Found: C, 68.4; H, 6.9; N, 7.4. $C_{21}H_{26}O_4N_2$ requires C, 68.1; H, 7.1; N, 7.4%)

Coupling with Diazotised Sulphanilic Acid.

A number of compounds were coupled with diazotised sulphanilic acid.

a) Indole - immediate brown precipitate, changing to red. This precipitate showed indicator action - red in acid, yellow in alkali.

b) 1:7-dimethylindoline - immediate precipitate of red dye. Red in acid, yellow in alkali.

c) Yohimbic Acid - red colour formed immediately but no precipitate. Red in acid, yellow in alkali.

d) Mitraphylline - Pale yellow colour darkening slightly on standing a few weeks but not changing further even after several months.

Zinc Dust Distillation of Mitraphylline.

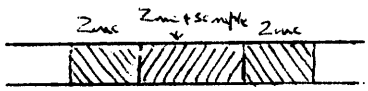
The distillation of mitraphylline from zinc dust was carried out by three separate procedures.

1) Distillation of mitraphylline at atmospheric pressure.

2) Distillation of mitraphylline at "mercury pump" pressures.

3) Distillation of the amorphous hydrochloride of mitraphylline at "mercury pump" pressures.

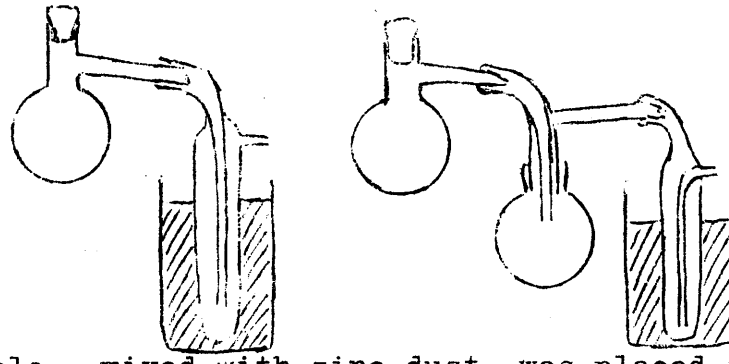
1) Mitraphylline (200mg.) was mixed with zinc dust (5g.) and placed in a Pyrex tube as shown in sketch. Zinc dust was placed in the tube on each side of the sample.



The tube was heated strongly by a bunsen burner at the point which contained the sample, and a second burner

was used to drive the distillate towards the ends of the tube. When the distillation was complete, the burners were removed and the tube was allowed to cool. When cold, the tube was cut and the products extracted with ether. The ether solution was extracted with dilute hydrochloric acid; the aqueous layer separated and made alkaline with ammonia then extracted with ether. The ether solutions of bases and neutral substances were dried over sodium sulphate. Indoles were recognised in the neutral fraction by their smells andby their reaction with Ehrlich's reagent. No crystalline product was obtained from this experiment.

2) The apparatus used in this and series (3) is shown below.



The sample , mixed with zinc dust, was placed in the distilling flask and the distillate was received in the liquid-air cooled receiver. In (b) the simple receiver was replaced by two receiving flasks, the first at room temperature and the second cooled in liquid-air. Apparatus (b) was necessitated by the frequent blocking of the receiver in (a).

Mitraphylline (1g.) was ground and mixed thoroughly with zinc dust (25g.) and placed in the distilling flask then covered with more zinc dust (5g.). The apparatus was evacuated to about 10^{-4} mm. Hg. and heated in an air-bath to 400°. At about 200°, fumes appeared and a red oily distillate collected in the receiver. When distillation was complete, the apparatus was allowed to cool and the contents of the receiver extracted with ether. A portion of the distillate did not dissolve and this on crystallisation from methanol formed white needles m.p. 263° undepressed on admixture with mitraphylline. The ether solution was extracted with dilute caustic soda solution. The ether solution of neutral substances was dried over sodium sulphate, filtered and evaporated to dryness. The hydrochloric acid extract contained bases which were precipitated with ammonium hydroxide then dissolved in ether, dried over sodium sulphate, and recovered from the filtered solution by evaporation of the solvent. The caustic soda extract contained a negligible amount of acidic material.

A crystalline substance was observed in the neutral fraction and some of the bases yielded crystalline picrates. These crystalline materials are described below.

3) Mitraphylline (1g.) was dissolved in chloroform (50ml.) and dry hydrogen chloride bubbled through the solution. There was some precipitation of the amorphous hydrochloride of mitraphylline. The chloroform was evaporated off under vacuum and the amorphous product mixed thoroughly with zinc dust (25g.), placed inthe distilling flask, and covered with more zinc dust (5g.). The distillation wascarried out as above. The product still contained a small amount of mitraphylline. A volatile fraction was found to be chloroform (carbylamine test). The main products were similar to those obtained in experiment (2), viz. a neutral fraction (containing a crystalline substance) and a basic fraction (affording crystalline picrates).

Separation and Characterisation of the Neutral Fraction.

The neutral fraction was dissolved in benzene and chromatographed on alumina. Elution with benzene afforded indolic substances and elution with chloroform gave a crystalline substance, crystalling in stout white hexagonal rods from ethyl acetate m.p. 179-81° (micro-m.p. 175-7°). (Found: C, 75.4; H, 5.7; N, 8.4. $C_{10}H_9ON$ requires C, 75.4; H, 5.65, N, 8.8%.)

This compound sublimes at 110-5° at 12mm.; it is insoluble in cold alkali, but dissolves in boiling dilute alkali and is reprecipitated unchanged by dilute acid.

Yield: The yield at the beginning of the series of zinc dust distillations was 1mg. per 1g. but rose finally to 20mg. per 1g.

Micro-hydrogenation showed that a sample dissolved in acetic acid absorbed 1.02 mol. hydrogen in 15 mins. over Pd black catalyst, indicating the presence of a double bond in the molecule.

Pure degradation product (20mg.) was hydrogenated in acetic acid over Pd black catalyst for 75 mins., filtered from catalyst, and the acetic acid evaporated off in vacuo. Ether was added to dissolve the product and the ether solution was extracted with dilute sodium carbonate solution to remove residual acetic acid, then dried over sodium sulphate. The sodium sulphate was filtered off and the solution evaporated to dryness. The hydrogenation product was crystallised from ether at -30°. micro-m.p. 93-7° mixed micro-m.p. with authentic 3-ethyloxindole 93-8°. Micro-m.p. of pure 3-ethyloxindole 96°.

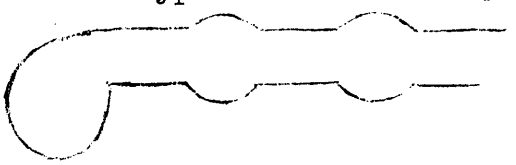
The identity of the hydrogenated product with 3-ethyloxindole was confirmed by comparison of their X-ray diffraction patterns. These X-ray photographs

are identical in each of 13 lines.

The degradation product is therefore either 3-ethylidene- or 3-vinyl-oxindole. U.V. spectra of the degradation product and of 3-ethyloxindole suggest that the product is 3-vinyloxindole.

Separation and Characterisation of the Basic Fraction.

The basic fraction was dissolved in ether, filtered, evaporated and redissolved in ether repeatedly to remove all traces of mitraphylline. The bases were placed in a flask of the type shown in the sketch



and distilled at 0.8mm. pressure in a carefully regulated air-bath. The fractions were collected at 60°, 60-95°, 100-20°, and 120-30°. A viscous material remained in the flask at the end of the distillation. With the exception of the first fraction, all the distillates gave oily picrates. The first fraction (micro-b.p. by Siwoloboff method 239-45°.) was dissolved in ether and a few drops of a saturated solution of picric acid in ether added. There was an immediate precipitate of a crystalline picrate. This was crystallised from methanol m.p. 202-4° Mixed m.p. with 1:2:3:4-tetrahydroisoquinoline picrate (m.p. 200°) 180-5°. The picrates are not identical.

The fractional crystallisation of the picrates is shown in the accompanying table.

Methanol: Fraction 1 1st crystallisation m.p. 202°
2nd m.p. 215°
3rd m.p. 222°
Fraction 2 m.p. 219-20°
Fraction 3 m.p. 190-215°

Acetone: Fraction 4 m.p. 134-42°(micro)
Fraction 5 m.p. 123-6° (micro)
- Fractions 5 and 6 were combined and crystallised twice m.p. 123-6° (micro) - "A"

Ethanol: Fraction 6 m.p. 95-106° (micro)
recryst 107-18°
recryst. 112-8°
recryst. 120-7°
recryst. 124-6° - "B"

"A" and"B" were combined and crystallised m.p. 127-9°(micro). Mixed m.p. with picric acid 85-120°(micro).

Fraction 1. Found:C, 50.4; H, 3.1. $C_9H_7N.C_6H_3O_7N_3$ requires C, 50.3; H, 2.8%. Mixed melting ppint with authentic specimen of isoquinoline picrate showed no depression.

The picrate of the degradation base (fraction 1) was dissolved in water and a drop of dilute caustic

soda solution added. The base thus regenerated was extracted with ether and the solution dried over sodium sulphate. Asolution of styphnic acid in ether was added to the filtered solution of the base, precipitating the styphnate of the base. This was filtered off and crystallised from methanol m.p. 188-91° (micro). No depression on admixture with an authentic specimen of isoquinoline styphnate.

Preparation of Isoquinoline Styphnate.

A saturated solution of styphnic acid in ether was added a solution of isoquinoline in ether. The precipitate was filtered, washed with ether and crystallised from methanol m.p. 193° (micro-m.p. 188-90°).

(Found: C, 48.4; H, 2.9; N, 15.0. $C_9H_7N.C_6H_3O_8N_3$ requires C, 48.2; H, 2.7; N, 15.0%.)

Lithium aluminium hydride Reduction of Mitraphylline.

a) In ether.

Lithium aluminium hydride (1.0g.) was powdered and refluxed with dry ether (20ml.) for 30 mins. Mitraphylline (1.0g.) was added to the cooled solution, whereupon slight effervescence was noted. The cold reaction mixture was stirred for 30 mins. and then refluxed for 3 hours. The flask and its contents were cooled and ethyl acetate (10ml.) added, then water(2ml.) and alcohol (30ml.). The paste thus formed was filtered and

the residue extracted three times with chloroform. The filtrates were combined and evaporated to dryness. The residue was extracted with chloroform and filtered. This filtrate was evaporated to dryness yielding a crystalline solid. This solid was fractionally crystallised, and yielded mitraphylline m.p. 263° (0.9g.) and an amorphous basic material (45mg.). This amorphous substance did not yield any crystalline derivatives.

Lithium aluminium hydride Reduction - Contd.

b) In Dioxan.

Mitraphylline (0.5g.) was added to lithium aluminium hydride (0.5g.) in dioxan (20ml.) and boiled for 3 hours. Ethyl acetate (10ml.) was added to the cooled solution, followed by water (2ml.) and alcohol (10ml.). The paste thus formed was filtered and the residue extracted with chloroform and the chloroform solution filtered. The combined filtrates were evaporated to dryness under reduced pressire on a steam bath and the residue triturated with chloroform. The chloroform solution was evaporated to dryness and the residue crystallised from methanol. A small quantity of mitraphylline m.p. 263° was recovered and the rest of the product was an amorphous base (0.3g.).

Action of Liquid Ammonia on Mitraphylline.

Mitraphylline (100mg.) was placed in a small Dewar flask and liquid ammonia (60ml.) added and the whole stirred. After 4 hours the liquid ammonia (60ml.) was decanted off, and fresh liquid ammonia (60ml.) added. After another 4 hours this was repeated and the reaction mixture was allowed to stand overnight. The liquour was then decanted and the combined liquours evaporatedto dryness. The product melted at 263° and proved to be mitraphylline. The residual solid in the Dewar flask was crystallised from methanol and also proved to be mitraphylline.

Action of Conc. Sulphuric Acid on Mitraphylline.

Mitraphylline (0.5g.) was placed in a small test-tube and concentrated sulphuric acid (1ml.) added. There was an immediate evolution of carbon dioxide which was identified by the precipitate of barium carbonate that it produced on reaction with barium hydroxide. When the gas was tested with palladium chloride paper there was no darkening of the colour of the paper showing that there was no appreciable quantity of carbon monoxide present. After the reaction mixture had been left for a few hours, a few drops of water were added to it causing the immediate predipitation of a white solid. The mixture was heated until this precipitate dissolved and then allowed to cool. On cooling the sol-

ution deposited crystals which were filtered off and recrystallised from methanol. These crystals lost water at about 200°, and decomposed at 260°. When admixed with an authentic specimen of mitraphylline sulphate there was no depression in melting point. The filtrate was made alkaline with ammonia and the white precipitate thus formed was extracted with chloroform . The chloroform solution was dried over sodium sulphate and filtered, then evaporated to dryness. The base obtained in this way could not be induced to crystallise nor form any crystalline derivative.

Effect of Boiling Mitraphylline with 30% Sulphuric Acid.

Mitraphylline (100mg.) was boiled with 30% sulphuric acid and the evolution of carbon dioxide was noted. When no more carbon dioxide was evolved (50 mins.), boiling was stopped, the solution cooled and ammonium hydroxide added. The white precipitate that formed was extracted with chloroform. Part of the precipitate dissolved and the chloroform solution was dried over sodium sulphate, filtered and the solvent evaporated off. This product could not be crystallised and did not form any crystalline derivatives.

The chloroform-insoluble part of the precipitate was filtered off and dissolved in methanol. The methanol solution was dried over sodium sulphate, filtered

and the methanol evaporated off on a steam bath. The product still did not dissolve in chloroform. It was also insoluble in acetone and redissolved in methanol only with difficulty. It did not yield crystals.

Effect of Acetic Acid/Sulphuric Acid on Mitraphylline.

Mitraphylline (20mg.) was heated to boiling with acetic acid/sulphuric acid (4:1 by volume). After one minute the solution had become dark brown in colour. Heating was continued for 5 minutes. No evolution of carbon dioxide was observed. A few drops of water were added to the solution but caused no precipitation. Ammonium hydroxide was added to precipitate the base. When shaken with chloroform part of this precipitate dissolved and the chloroform solution was dried over sodium sulphate, filtered and evaporated to dryness yielding an amorphous base.

The experiment was repeated heating the reaction mixture only to 100° on a water bath. In this case there was no chloroform-insoluble fraction.

The experiment was repeated using mitraphylline (100mg.) and acetic acid/sulphuric acid (19:1 by volume) and heating to 100° for 4 hours. From this experiment mitraphylline was recovered (59mg.) and amorphous base(25mg.).

Active Hydrogen Determinations.

In the first series the active hydrogens were determined by treating the compounds in pyridine solution with lithium aluminium hydride.

Results:

7-methylindole. mol. wt. =131

75mg. substance gave 13.5ml. H_2 at 293°A and 759mm. pressure.
therefore substance contains $\frac{13.5 \times 273 \times 759 \times 131}{75 \times 293 \times 760 \times 22.4}$ active hydrogens

= 0.98 active hydrogens.

3-ethyloxindole. mol. wt. =161

20mg. gave 5.9ml. H_2 at 294°A and 759mm.
therefore substance contains $\frac{5.9 \times 273 \times 759 \times 161}{20 \times 294 \times 760 \times 22.4}$ active hydrogens

= 1.99 active hydrogens.

Mitraphylline mol. wt. =370

121mg. mitraphylline gave 16.9ml. H_2 at 293°A and 766mm.
Therefore mitraphylline contains $\frac{16.9 \times 273 \times 766 \times 370}{121 \times 293 \times 760 \times 22.4}$

= 2.08 active hydrogens.

Repeat.

52mg. mitraphylline gave 7.2 ml. H_2 at 294° and 765mm.
therefore mitraphylline contains $\frac{7.2 \times 273 \times 765 \times 370}{52 \times 294 \times 760 \times 22.4}$

= 2.15 active hydrogens.

Rhynchophylline. mol. wt. = 384

69mg. rhynchophylline gave 8.0ml. H_2 at 294°A and 765mm.

therefore rhynchophylline contains $\frac{8.0 \times 384 \times 273 \times 765}{69 \times 294 \times 760 \times 22.4}$

= 1.86 active hydrogens.

Rotundifoline. mol. wt. = 398

57mg. rotundifoline gave 10.9ml. H_2 at 297°A and 765mm.

therefore rotundifoline contains $\frac{10.9 \times 273 \times 765 \times 398}{57 \times 297 \times 760 \times 22.4}$

= 3.14 active hydrogens

In the second series the number of active hydrogens in mitraphylline was determined by dissolving mitraphylline in boiling phenetole and adding methyl magnesium iodide in phenetole. Boiling was continued until no more methane was evolved. All measurements of volume were made at room temperature.

Results:

64.5mg. mitraphylline gave 7.8ml. CH_4 at 293°A and 760mm.

therefore mitraphylline contains $\frac{7.8 \times 273 \times 760 \times 370}{64.5 \times 293 \times 760 \times 22.4}$

= 1.9 active hydrogens.

61 mg. mitraphylline gave 7.3ml. CH_4 at 293°A and 760mm.

therefore mitraphylline contains $\frac{7.3 \times 370 \times 273 \times 760}{61 \times 293 \times 760 \times 22.4}$

= 1.85 active hydrogens.

The Action of Methyl Magnesium Iodide on Mitraphylline.

Mitraphylline (100mg.) was boiled with methyl magnesium iodide in phenetole. After three minutes boiling the solution was cooled and water added to decompose the excess methyl magnesium iodide then dilute sulphuric acid was added. The aqueous acid layer was separated from the phenetole layer and the latter extracted once more with sulphuric acid. The combined acid extracts were extracted with ether to remove all traces of phenetole, then made alkaline with ammonia. The precipitate thus formed was extracted with chloroform and dried over sodium sulphate. After filtering, the chloroform solution was evaporated to dryness affording a solid which crystallised from acetone in needles, m.p. 248-250° depressed on admixture with mitraphylline. (Found: C, 75.3; H, 8.0; N, 7.3. $C_{22}H_{28}O_2N_2$ requires C, 75.0; H, 8.0; N, 7.95%.)

Methoxyl determination on this compound gave a negative result.

9-Methyl-β-carboline. (9-methyl-norharman).

7-methyltryptophan (0.1g.) was dissolved in water (15ml.) and 40% formaldehyde (1 ml.) added. The solution was kept at room temperature for 3 hours then heated on a water bath for 2 hours (when a sample gave no colour on boiling with ninhydrin). Without separating the 9-methyl-2:3:4:5:tetrahydro-4-carboxy-β-carboline, 10% potassium dichromate solution (5 ml.) and acetic acid (1 ml.) were added and the solution was boiled for 3 minutes. The excess potassium dichromate was reduced by sodium sulphite and the 9-methyl-β-carboline extracted with ether after precipitation by aqueous sodium carbonate solution. The ether solution was dried over sodium sulphate then evaporated to yield 9-methyl-β-carboline(70mg.)

The product sublimed at 150° at 1mm. pressure and when crystallised from benzene afforded fluffy white needles changing rapidly in contact with the mother liquours to rosettes of stout needles m.p. 229-30°.(Found: C, 78.9; H, 5.5; N, 15.4. $C_{12}H_{10}N_2$ requires C, 79.1; H, 5.5; N, 15.4%.)

2:9-Dimethyl-β-carboline. (9-methylharman.)

2:9-Dimethyl-β-carboline, needles, m.p. 213° from benzene, was obtained by using acetaldehyde (0.3ml.) in place of formaldehyde as in the preceding experiment. (Found: C, 79.3; H, 6.4; N, 14.4. $C_{13}H_{12}N_2$ requires C, 79.5; H, 6.2; N, 14.3%.)

Chloroacetyl-N-methyl-o-toluidide.

Benzenesulphonyl-o-Toluidide was methylated in an excess of aqueous sodium hydroxide by methyl sulphate. The resultant benzenesulphonyl-N-methyl-o-toluidide, m.p. 78° from ethanol, (Found: C, 64.3; H, 5.7. $C_{14}H_{15}O_2NS$ requires C, 64.4; H, 5.8%.), was hydrolysed when heated (45 minutes) with a mixture of water and sulphuric acid (1:2, v/v). N-methyl-o-toluidine (yield 40% from o-toluidine) was recovered in steam from the basified solution. Chloroacetyl chloride (3.3g.) was slowly added to a solution of the base (3.6g.) in benzene (25ml.) and pyridine (2.4g.) and after 2 hours chloroacetyl-N-methyl-o-toluidide, m.p. 46° from light petroleum (60-80°) was recovered from the acid washed benzene solution (yield, 85%) (Found: C, 60.6; H, 6.2. $C_{10}H_{12}ONCl$ requires C, 60.8; H, 6.1%).

1:7-Dimethylindole.

A mixture of chloroacetyl-N-methyl-o-toluidide (7.5g.), aluminium chloride (7.5g.) and sodium chloride (1.5g.) was heated at 180-5° for 1 hour and the cooled powdered product was added to ice and extracted in benzene. After purification on alumina 1:7-dimethyloxindole was recovered and had m.p. 119-20° from light petroleum (60-80°)(Found: C, 74.7; H, 6.9; N, 8.9. $C_{10}H_{11}ON$ requires C, 74.5; H, 6.8; N, 8.7%). To a stirred suspension of this oxindole (3.5g.) in anhydrous ether (35ml.) lithium aluminium

hydride (1.2g.) was slowly added. After further stirring (10 minutes), water (10ml.) and then hydrochloric acid (10ml.,4%) were added and the ethereal layer was washed with acid, (1:7-dimethylindoline was recoverable in steam from the basified acid solution), dried and evaporated. A solution of the residue in light petroleum (60-80°) was passed through an alumina column and elution with the same solvent gave 1:7-dimethylindole as needles (1.5g.) m.p. 78° from light petroleum (40-60°) (Found: 82.5; H, 7.7; N, 9.6. $C_{10}H_{11}N$ requires C, 82.7; H, 7.6; N, 9.7%), whereas subsequent elution with benzene afforded unchanged 1:7-dimethyloxindole (1g.)

1:7-Dimethyl-3-dimethylaminomethylindole (1:7-Dimethylgramine).

To an aqueous solution of dimethylamine (3ml.,33%), maintained below 5°, acetic acid (3ml.) and then formaldehyde(1.5ml.,40%) were added and the whole was poured upon 1:7-dimethylindole (2.9g.) which dissolved when the mixture was shaken at room temperature. After 15 hours the solution was basified and extracted with ether. Since the base did not readily crystallise a portion of the ethereal solution was treated with a solution of picric acid in ether, affording 1:7-dimethylgramine picrate as orange needles, m.p. 142° from ethanol (Found: C, 53.0; H, 5.0; N, 16.4. $C_{13}H_{18}N_2.C_6H_3O_7N_3$

requires C, 52.9; H, 4.9; N, 16.2%). To the rest of the ethereal solution methyl iodide was added and the precipitated salt was dried *in vacuo* for use in the following experiment.

α-amino-*β*-(1:7-dimethyl-3-indolyl)-propionic Acid (1:7-Dimethyltryptophan)

A solution of the fore-going salt (0.86g.) and ethyl acetamidomalonate (0.54g.) in anhydrous ethanol (10ml.) containing sodium ethoxide (from 0.056g. of sodium) was heated under reflux for 26 hours. Addition to water (200ml.) afforded ethyl *α*-acetamido-*α*-ethoxycarbonyl-*β*-(1:7-dimethyl-3-indolyl)propionate which, after softening at 158°, had m.p. 162° from ethyl acetate (Found: C, 64.5; H, 7.2. $C_{20}H_{26}O_5N_2$ requires C, 64.2; H, 7.0%). It (3.5g.) was heated under reflux for 18 hours with a solution of sodium carbonate (3.5g.) in water (35ml.) and, after some oil was removed in ether, the acidified solution afforded *α*-acetamido-*β*-(1:7-dimethyl-3-indolyl)propionic acid, m.p. 181° from ethyl acetate (Found: C, 65.4; H, 6.7. $C_{15}H_{18}O_3N_2$ requires C, 65.7; H, 6.6%). The acetamido-compound (1g.) and water (50ml.) were heated at 200° for 6 hours. The solid obtained was extracted with dilute sulphuric acid from which 1:7-dimethyltryptophan was precipitated at pH 7 by dilute sodium hydroxide and formed colourless needles,

m.p. 218° from water (Found: C, 66.9; H, 7.1. $C_{13}H_{16}O_2N_2$ requires C, 67.2; H, 6.95%).

1:9-Dimethyl-β-carboline.

1:9-dimethyl-β-carboline was prepared from 1:7-dimethyltryptophan as described above for 9-methyl-β-carboline. M.p. 68-70° resolidifying and re-melting at 96°. Crystallised from benzene or methanol. (Found: C, 72.8; H, 6.7. $C_{13}H_{12}N_2.H_2O$ requires C, 73.1; H, 6.5%.)

1:9-Dimethyl-β-carboline was also prepared by N-methylation of 9-methyl-β-carboline by the following method. A suspension of 9-methyl-β-carboline was heated under reflux in benzene with methyl sulphate for 1 hour whereupon the mixture became pasty and yellow. After standing overnight 3:9-dimethyl-β-carbolinium methosulphate was filtered off and crystallised from methanol in yellow needles, m.p. 228° (Found: C, 54.4; H, 5.2. $C_{14}H_{16}O_4N_2S$ requires C, 54.5; H, 5.2%). Addition of dilute aqueous sodium hydroxide to a solution of the methosulphate in water caused the precipitation of 3:9-dimethyl-β-carbolinium hydroxide which crystallised in yellow needles from water m.p. 165° resolidifying and decomposing above 230° (Found C, 60.35; H, 7.5. $C_{13}H_{14}ON_2.3H_2O$ requires C, 60.0; H, 7.7%). This hydroxide was heated at 100°/15mm. for 1 hour then cooled and suspended in dry

benzene. A few drops of methyl iodide were added and the mixture heated at 45-50° for 3 hours. After standing overnight the solution was evaporated to dryness affording 1:3:9-trimethyl-β-carbolinium methiodide which crystallised in needles decomposing above 320°(from water) (Found: C, 49.8; H, 4.3. $C_{14}H_{15}N_2I$ requires C, 49.7; H, 4.5%). The methiodide was heated in a sublimation tube at 300°/15mm. and a yellow sublimate was obtained. This sublimate was resublimed giving white plates m.p. 70° resolidifying and re-melting at 96°, no depression on admixture with the above 1:9-dimethyl-β-carboline (Found: N, 13.5. $C_{13}H_{12}N_2$ requires N, 13.1%).

1:2:9-Trimethyl-β-carboline.

1:2:9-Trimethyl-β-carboline was prepared from 1:7-dimethyltryptophan as described above for the preparation of 2:9-dimethyl-β-carboline from 7-methyltryptophan. The 1:2:9-trimethyl-β-carboline thus formed crystallised in prisms from benzene and sublimed at 150°/15mm. m.p. 165-8°.

1:2:9-trimethyl-β-carboline was also prepared by N-methylation of 2:9-dimethyl-β-carboline as described above for the preparation of 1:9-dimethyl-β-carboline from 9-methyl-β-carboline. 1:2:3:9-tetramethyl-β-carbolinium methiodide m.p. 305-10° was prepared via 2:3:9-trimethyl-β-carbolinium methosulphate and hydroxide m.p.'s 218° and 165°(resolidifying and decomposing above

230°) respectively. By heating the methiodide at 300°/15mm. a yellow sublimate was obtained which when resublimed gave needles of 1:2:9-trimethyl-β-carboline m.p. 168-9° not depressed on admixture with a sample of 1:2:9-trimethyl-β-carboline obtained above. (Found: C, 80.1; H, 6.9. $C_{14}H_{14}N_2$ requires C, 80.0; H, 6.7%.)

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