Chemistry of The MITRAGYNA GENUS

With a supplementary section on:

The Zerewitinoff Estimation of
Active Hydrogen Atoms

and

Additional papers on:

Urea Alkyl Sulphates
Alkyl iso-Ureas
Chemistry of the Mitragyna Genus (Part I).

T H E S I S

for the Degree of

Doctor of Philosophy

of the

University of Glasgow

by

Patrick A. Ongley, B.A., M.Sc.(N.Z.).

October, 1950.

ProQuest Number: 13850817

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

ProQuest 13850817

Published by ProQuest LLC (2019). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Acknowledgments.

I wish to acknowledge my indebtedness to my supervisors, Dr. G.M. Badger, and Professor J.W. Cook, F.R.S., without whose assistance and encouragement this work would never have been completed. I have also to thank sincerely Dr. S.T.R. Stotherd Mitchell for advice on the measurements of optical activity and ultra-violet spectra; Dr. Irene M. McAlpine for advice on the Zerewitinoff estimation of active hydrogen atoms; and Miss R.H. Kennaway and Mr. J.M.L. Cameron for micro-analyses. The plant materials were obtained through the courtesy of Sir John Simonsen, F.R.S., Director of the Colonial Products Research Council, and of the Chief Conservator of Forests, Nigeria, and the bulk extractions were carried out by Messrs. Duncan Flockhart, Ltd., Edinburgh. Drs. E. Dyer, W. Klyne, and L.J. Sargent, Professors D. Karrer and L. Ruzicka, M. Raymond-Hamet and Mrs. E. Stedman were most generous with gifts of material for investigation and of authentic specimens. Finally, I must thank sincerely my many friends, both in Glasgow and elsewhere, for their unfailing encouragement and most helpful advice.

Table of Contents.

Summary.

Previous work on the Mitragyna alkaloids was largely haphazard, and very little attempt had been made to correlate the known properties. The first task in this investigation, therefore, was to do this. The properties of mitragynine, mitragynol, mitraphylline, rhynchophylline, and rotundifoline, and of formosanine, from a species of the closely-related genus Ourouparia, have now been more completely examined.

Concerning the occurrence of the alkaloids, several interesting results were encountered. There has been controversy as to whether or not mitrinermine from M.inermis and "rhynchophylline" from O.rhynchophylla are the same alkaloid. It has now been shown conclusively that they are the same. The hitherto unknown d-form of mitraphylline has been found in the bark of M.rubrostipulacea; and in M.ciliata, previously uninvestigated, rotundifoline has been found in the leaves, and rhynchophylline in the bark. -sitosterol has been identified in the bark of M.inermis, and quinovic acid in the bark and leaves of various Mitragyna species.

As far as functional groups are concerned, it is now known that the alkaloids contain no N-methyl groups, but are methyl esters and often contain additional methoxyl groups.

Although neither mitraphylline nor rotundifoline gives hydroxyl or keto derivatives, acetyl-rhynchophylline is known. While neither mitragynine, mitraphylline, rotundifoline, nor formosanine reacts with diazomethane, rhynchophylline does to give a methyl derivative.

Mitragynol deserves special mention. This alkaloid, because of its solubility in sodium hydroxide, formation of azo dyes, and giving of several phenolic colour reactions, seems to be phenolic, but several reactions are difficult to explain on this hypothesis. Diazomethane, acetic and propionic anhydrides, and acetyl chloride with mitragynol all give the same reaction product, possibly the result of isomerisation. Such isomerisation occurs in the hydrolysis and re-esterification of ester alkaloids, e.g., other Mitragyna alkaloids, and Yohimbé and Senecio alkaloids.

Two known degradation products have been re-examined. The base $C_9H_{13}N$ resulting from the selenium degradation of rotundifoline has been shown to be 3:4-diethylpyridine, and the neutral compound $C_{10}H_9NO$ formed in the calcium oxide distillation of rhynchophyllic acid and formerly considered a carbostyril is shown by its spectrum to be an indole. The various known properties of the compound suggest that it may be a 2:3-dimethyl-hydroxy-indole.

From a detailed study of colour reactions and of ultra-violet spectra, it is concluded that the alkaloids are

indolic. If this is so, and if they have the skeleton of any known indolic alkaloid, it is possibly that of the Pseudocinchona, but more probably that of the Yohimbé type.

Since at lower temperatures there was difficulty in determining the number of active hydrogen atoms in the various alkaloids, an investigation was made of the effect of temperature on the determination of active hydrogen atoms. Examination of a range of 38 compounds each at the temperatures: room 50-60°, 100°, and 160° in phenetole showed that at elevated temperatures no abnormal values resulted. Low values were met in fluorene compounds. A smaller range of compounds, where low values had been either reported in the literature or encountered in this investigation, was investigated also in butyl ether and in mixed solvents. In these cases the expected values were obtained, if not at room temperature, then at least at higher temperatures. Although some hydrogen atoms, e.g., the second in an amino group, do become active only at higher temperatures, it is felt that many low values reported are due not to inactivity but either to insolubility of reactant preventing complete reaction, or insolubility of reaction product adsorbing the methane produced. In one case where this could happen, mechanical stirring during the reaction led to the expected value being obtained.

Foreword.

The purpose of this investigation is the examination of the chemistry of the genus Mitragyna and, to a minor extent, of the closely related genus Ourouparia. After a brief discussion of the botany of the plants concerned, it is proposed to deal first with the known chemistry of the Mitragyna and Ourouparia alkaloids, secondly with the isolation of the various products, both alkaloidal and non-alkaloidal, thirdly with the new information obtained and its correlation with that already known. Finally, an attempt will be made to examine possible skeletal structures for the alkaloids, and to indicate possible lines along which future investigation might proceed. Since much of the earlier work was unsystematic and even, in part, erroneous, much of the present work has been concerned with the correction of these errors, and with characterising more fully the various alkaloids of the group.

Introduction.

Botanical Classification.

Three points in the botanical classification call for comment:- first, an apparent confusion between the genera Mitragyna and Mitragyne; secondly, the postulated identity of M.diversifolia with M.rotundifolia; and thirdly, the relationship of the genera Mitragyna and Ourouparia to the various genera known to contain indole alkaloids. In some

of the earliest papers on the Mitragyna alkaloids, e.g. by Miss Field[1] on the alkaloids of M. diversifolia, and of Ing & Raison[2] on the mitragynine from M. speciosa, the genus is called "Mitragyne". Later workers not only describe the alkaloids of various members of the "Mitragyna" genus, but even refer specifically to "Mitragyna" speciosa and "Mitragyna" diversifolia. Now while the genus "Mitragyne" belongs to the Loganiaceae, the genus "Mitragyna" is one of the admittedly closely related, but nevertheless totally distinct Rubiaceae. A search of the Index Kewensis shows that, although none of the species described in the alkaloid papers as "Mitragyne" is listed under that genus, all do appear as members of the genus "Mitragyna". The classification in these earlier chemical papers is therefore incorrect.

Although Miss Field[1] describes the isolation of mitraversine from M. diversifolia, Barger, Dyer & Sargent[3] describe this as identical with M. rotundifolia, which contains rhynchophylline, rotundifoline and two uncharacterized alkaloids. As will be mentioned later, it is not unknown for the nature of the alkaloid in a species to vary with the season of the year and the age and locality of the plant. It would be remarkable, however, if the same species contained one alkaloid at one time and four totally different alkaloids at another. Careful consideration of the properties of

mitraversine shows there is no possibility of it being identical with any of the four alkaloids found in M. rotundifolia. Two possible explanations of the discrepancy remain:- either that there has been a mistake in botanical identification, or that the two species are distinct. Although without specimens of the actual plants concerned one cannot comment on the accuracy of the identifications, on the whole the latter possibility seems the more probable. Since the Index Kewensis lists M. diversifolia and M. rotundifolia as two separate species, it is unlikely that they are in fact the same.

The remaining botanical problem is the relationship of the general Mitragyna and Ourouparia to the various genera containing indolic alkaloids, for, as will be shown later, the Mitragyna and Ourouparia alkaloids are almost certainly indolic. The various genera, their orders, and cohorts are shown in Table 1.

Table 1.

Typical Alkaloid	Genus	Order	Cohort
Rhynchophylline	Mitragyna	Rubiaceae	Rubiales
	Ourouparia	"	"
Yohimbine	Corynanthe	"	"
Cinchonamine	Pseudocinchona	"	"
Strychnine	Strychnos	Loganiaceae	"
Sempervirine*	Gelsemium	"	"
Harmine	Peganum	Rutaceae	Rutales
Rutecarpine	Evodia	"	"

Table 1 (Contd.)

Typical Alkaloid	Genus	Order	Cohort
Calycanthine	Calycanthus	Calycanthaceae	Rosales
Physostigmine	Physostigma	Leguminosae	"
Erythraline	Erythrina	"	"
Ergotamine	Claviceps	a fungus.	

*Gelsemine is perhaps more typical of the Gelsemium alkaloids than is sempervirine. On the other hand, the latter is the only one of these of which the structure is known, and since one is concerned here with possible skeletal formulae for the Mitragyna alkaloids, sempervirine is the most suitable to quote.

Historical.

In this section it is proposed to review the present knowledge of the chemistry of the Mitragyna and Ourouparia alkaloids. The reason for including the alkaloids of the closely related genus Ourouparia (also called Uncaria) is that not only are they very similar to those of the genus Mitragyna, but also that one alkaloid, rhynchophylline, is common to both genera. As far as is known the occurrence of the same alkaloid in different genera is most unusual. On the available evidence it would seem that these alkaloids:- mitragynine, mitragynol, mitraphylline, mitraspecine, mitraversine and

rotundifoline (occurring in the genus Mitragyna), formosanine, gambirine, handamine and isorhynchophylline (in the genus Ourouparia), and rhynchophylline (in both genera) are probably indolic esters each with two nitrogen atoms.

Mitraphylline

Mitraphylline, $C_{20}H_{23}O_3N_2OMe$, which occurs in the bark of M. macrophylla[1,2] and M. stipulosa[6] and in the bark, leaves and fruit of M. rubrostipulesacea (= Adina rubrostipulata[7,8,9]), has been described as melting at 262-3°[5], 258-267°[6] and 270°[10], and having specific rotations varying from 0° to -22°[9,11]. The absorption spectrum shows λ max. at 2841 and 2420 A°[9,12]. A number of salts of mitraphylline - no analyses and few melting points are given - are known[9,10], and many colour reactions also[4,7,12]. The discrepancy in rotation may be due partly to a concentration effect, and partly either to racemisation and/or separation during crystallisation of the l-form and a true racemate. Although the alkaloid from A. rubrostipulata was first thought to be a distinct alkaloid, m.p. 306°[9], Raymond-Hamet[11], by means of analyses, rotations, melting points and mixed melting points showed that "rubradinine" is identical with mitraphylline. The ultra-violet spectrum shows λ max 2820, 2430 Å.

Mitragynine.

Mitragynine, occurring in the leaves of M.speciosa Korth is an amorphous alkaloid which melts at 105-115°, and boils at 230-240°/5 mm.[1,2]. It has been characterised as the picrate[1,2], acetate[1,2,6], trichloracetate[1], cinnamate[1], hydrogen fumarate[1], hydrogen maleate[1], and trinitrobenzene complex[1].

Formula.

Concerning the formula of mitragynine, there is doubt over first the nitrogen, secondly the carbon and hydrogen, and thirdly the methoxyl content.

As the result of a picrate analysis Miss Field[1] gives the formula as $C_{22}H_{31}O_5N$, Ing & Raison[2] basing their conclusion on analyses of six derivatives, suggest $C_{22}H_{30-32}O_4N_2$[2]. A recent analysis in this laboratory gave a result agreeing with the latter. Further support for the second formula is the occurrence of a degradation product $C_{14}H_{14}ON_2$[2]. It would seem therefore that the molecule contains two nitrogen atoms.

Although Ing & Raison suggest $C_{22}H_{30-32}O_4N_2$ as the empirical formula for mitragynine, the analytical figures, as is shown in Table 2, agree equally well with the formula $C_{23}H_{32-4}O_4N_2$.

Table 2.

Compound	Found (average of lit. values)				Calc. for $C_{22}H_{30-32}O_4N_2$				Calc. for $C_{23}H_{32-4}O_4N_2$			
	C	H	N	Misc	C	H	N	Misc.	C	H	N	Misc.
Base	-	-	-	e 399				386 388				400 402
Acetate	64.8	7.3	6.25	-	a64.6 b64.3	7.6 8.0	6.3 6.3	- -	c65.2 d64.95	7.8 8.2	6.1 6.1	- -
Picrate	54.25	5.45	11.1	f35.45	a54.6 b54.45	5.4 5.7	11.4 11.3	37.2 37.1	c55.3 d55.1	5.6 5.9	11.1 11.1	36.4 36.2
Trinitro-benzene deriv.	57.6	5.5	12.2	-	a56.1 b55.9	5.5 5.8	11.7 11.7	- -	c56.8 d56.4	5.7 6.0	11.4 11.4	- -
Cinnam-ate	68.4	7.2	5.05	-	a69.7 b69.4	7.1 7.5	5.2 5.2	- -	c70.0 d69.8	7.3 7.6	5.1 5.1	- -
Meth-iodide	52.3	6.5	5.1	g23.95	a52.3 b52.0	6.25 6.6	5.3 5.3	24.0 24.0	c53.1 d52.9	6.5 6.7	5.2 5.15	23.4 23.3
Hydrogen Fumarate	-	-	5.7	-	-	-	a6.1 b6.0	- -	-	-	5.9 5.9	-

a. for $C_{22}H_{30}O_4N_2$

b. for $C_{22}H_{32}O_4N_2$

c. for $C_{23}H_{32}O_4N_2$

d. for $C_{23}H_{34}O_4N_2$

e. equivalent

f. picric acid

g. iodine.

Ignoring methoxyl values, which will be discussed later, and taking as criterion of incompatibility a difference of 0.5% or greater for carbon, hydrogen, nitrogen and iodine, one may analyse the analytical results statistically as follows:

No. of types, e.g., C, H etc., in which the mean experimental value was:

Nearer that reqd. for C_{22}	Nearer that for C_{23}	Compatible w. that for C_{22}	Compatible w. that for C_{23}
10	8	13	12

Incompatible with that for C_{22}	Incompatible with that for C_{23}
5	6.

Statistically, therefore, the results agree as well with those required for the C_{23} as for the C_{22} formulae.

As far as methoxyl groups are concerned, although, as is shown in Table 3, the experimental values are nearer those required for three methoxyls than for two, nevertheless there is a possibility that only two are present.

Table 3.

A. for $C_{22}H_{30}O_4N_2$

Compound	No. of Detns.	Exptl. Value	Calc. for 2 x OMe	Devn. from Exptl. Values	Calc. for 3 xx OMe	Devn. from Calc. Exp. Vals
Base	1	21.8	15.9	+5.9	23.9	-2.1
Picrate	4	13.9	10.1	3.8	15.1	1.2
Acetate	4	18.0	13.8	4.2	20.8	2.8
Cinnamate	1	15.8	11.6	4.2	17.4	1.6
Mean	-	-	-	4.1	-	2.0

Table 3 (Contd.)

B. for $C_{23}H_{34}O_4N_2$

Compound	No. of Detns.	Exptl. Value	Calc. for 2 x OMe	Devn. from Exptl. Vals.	Calc. for 3 x OMe	Devn. from Exp. Vals.
Base	1	21.8	15.5	6.3	23.3	1.5
Picrate	4	13.9	9.9	4.0	14.8	0.9
Acetate	4	18.0	13.5	4.5	20.2	2.2
Cinnamate	1	15.8	11.3	4.5	16.9	1.1
Mean	-	-	-	4.5	-	1.5

It would seem therefore that Mitragynine contains two nitrogen atoms per molecule, and probably three methoxyl groups. As to whether there are 22 or 23 carbon atoms, one cannot be certain.

Degradation.

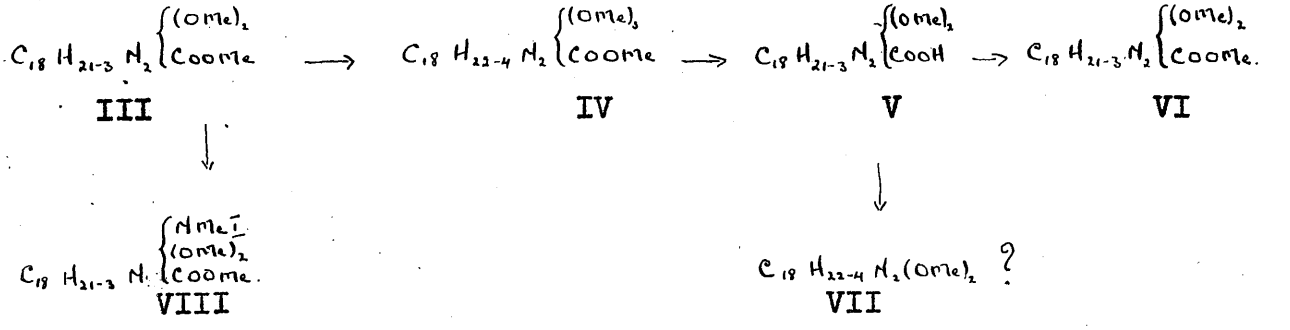
Although Miss Field[1] suggests ethanolic sodium hydroxide hydrolyses mitragynine I to a dibasic acid II, her only analysis of the acid is a methoxyl determination, and

$C_{17}H_{22}N\{(COOMe)_2 OMe \longrightarrow C_{17}H_{22}N\{(COOH)_2 OMe$

I II

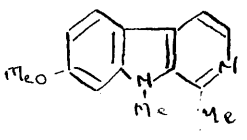
she makes no attempt at reesterification. Ing & Raison[2], on the contrary, hold that ethanolic potassium hydroxide gives two products - a tetra-methoxy compound IV corresponding to the addition of one mole of methanol to one of mitragynine III, and a monobasic acid V. Further heating converts IV, doubtless an intermediate product, to V. Esterification of the acid gives not mitragynine, but an isomer VI. These changes are summarised in Table 4.

Table 4.

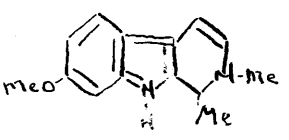


Calcium oxide distillation of V gives a green oil[1], which is possibly the decarboxylation product VII, or a degradation product of it. When refluxed with methyl iodide in acetone, mitragynine gives a monomethiodide VIII[2]. This, together with the result of potentiometric titration[1] and the formulae of the various salts, suggests that mitragynine is monobasic. On selenium dehydrogenation no crystalline product was isolated, but the evolution of dimethyl selenide suggested demethylation.

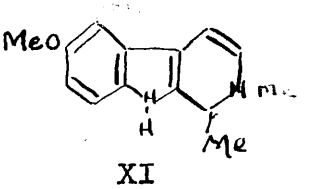
By the zinc dust distillation of mitragynine, Ing & Raison[2] obtained a base, $C_{14}H_{14}ON_2$, containing one N-methyl, one methoxyl, and one active methylene group. Because of the marked fluorescence in acid solution, it was suggested that the compound may be a β-carboline. Ing & Raison found the substance is not identical with 1-or 3-N-methyl harmine IX & X,



IX

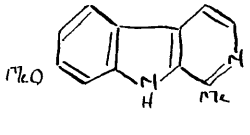


X

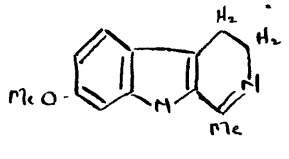


XI

and McCloskey[13] has now found the base not to be the 1-N-methyl-7-methoxy compound, XI. Further similarities of the degradation product with the harmines are the formation of a p-nitrobenzylidene derivative and the melting points of this, the base and the picrate with those of the corresponding harmine derivatives. As already mentioned, the base contains a methoxyl and an N-methyl group, and an active hydrogen atom. Since 3-methylation of β -carbolines gives coloured compounds of quinonoid structure[14], and since the base is colourless, if it is a β -carboline, the N-methyl group is probably in position 3. Since all the known β -carboline alkaloids which contain more than three rings form the fourth at C_2 and N_3, the C-methyl group is probably at position 2 as in the harmine XII and harmaline XIII. The active hydrogen is



XII

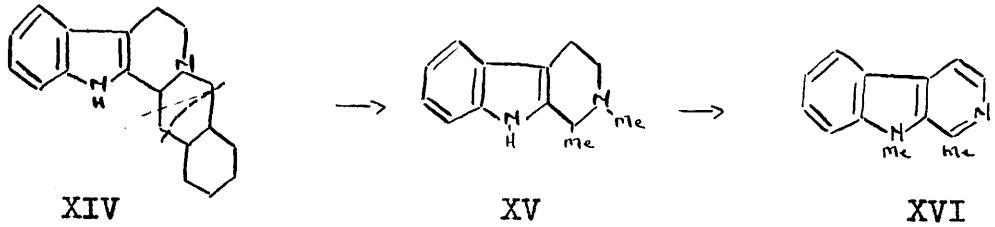


XIII

probably in the C-methyl group. Since the methoxyl group does not occur at C_6 or C_7, it must be in one of the four remaining free positions, i.e., at C_4, C_5, C_6 or C_9. I am deeply indebted to Mr. P. McCloskey, B.Sc. of this laboratory, for a very helpful discussion of the various β -carboline formulae possible for this degradation.

It is doubtful, however, if the base is necessarily a β -carboline. Since the Mitragyna resemble the harmala

alkaloids, the occurrence of β-carboline as a degradation product of a Mitragyna alkaloid would not be surprising. On the other hand if, as is quite likely, mitragynine is built up from tryptophene via the harmala alkaloid structure, one would expect the methoxyl group of the alkaloid, and therefore also of the degradation product, to be in the usual position. As already mentioned, the methoxyl group is not in this position. Again, since as will be shown later, the Mitragyna alkaloids are probably of the Yohimbine (XIV) type, it is hard to see how a 1-methyl compound could arise in degradation. Although methyl migration is possible (XV, XVI), it is by no means probable.



XIV XV XVI

Since the main evidence for the presence of a β-carboline structure is the blue fluorescence - given also by such non-carbolinic alkaloids as strychnine and the ergot alkaloids - it is doubtful if one is justified in assuming the presence of a β-carboline structure.

Mitraversine.

Mitraversine, m.p. 237°, is a phenolic alkaloid occurring in the leaves of M. diversifolia[1]. Although the suggested formula is $C_{22}H_{26}O_4N_2$, as is shown in Table 5,

five other formulae fit the analytical figures at least as closely.

Table 5.

Found		C, 68.4	H, 7.0	N, 7.6	
Calc. for	$C_{21}H_{24}O_4N_2$	68.6	6.6	7.6	1
	$C_{21}H_{26}O_4N_2$	68.2	7.1	7.6	2
	$C_{21}H_{28}O_4N_2$	67.8	7.6	7.5	3
	$C_{22}H_{26}O_4N_2$	69.2	6.9	7.3	4
	$C_{22}H_{28}O_4N_2$	68.8	7.35	7.3	5
	$C_{22}H_{30}O_4N_2$	68.5	7.8	7.2	6

If any reliability may be placed on a single analysis, the most probable formulae would seem to be 2, 3 and 5 in that order. Although similarity in melting point and a possible similarity in empirical formula suggest the identity of mitraversine with mitraspecine (q.v.), the fact that while mitraversine is a phenol, mitraspecine is non-phenolic precludes the identity. As will be discussed later, identity of mitraversine with mitragynol is likewise precluded.

Mitraspecine.

Mitraspecine, m.p. 244-5°, $[\alpha]_D$ = +59.15° (chloroform c = 5.3) occurs in the bark and the wood of M. speciosa. Colour reactions and derivatives are described, although neither melting points nor analytical data are given for these[15]. Although Denis[15] suggests $C_{25}H_{27}O_2N_2(OMe)_3$

as the empirical formula, it is extremely doubtful if his analytical data (C, 68.2, 70.7; H, 7.40, 7.60; N, 6.40, 6.46; OMe, 17.2%) warrant the deduction of any formula. If one accepts the lower figures for C and H, the analytical figures fit moderately well the formulae $C_{20}H_{22}O_2N_2(OMe)_2$ (requires C, 68.8;, H, 7.35; N, 7.3; OMe, 16.2%) or $C_{20}H_{24}O_2N_2(OMe)_2$ (requires C, 68.5; H, 7.8; N, 7.2; OMe, 16.1%

Formosanine.

Formosanine, m.p. 202-218°, $[\alpha]_D$ = +91.3° (in chloroform), and +80.3° (in ethanol) occurs in O. formosana Matsumura and Hayata. The formula is probably $C_{20}H_{21-23}O_3N_2OMe$[16,17]. The absorption spectrum shows λ max. 2830, 2443 Å[12].

Hanadamine.

Hanadamine, m.p. 187°, formula $C_{19}H_{20}ON_2\overset{OH}{C}COOMe$, occurs in O. Kaw-akamii and Hayata[18]. Although, as Raymond-Hamet points out[17], formosanine and handamine are isomeric, there seems never to have been any detailed examination to see if the two alkaloids are not in fact the same.

Iso-rhynchophylline.

Isorhynchophylline, $[\alpha]_D$ = +8.3° (in ethanol c = 1.9), dec.pt. perchlorate, 150°, as an amorphous alkaloid which occurs in O.rhynchophylla and is isomeric with rhynchophylline and identical with the methylation product of

rhynchophyllic acid[19] (vide rhynchophylline, p.16).

Gambirine.

Gambirine occurs in O.gambir[20]. Although the pharmacology is discussed in several papers[20,21,22], there is no record of any empirical formula for any physical or chemical properties of gambirine.

Rotundifoline.

Rotundifoline, m.p. 233-4°, $[\alpha]_D$ = +124° (c = 2.1 in chloroform) has the formula $C_{20}H_{19}O_2N_2\begin{cases}OMe\\COOMe\end{cases}$, and, together with mitragynol, rhynchophylline and an uncharacterised alkaloid, occurs in the leaves of M.rotundifolia[3]. Two significant colour reactions are a garnet-red colour with ferric chloride in non-alcoholic solvents, and a deep orange with sodium nitrite in acetic acid. No semicarbazide is formed, and, despite the indication of an enolic group given by ferric chloride, no acetyl derivative has been obtained. When refluxed with alcoholic potash, rotundifoline (XVII) hydrolyses to rotundifolic acid (XVIII), which, when distilled from calcium oxide, decarboxylates to the base $C_{20}H_{24}O_3N_2$ (XIX). These changes are summarised in Table 6.

Table 6.

$C_{20}H_{23}O_3N_2COOMe$	⟶	$C_{20}H_{23}O_3N_2COOH$	⟶	$C_{20}H_{23}O_3N_2H$
XVII		XVIII		XIX

With rotundifoline 30% sulphuric acid eliminates

carbon dioxide to give a product similar to that obtained by the action of the acid on rhynchophylline. Selenium dehydrogenation gives a mixture of bases including one, $C_9H_{13}N$, now identified as 3:4-diethylpyridine, and several neutral substances containing selenium. Degradation of rotundifoline with soda-lime gives a mixture of indoles, ammonia and pyridine-like bases.

Mitragynol.

Mitragynol has been described by Badger as a phenolic alkaloid, m.p. c.125°, empirical formula $C_{21}H_{26}O_5N_2$ [23], and occurs in the leaves of M.rotundifolia.

Rhynchophylline.

Rhynchophylline occurs in the bark of M.inermis [24] and M.stipulosa [25], and in the leaves of M.rotundifolia [3], and in O.rhynchophylla [19,26,27]. Raymond-Hamet [28] suggests rhynchophylline is identical with crossoptine, which occurs in Crossopteryx kotschyana Fenzl [29]. The formula is either $C_{19}H_{21}N_2$(OMe, OH, COOMe) [24,25] or $C_{19}H_{17\text{-}19}N_2$(OMe, OH, COOMe) [19]. Rhynchophylline forms a crystalline chloroaurate and chloroplatinate [26]. When an attempt is made to acetylate the crude base isorhynchophylline (XX) a mixture of rhynchophylline and acetyl rhynchophylline (XXI) is obtained [19]. Treatment with alcoholic potash hydrolyses rhynchophylline (XXIV) to rhynchophyllic acid (XXII), which on calcium oxide distillation gives a

product $C_{10}H_9NO$, formerly thought to be a methyl carbostyril[3], but is now shown to be probably a hydroxy-2:3-dimethylindole (XXIII). Methylation of rhynchophyllic acid gives not rhynchophylline, but the naturally occurring isomer isorhynchophylline (XXIV). These changes are summarised in Table 7.

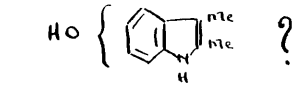
Table 7.

$C_{20}H_{24}ON_2$ {OH, COOMe} (XX) ⟷ $C_{20}H_{24}ON_2$ {OH, COOH} (XXII) ⟷ $C_{20}H_{24}ON_2$ {OH, COOMe} (XXIV)

XX ↓

$C_{20}H_{24}ON_2$ {OCOMe, COOMe} (XXI)

XXII ↓



(XXIII)

Degradation of rhynchophylline with soda-lime gives a mixture of oxygenated indoles, ammonia and a pyridine-like base of probable formula $C_8H_{9-11}NO$[3]. With 30% sulphuric acid, rhynchophylline splits off carbon dioxide to give a substance which "in its insolubility resembles that formed by the action of acids on mitragynine"[3]. While ethereal ferric chloride gives no colour with rhynchophylline, an excess of sodium nitrite in acetic acid gives an oily nitroso derivative which, in turn, gives a positive Liebermann reaction[3]. The ultra-violet spectrum shows λ_{max} 2798, 2447 Å[12].

Formerly the alkaloids from O.rhynchophylla, on the one hand, and M.inermis and M.stipulosa on the other, were considered to be different alkaloids called rhynchophylline

and mitrinermine respectively. More conclusive evidence of identity is that of Barger, Dyer & Sargent[3], who in 1939 showed that the rhynchophylline occurring in the leaves of M.rotundifolia had approximately the same melting point as both mitrinermine from M.inermis or M.stipulosa and rhynchophylline from O.rhynchophylla, and gave no depression on mixing with either specimen. Nevertheless, Millat[31] has recently stated that, because of alleged differences in empirical formula, melting points, rotations, and colour reactions, mitrinermine and rhynchophylline are distinct. As will be shown later, however, all doubt about the identity has now been removed.

Occurrence of Alkaloids in the Various Species of Mitragyna and Ourouparia.

These may be summarised as follows in Table 8.

Table 8.

Species	Part of Plant	Alkaloids	Ref.
M.diversifolia	leaves	mitraversine	1
M.inermis	bark	rhynchophylline	24
M.macrophylla	bark	mitraphylline	4,5
M.rotundifolia	leaves	mitragynol, rhynchophylline, rotundifoline	3
M.rubrostipulacea	bark, leaves, fruit, wood	mitraphylline	7,8,9
M.speciosa	bark & wood	mitraspecine	15
	leaves	mitragynine	1,2
M.stipulosa	bark	mitraphylline	6
		rhynchophylline	25
O.formosana		formosanine	16,17

Table 8 (Contd.)

Species	Part of Plant	Alkaloids	Ref.
O.gambir		gambirine	20
O,kawakamii		handamine	18
O.rhynchophylla		rhynchophylline	19,26,27
		isorhynchophylline	19.

Discussion.

Isolation and Identification of Material.

Quinovic Acid.

The plant materials remaining after extraction of the alkaloids from the bark of M.inermis, and the bark and leaves of M.ciliata and M.rubrostipulacea were further extracted with 10% sodium carbonate solution. Addition of concentrated hydrochloric acid to these extracts precipitated in every case quinovic acid, identified as the acid itself and as its acetate and methyl ester. The individual physical constants obtained are given in Table 21, p. 100.

Only one result among these calls for comment. Two specimens separately extracted from the bark of M.rubrostipulacea had specific rotations not of 87° but of 43° and 44°. Three explanations are possible:-

1. Racemisation of the acid during extraction and/or purification. Since no specimen of acid from any of the other plant materials examined showed any tendency to racemise, this explanation is unlikely.

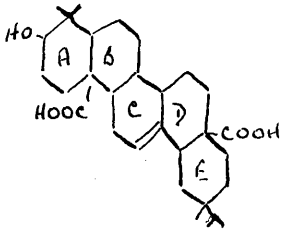
2. Occurrence of an impurity of high negative rotation. Although this is more likely, it is hard to reconcile the presence of sufficient impurity to halve the rotation with the obtaining of a correct melting point.

3. The occurrence of a certain amount of d and/or dl-acid. This seems the most probable explanation.

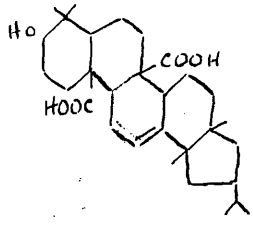
Nature of Quinovic Acid.

Quinovic (chinovic) acid is a triterpene acid which occurs in the Cinchona bark as the glucoside quinovine[32], (The genera Cinchona and Mitragyna both belong to the natural order Rubiaceae) and in the unrelated Egyptian plant Zygophyllum coccineum[33]. Quinovic acid is a monohydroxy dibasic acid with one double bond which cannot be hydrogenated over Adams' catalyst. As the result of a long series of degradations by Wieland, Ruzicka and their co-workers, not only of quinovic acid but of such derived substances as novic and pyroquinovic acids, the structure has been almost completely elucidated. This work has been reviewed by Schmitt & Wieland[34].

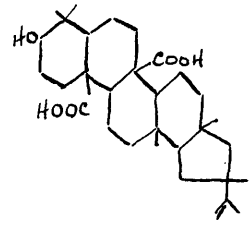
The one point now in dispute is whether the ring[5] is five- or six-membered. While Schmitt & Wieland[34] suggest that ring E is six-membered and that the formula is as in XXV, Ruzicka & Anner[35] suggest a five-membered ring and the structure XXVI or XXVII. Two reasons are given in support of



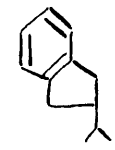
XXV



XXVI

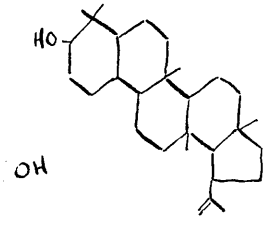


XXVII



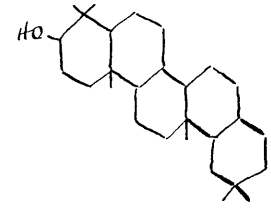
XXVIII

structure XXVI or XXVII. First, pyrolysis of quinovic acid gives as product, XXVIII, which is held to come from rings D and E. Secondly, while XXVI and XXVII can be split into isoprene units, XXV cannot. On the other hand, ring E might easily have changed from a six-membered to a five-membered ring on pyrolysis and, as Noller points out, although all the triterpene acids of known structure fit one of the three types, lupeol (XXIX), or α - (XXX) or β -amyrin (XXXI), quinovic acid may well be in a class by itself[36]. It would seem that at present a considerable amount of evidence



XXIX

unknown constitution



XXXI

XXX

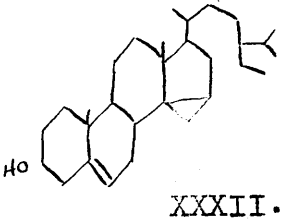
might be advanced in support of either structure. The ultimate solution of the problem may well come through crystallographic examination.

β-Sitosterol.

Through the courtesy of Dr. Raymond-Hamet I was able to examine a substance extracted from the bark of M.inermis and thought to be a sterol. After recrystallisation from methanol the substance melted at 134-5°, and had a specific rotation, $[\alpha]_D$ = -35.6° (c = 1.5 in chloroform). Methanolic and chloroform solutions gave typical sterol colour reactions. Examination of the physical constants of the substance and of its acetate and 3:5-dinitrobenzoate showed it to be β-sitosterol. The constants obtained are compared with those of β-sitosterol in Table 22, p.102.

Nature of β-Sitosterol.

β-Sitosterol (XXXII) occurs in the oils of cotton seed[37],



XXXII.

Calycanthus[38], and wheat germ[39,40] and in tall oil[41], and crepe rubber[42], and apparently in rye germ[43] and corn[39,40] oils. Most significant, however, is the occurrence of it in Cinchona bark or wax[44-48]. Thus quinoric acid and β-sitosterol occur in both the Cinchona and the Mitragyna species.

Alkaloids.

Apparatus.

The apparatus used for the extraction of alkaloids and shown in Fig.1 was adapted from that of Wester[49]. The main modifications were:-

1. Placing the distillation flask directly under the extraction vessel. This gave a more compact apparatus.
2. Making the siphon from three pieces of glass tubing instead of from one. The two advantages of this were the less risk of breaking the siphon tube, and the possibility, if the extract is reluctant to siphon, of starting siphoning by manipulation of the top of the siphon tube.
3. Making the top elbow of the siphon as sharp as possible. By reducing the horizontal cross-section at the head of the siphon tube, this aids siphoning.
4. Drawing out the already narrower siphon tube to a fairly small jet. By reducing the area on which back pressure is exerted, this also aids siphoning.

Methods.

Various extraction methods were first tried with the bark of *M. inermis*. The method showing greatest efficiency, utility and economy was to mix the moistened ground plant material with lime, air dry, and then to extract with chloroform in the modified Soxhlet apparatus.

Difficulty was experienced in working up the mitraphylline extracts. With 2% hydrochloric acid, the concentrated chloroform extracts gave emulsions which could not be broken by any of the usual methods, e.g., addition of sodium chloride, water, or chloroform: evaporation to dryness left a very hard residue which could not be ground. Ultimately it was found that the best method was to evaporate the extract almost to dryness, mix the resulting paste with sand that had previously been well washed with dilute hydrochloric acid, and then grind the mixture with successive quantities of 2% hydrochloric acid.

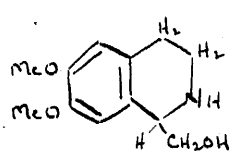
Concerning the occurrence of particular alkaloids, several points are noteworthy.

Variability of Alkaloid Content of the Same Material.

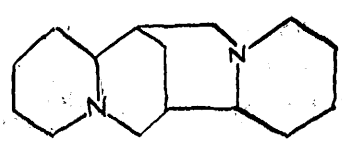
Although Raymond-Hamet & Millat[24] describe the yield of rhynchophylline from M.inermis bark as 0.23%, I found only 0.003%. While the bark used by Raymond-Hamet & Millat came from French Equatorial Africa, that used by me came from Nigeria. Again in this present investigation, while the first batch of leaves of M.rubrostipulacea contained 0.5% mitraphylline, the second batch gathered probably six months later, contained only 0.05%.

Variability of alkaloid content is by no means uncommon, and may be due to several factors. If the alkaloid

is manufactured mainly at a definite time of the year and in a definite part of the plant, and migrates from there to various other parts, obviously the alkaloid content of each part will vary considerably according to the season of the year. This seasonal variation possibly accounts for the use as fodder of such alkaloid-rich plants as Lupinus angustifolius L. ("blue lupin"). In the case of calycotomine, XXXIII, White[50] has found that the alkaloid content of the plant varies from day to day. In 1944 White[51] described experiments on the s parteine XXXIV content of broom stems (Cytisus scoparius Link). He worked worked on the same three patches of broom in Wellington, New



XXXIII



XXXIV

Zealand, about three miles apart. His figures, quoted in Table 9, show clearly the differences not only between old and young plants and between the same plant at different seasons of the year, but even between patches growing only about two miles apart.

Henry[52] cites work showing how the amount and nature of alkaloid in Duboisia myoporoides in Australia varies with both the season of the year and the age and geographical distribution of the plant.

Table 9.

	Oct.	Mar.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Lot 1 old	0.85	0.60	0.50	0.50	0.49	0.44	0.37	0.51	0.56	0.57	0.58	0.52
young	-	0.17	0.31	0.33	0.54	0.70	0.89	0.89	0.91	0.97	1.02	1.12
Lot 2 old	1.30	1.13	0.37	0.43	0.90	0.66	0.55	1.00	0.71	0.72	0.72	1.22
young	-	0.29	0.70	0.70	0.97	1.21	1.54	1.56	1.46	1.44	1.35	1.44
Lot 3 old	1.80	1.06	1.27	0.81	0.63	0.78	0.73	0.66	0.97	0.71	0.59	0.37
young	-	0.33	0.81	0.89	1.04	1.24	1.52	1.07	1.44	1.35	1.40	1.44

So far, variations in alkaloid content with variations in geographical locality of the plant, season of the year and age of the plant have been discussed.

The fourth type of variation is due to loss on storage. According to Aston[53], if the bark of the puketea (Laurelia Novae Zealandiae) "was allowed to remain in a dry room some months before extraction the yield (of puketaine) was very small".

The variations in alkaloid content of different specimens of the same species of Mitragyna are by no means uncommon and may be due to either a variation in the age of the plants or the season in which they were gathered, or loss of alkaloid on storage. Since all specimens concerned came from more or less the same locality, geographical considerations are unlikely to have influenced the alkaloid content. With regard to these factors, James[54] goes so far as to say that unless the season of the year as well as the locality

of the plant source is recorded, figures dealing with the amount of alkaloid in a given part of a plant are meaningless.

The Alkaloids of M.ciliata.

While the leaves of the hitherto unexamined M.ciliata were found to contain 0.15% rotundifoline by dry weight, the bark was found to contain 0.02% rhynchophylline.

Although the occurrence of two different alkaloids in two different parts of the same plant is unusual, it is by no means unknown. Examples are given in Table 10. Since sparteine (XXXIV) and calycotomine (XXXIII) are totally dissimilar, the case of Cytisus proliferus is particularly interesting.

Table 10.

Species of Cytisus	Alkaloid in Seeds	Alkaloid in Top Shoots	Reference
linifolius	cytisine	anagyrine	54
monspessulanus	cytisine	methyl cytisine	55
proliferus	sparteine	calycotomine	50

There is, of course, the further case of M.speciosa with mitraspecine in the bark and wood[15] and mitragynine in the leaves[1,2].

d- and l-Mitraphylline.

Although, as is shown in Table 11, the recorded values for the specific rotation of mitraphylline vary

considerably, nobody has hitherto reported the d-form now isolated from the bark of M.rubrostipulacea. The alkaloid in the leaf, however, was found to be the usual l-form.

Table 11.

Species	Part of Plant	in chloroform	Reference
M.macrophylla	bark	0^{o}	5
M.stipulosa	bark	-7.7	6
M.rubrostipulacea	doubtful	-22.7	9
M.rubrostipulacea	bark	-9.8	11

Although variation in values of specific rotation in different species might be due to the occurrence of varying amounts of d- and d-l- or r-form in the different species, and although even variation in the same species might be attributed also to varying degrees of racemisation, the change in sign of the alkaloid now found in the bark as compared both with that in the leaves and with that found by other workers in the bark, means that both the d- and the l-forms must exist in the plant.

Occurrence of two optically active forms of a natural product is not unknown. Examples among the alkaloids include d and dl-laudanine and -laudanidine, l- and dl-hyoscine, and d- and l-N-methylconiine, and among non-alkaloidal products, d- and l-phellandrene, -limonene, -menthone, -camphor, -borneol, and -fenchone, and d-, l- and dl-carvone. More

unusual, however, is the occurrence of the two forms in the same plant, but examples are not lacking, e.g., d-, l- and dl-lupinine in Lupinus angustifolius[56], l- and dl-lupanine in Podalaria buxifolia[57], d- and dl-lupanine in P.sericea[58], and "the predominantly active form in green parts, and more or less dl- in seeds of Lupinus hartwegii and Podalaria calyptrata.

Properties of Individual Alkaloids.

In this section it is proposed to deal especially with the factual results of the investigation. Although certain theoretical aspects peculiar to individual alkaloids will also be discussed here, aspects common to several alkaloids will be discussed in the next section.

Mitraphylline.

On analysis mitraphylline was found one methoxyl group (in agreement with the results of Raymond-Hamet & Millat[6], Devis[10], and Raymond-Hamet[11]), and four active hydrogen groups, but no N-methyl group. Potentiometric titration showed that mitraphylline is a monoacidic base with p^{K_B} = 9.66. It is thus a fairly weak base, of strength intermediate between that of aniline, p^{K_B} = 9.3, and chloraniline, p^{K_B} = 10.3. The monoacidity is in agreement with the formation - as shown by analysis - of a monopicrate. As already mentioned,

although the alkaloid extracted from the leaves was laevo-rotatory in chloroform ($[\alpha]_D = -7.4^\circ$), that from the bark was dextro-rotatory ($[\alpha]_D = +7.1^\circ$). A solution of the base in 2N hydrochloric acid was dextro-rotatory - $[\alpha]_D = +26.7^\circ$ (c = 2.70), 21.5° (c = 1.35). The ultra-violet spectrum, almost identical with that found by Raymond-Hamet[12] for mitraphylline, was similar to those of both the other Mitragyna and Ourouparia alkaloids, and yohimbine and related alkaloids (cf. Pruckner & Witkop[59]). Colour reactions, summarised in Table 1, pp.65-66 and discussed on pp.63-72, indicated the presence of an indole nucleus, a double bond, and an imino group, but were negative for a methylene dioxy or enolic group.

Derivatives.

Attempts to prepare various derivatives of mitraphylline were mainly abortive. There was no reaction with acetic anhydride, acetyl chloride, 3:5-dinitrobenzoyl chloride, 2:4-dinitrophenylhydrazine, hydroxylamine hydrochloride, diazomethane or a weakly acid solution of diazotised sulphanilic acid. No colour change was observed on refluxing mitraphylline in methanol with freshly distilled benzaldehyde. Although, because of the small quantities used, mitraphylline could not be recrystallised from the reaction mixture, it is so extremely rare for a benzylidene derivative to be colourless

that one may say no such derivative was formed.

While hydrogenation over palladium gave an uptake of hydrogen equivalent to one double bond, with Adams' catalyst a hexahydro derivative, m.p. 252-3°, was formed. Bromine in chloroform gave a dibromo polybromide which on refluxing with acetone gave dibromomitraphylline, m.p. 195-8°, chloroplatinate dec.pt. 230°. Methyl iodide gave an amorphous methiodide, m.p. 85-7°.

Degradation Reactions.

Hydrolysis of mitraphylline with 4N methanolic potassium hydroxide gave an amorphous amphoteric acid, m.p. 173°. Re-esterification with ethereal diazomethane gave an amorphous base, m.p. 152°. Since all attempts to recrystallise this failed, it was purified by repeated solution in dilute hydrochloric acid and precipitation with concentrated ammonia. While the ultra-violet spectrum of the acid showed λ_{max} 2800, 2390 Å, that of the methyl ester showed λ_{max} 2740, 2410 Å. It would seem therefore that the esterification product of mitraphyllic acid is different from mitraphylline. Since neither mitraphyllic acid nor its methyl ester could be crystallised, it was felt that they were not sufficiently pure for analysis. When the amorphous rhynchophyllic acid was analysed, for example, the carbon values were 0.9 and 1.6% low[3]. One cannot therefore be sure that

mitraphylline is a methyl ester. Since, however, alkaloids that are esters of a simple alcohol are always methyl esters, it is probably safe to assume that mitraphylline is a methyl ester. The formation of an isomer on re-esterification of an acid obtained by hydrolysis of an alkaloid is typical not only of the Mitragyna alkaloids, c.f., mitragynine[2] and rhynchophylline[19], but also of the Yohimbé alkaloids.

Caustic potash fusion of mitraphylline gave no identifiable products.

Mitragynine.

Because of the two postulated formulae, $C_{22}H_{31}O_5NO$ and $C_{22}H_{30-32}O_4N_2$[2] for mitragynine, a nitrogen determination was done on a specimen of mitragynine picrate. The result supported the formula $C_{22}H_{30-32}O_4N_2$. By analogy with the other Mitragyna and Ourouparia alkaloids, one would expect this formula rather than the other to be correct. Zerewitinoff estimation showed the presence of three active hydrogen atoms. In chloroform mitragynine was dextro-rotatory. $[\alpha]_D$ = 49.8° (c = 1.50), and 58.7° (c = 0.75). Although the ultra-violet spectrum of mitragynine acetate was typical (λ_{max} 2900, 2480 Å) of these alkaloids and their salts, that of the free base (λ_{max} 3555, 2910 Å) was abnormal. While the appearance of a peak at 3555 Å may be linked with the appearance of colour in the free base, the disappearance of the typical lower peak of the alkaloids is less easily explained. The

colour reactions were similar to those given by mitraphylline and are described in detail on p. 129.

Derivatives.

Although mitragynine is described as failing to take up hydrogen in acetic acid over either platinum oxide or palladium, a chloroform solution of the base decolourised a chloroform solution of bromine acid without the evolution of fumes of hydrogen bromide. This might, however, be due to perbromide formation, c.f. mitraphylline. It is significant that the Ipatieff test for unsaturation was negative[60]. There was no reaction with 2:4-dinitrophenylhydrazine, diazomethane or a weakly acid solution of diazotised sulphanilic acid.

Neither selenium dehydrogenation (cf. Ing & Raison[2]), nor potash fusion gave any isolable products.

Mitraversine.

Mitraversine showed specific rotations of +37° (c=2.00) and 38° (c = 1.00) in chloroform and +2.7° (c = 1.00) in 2N hydrochloric acid. The ultra-violet spectrum (λ_{max} 2885, 2400 Å) is of the usual type for Mitragyna alkaloids.

Since it has been suggested that M.diversifolia, from the leaves of which mitraversine has been extracted, is identical with M.rotundifolia, from the leaves of which mitragynol, rhynchophylline, rotundifoline and another non-phenolic amorphous alkaloid have been extracted, it is necessary to

consider if mitraversine might be identical with any of these. Examination of the data listed in Table 12 shows this to be impossible.

Table 12.

Alkaloid	Mitraversine	Mitragynol	Rhynchophylline	Rotundifoline.
m.p.	237°	125-130°	213-215°	232-233°
$[\alpha]_D$ (in $CHCl_3$; c=2.1)	+37°	-23 to -25°	+124°	-7.6°
$[\alpha]_D$ (in 2N HCl; c=2.1)	+2.7°	+3.0°	+70.6° (c=2.5)	+42.9° (c=3.8)
NaOH	soluble	soluble	insoluble	insoluble
Formula (Most probable)	$C_{21}H_{26-8}O_4N_2$	$C_{22}H_{28}O_5N_2$	$C_{22}H_{26-8}O_4N_2$	$C_{22}H_{28}O_5N_2$

Formosanine.

Formosanine was found to have specific rotations +90.0° (c = 2.00) and +90.7° (c = 1.00) in chloroform, and in 2N hydrochloric acid +25.3° (c = 1.00) and +26.7° (c=0.50). Colour reactions (described in detail on p.134) were positive for an indole nucleus and an imino-group, and a double bond, but negative for enolic and methylenedioxy groups. There was no reaction with either diazomethane or 2:4-dinitrophenylhydrazine. Over Adams' catalyst in acetic acid, one equivalent of hydrogen was taken up very slowly.

Rotundifoline.

On analysis rotundifoline was found to contain two methoxyl groups (in agreement with the result of Barger, Dyer & Sargent[3]), and three active hydrogen atoms, but no

N-methyl groups. The formula of the chloroplatinate, which decomposed at 232-4°, shows that rotundifoline is a monoacidic base. Determinations of specific rotation in chloroform at several concentrations showed there was little change in specific rotation with concentration. In 2N hydrochloric acid the specific rotation was found to be $[\alpha]_D$ = 60.9° (c = 2.48). The ultra-violet spectra in methanol (λ_{max} 2925 Å, λ inflexion 2390 Å) and in very dilute hydrochloric acid (λ_{max} 2900, 2470 Å) were typical of the Mitragyna alkaloids. Except that a positive enolic test was obtained with ethereal ferric chloride, the colour tests, described on p. 137, were similar to those given by mitraphylline. Although there was no absorption of hydrogen over palladium in acetic acid, over Adams' catalyst, one mole was taken up to give dihydrorotundifoline, m.p. 233°; $[\alpha]_D$ = 123.7° (c = 2.4 in chloroform). That the specific rotation of dihydrorotundifoline and rotundifoline are, within experimental error, identical, indicates that the double bond reduced was unconjugated. Dihydrorotundifoline will be discussed later in connection with the product formed by the action of diazomethane on mitragynol. Rotundifoline failed to react with benzaldehyde and diazomethane, and with reagents for keto and hydroxyl groups, c.f. the failure of Barger, Dyer and Sargent[3] to obtain either a semicarbazone or a definite acylation product. The base $C_9H_{13}N$ obtained by Barger,

Dyer and Sargent by selenium dehydrogenation of rotundifoline has now been identified as 3:4-diethyl pyridine. As will be seen later, this identification is important in the examination of possible skeletal structures of the Mitragyna alkaloids.

Mitragynol.

Like rotundifoline, mitragynol contains an imino but no N-methyl group, and fails to react with benzaldehyde. The colour reactions of the base, and the ultra-violet spectra of both base and hydrochloride are typical of those of the Mitragyna alkaloids. Unlike rotundifoline, however, mitragynol gives neither a dibromo nor a dihydro derivative and no Ipatieff test for unsaturation. There are present four active hydrogen atoms per molecule. Specific rotations, given in detail on p.146, vary not only with concentration but also, for those in acid solution, with the nature and concentration of the acid. Solutions in 2N hydrochloric acid and 2N potassium hydroxide showed no sign of mutarotation over a period of 24 hours. Although fairly unusual, this behaviour is by no means unknown[61].

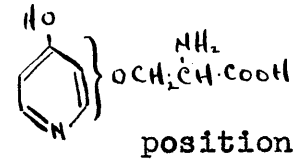
Phenolic Nature of Mitragynol.

As to whether mitragynol is phenolic, the evidence is conflicting. Mitragynol not only is soluble in sodium hydroxide, but insoluble in sodium carbonate and ammonium hydroxide, but also gives several characteristic phenol colour

tests with ethereal and alcoholic ferric chloride, Millon's reagent and phthalic anhydride, and forms azo-dyes with diazotised aniline and p-toluidine in alkaline solution. It is realised, of course, that the reaction with ferric chloride might, as with rotundifoline for example, be due to an enolic group and that the solubility properties could be those of a somewhat inert carboxyl, or very acidic imino group. Again, certain substances other than phenols will couple with diazotised aniline and toluidines. The implication to be drawn from the cumulative evidence, however, is that mitragynol is phenolic.

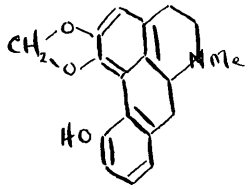
Contra-indicating the phenolic nature of the alkaloid is the peculiar product of the action of diazomethane, acetic and propionic anhydrides and acetyl chloride. Since compound "A", prepared by the action of diazomethane has the same melting point (no depression on mixing), specific rotation and ultra-violet spectrum as has compound "B" prepared by the action of acetic anhydride, it would seem that "A" and "B" are identical. (Results of mixed melting point determinations in accord with the identity of "B" with the products from acetyl chloride and propionic anhydride.) The only explanation of the formation of "A" and "B" (called "A" in future discussion) is that in each case there has been an intramolecular rearrangement. One would expect a phenolic group to be methylated or acetylated under the conditions. Although

in this respect some phenolic groups are inactive, e.g., leucenol (XXXV) cannot be acetylated[62], and puketaine (XXXVI)



position of side chain uncertain

XXXV



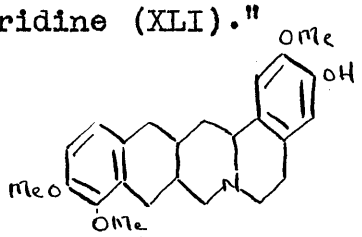
XXXVI

does not react with diazomethane[63], there is a considerable difference between non-activity and the type of reactivity shown by mitragynol.

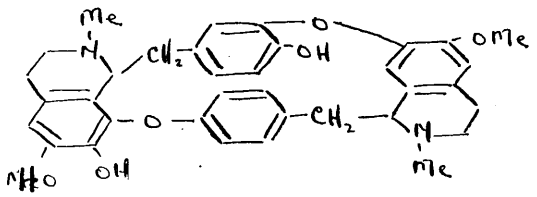
Some comment is necessary on the phenolic colour reactions given.

With Millon's reagent, mitragynol gave a yellow colour. With monohydric phenols having at least one free ortho-position the reagent is said to give a red colour[64]. King[65] discusses fully what types of phenols will give a positive test: "A positive Millon test is given by phenols with one other substituent, e.g. guaiacol and the cresols." On the introduction of another substituent the reaction becomes more specific, for it is not given by thymol, carvacrol, α-naphthol, or isovanillic acid, which have at least one free position but in which the para-position is occupied. When there are three substituents besides the phenolic group, the reaction is not given in the case of corybulbine (XXXVII). A positive reaction is given however by the benzylisoquinoline alkaloids bebeerine (XXXVIII) and tubocurarine chloride

(XXXIX) and by protocuridine (XL), but not by neoprotocuridine (XLI)."

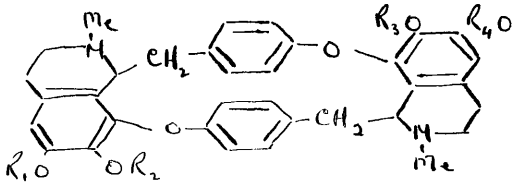


XXXVII



XXXVIII

XXXIX is a diastereoisomer of XXXVIII.



XL - one OH at OR_2 or OR_3 position of other unknown.

XLI - $R_1 = R_4 = H$; $R_2 = R_3 = Me$

Although mitragynol does not give the classical red colour, it does give a definite yellow. It seems probable, from this evidence, that mitragynol has a phenolic group with at least one free ortho-position.

With concentrated sulphuric acid followed by sodium nitrite, mitragynol gives a vivid red colour tinged with purple. Pouring the mixture into five times the volume of water destroys the colour. Under these conditions phenol is said to give a blue colour, which, when the mixture is poured into water, becomes purplish red. Although imino groups react with nitrous acid to give coloured products, the colours are not as vivid as those given by phenol[64]. It is extremely unlikely, therefore, that the colour given by mitragynol is due merely to an imino group.

When mitragynol was fused with phthalic anhydride and anhydrous zinc chloride and sodium hydroxide solution then

added, a yellow-green colour developed. Under these conditions, although most phenols give purple, red or green colours, para-substituted phenols do not give this test[66].

Potentiometric Titration of Mitragynol.

Potentiometric titration showed that mitragynol was monobasic and monoacidic. As an acid mitragynol is more acidic than is normal for phenols, but weaker than most carboxylic acids. As a base it is much weaker even than aniline.

Existence of Dihydrorotundifoline and its Identity with Compound "A".

The problem of the formation of dihydrorotundifoline is linked with the identity of compound "A" described earlier. While the evidence is very strong that "A" is identical with the alleged reduction product of rotundifoline, the more difficult problem is whether this product is really not unchanged rotundifoline. At first sight there seems to be considerable evidence for non-reduction. This evidence is based on the similarity of melting points of both bases and chloroplatinates and the absence of depressions of mixed melting points, the specific rotations, the ultra-violet spectra both in methanol and in very dilute hydrochloric acid, and numerous colour reactions.

Much may be said to nullify this evidence. Similarity of melting and decomposition points of closely related alkaloids

and the absence of depressions of mixed melting points is not unknown. An example is the case of solasonine and solauricine[67], where over a range of the two bases and twelve derivatives of each there is only one significant difference in a decomposition point, none in melting points, and no depressions whatsoever. Disinomenine and ψ-disinomenine[68] also show similarity of melting points of bases and derivatives - one significant difference among six pairs of constants. Similarity of rotation may quite well be due to the reduction of an isolated double bond[69]. Similarity of spectra is likewise no proof of identity. Strychnine and dihydrostrychnine have identical spectra, as do strychninolone and dihydrostruchninolone[70]. A third example is the case of cinchonamine and dihydrocinchonamine[71]. With regard to colour reactions, one may quote the case of corynanthine and yohimbine giving identical colours with a series of reagents[72].

On the other hand, much evidence has been found for the existence of dihydrorotundifoline and its identity with compound "A". On quantitative hydrogenation, rotundifoline absorbed one molecular equivalent of hydrogen. Since the attempted reduction of mitragynol was done in the same batch of solvent and with the same batch of catalyst as was used in the successful hydrogenation of rotundifoline, it would seem as if the catalyst was active and the solvent pure. Under similar conditions "A" likewise showed no uptake of

hydrogen. Similarly the Ipatieff test was positive for rotundifoline, but negative for mitragynol and compound A. Although with bromine in chloroform, rotundifoline forms the dibromide, both dihydrorotundifoline and "A" failed to brominate. Again although the observation of Barger, Dyer & Sargent[3] that rotundifoline gives a garnet red colour with ethereal ferric chloride, it was found that neither "A" nor dihydrorotundifoline gave any colour. Similarly antimony trichloride in dry ether gave with rotundifoline a flocculent precipitate coagulating on standing, but with dihydrorotundifoline and "A", only a transient haziness.

It might be suggested that an impurity present in the rotundifoline and removed in the purification of the alleged dihydrorotundifoline and non-existent in mitragynol and therefore in "A" would account for the differences. Although this might well account for the differences in colour and precipitation reactions, an impurity, even highly unsaturated, would be unlikely to occur in sufficient amount to give high yields of mixtures analysing (after purification) as the dihydro- and dibromo-compounds, and in just such an amount as to give an uptake of hydrogen equivalent to one mole per mole of rotundifoline. That the rotundifoline from M.ciliata has the same properties as that from M.rotundifolia indicates that it is extremely unlikely that even the colour reactions, much less the reactions of the double bond, may be

attributed to impurity. It is obvious therefore that "A" is dihydrorotundifoline and not rotundifoline itself. The relevant experimental data is summarised in Table 13.

Table 13.

	Rotundifoline from M. rotundifolia	Rotundifoline from M. ciliata	Dihydrorotundifoline	Compound A
Points of Similarity of Dihydrorotundifoline and "A" with rotundifoline				
m.p.	233-4°	233°	233°	233°
($CHCl_3$ c=2)	122.4°	123.3°	123.7°	122.6°
Ultra-violet spectrum in EtOH				
λ max	2925Å (log ε=3.47)	-	-	2915Å (log ε =3.46)
λ min	2770Å (log ε =3.29)	-	-	2755Å (log ε =3.30)
λ inflexion	2390 Å (log ε =4.23)	-	-	2395 Å (log ε =4.14)
Ultra-violet spectrum in very dilute HCl				
λ max	2900 Å (log ε = 3.54)	-	-	2900 Å (log ε_o=3.46)
	2470 Å (log ε = 4.25)	-	-	2450 Å (log ε_o=4.09)
λ min	2760 Å (log ε = 3.38)	-	-	2760 Å (log ε =3.39)
$B_2H_2PtCl_6$ dec.pt.	232-4°	-	229-30°	224-6°
Methoxyl content	2	2	2	2
Colour reactns.	Identical			As for rotundifoline w. the exceptns. given below.
Points of Differentiation.				
Crystalline form	Prisms	Prisms	Needles	Needles
Ipatieff Test	Permanganate decolourised	-	No reaction	No reaction

Table 13 (Contd.)

	Rotundifoline from M. rotundifolia	Rotundifoline from M.ciliata	Dihydrorotundifoline	Compound A
$SbCl_3$	Precipitate	Precipitate	Nil	Nil
Et_2O-$FeCl_3$	Garnet red	Garnet red	Nil	Nil
Kiliani's Reagent	Orange pink	Orange pink	Nil	Nil
HNO_3 conc.	Pale yellow; slight green fluorescence		Pinkish orange fading to orange; no fluorescence	
H_2 uptake over PtO_2	One mole	One mole	Nil	Nil

Nature of Compound A.

Since, as has just been shown "A", non-phenolic and prepared by the action of diazomethane on the phenolic mitragynol is identical with dihydrorotundifoline, one is tempted to assume that the reaction has been ether formation.

$C_{21}H_{26}O_5N_2$ (mitragynol) $\xrightarrow{CH_2N_2}$ $C_{22}H_{28}O_5N_2$ "A" or dihydrorotundifolone $\xleftarrow{H_2/PtO_2}$ $C_{22}H_{26}O_5N_2$ rotundifoline

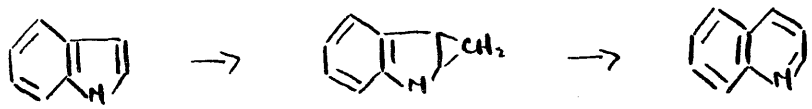
Since, however, "A" is also formed by the attempted acylation of mitragynol, the reaction with diazomethane is by no means as simple as ether formation.

Diazomethane could react in any of four ways:-

(a) R-O-H $\longrightarrow$ R-O-Me

(b) >NH $\longrightarrow$ >N Me

(c) >C = C< $\longrightarrow$ >C — C< (with CH_2 bridge)

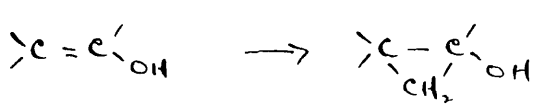
(d) 

In the case of mitragynol, however, each of these mechanisms is open to serious objection.

(a). Mitragynol, "A", and rotundifoline have each two methoxy groups. Although,as will be discussed later on p. 77, methoxyl groups may sometimes be undetected in Zeisel estimation, this is very unusual. A minor objection is that while ether formation usually lowers the melting point of a phenol, in this case the melting point has been raised by 100°. Thus, although the loss of phenolic properties and of one active hydrogen atom would be explained by ether formation, it is unlikely that this could have taken place.

(b). The two arguments against N-methylation are the failure to detect an N-methyl group in "A" and its lack of phenolic properties. Although, as will be mentioned later on p. 77, N-methyl groups may occasionally pass undetected in Zeisel estimations, this is very unusual.

(c). If in the phenolic ring the methylene group of the diazomethane is added across a double bond attached to the carbon atom bearing the hydroxyl group, this would explain the loss of both double bond and phenolic properties. Although



the new secondary alcoholic group should be detected by

hydroxylic reagents, and rotundifoline itself gives no derivative with either acetic anhydride or 3:5-dinitrobenzoyl chloride, such a group may, as will be discussed later on p. 79, fail to react. On the other hand the enolic nature of rotundifoline has been destroyed in the formation of dihydrorotundifoline, presumably by saturation of a double bond in an already partially reduced ring:-

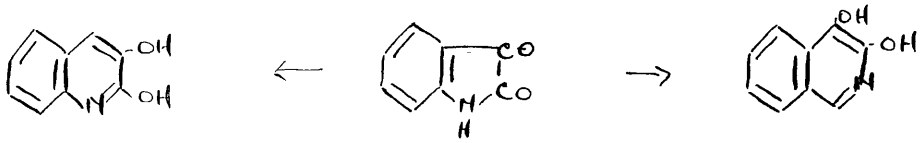
$$>C=C<_{OH} \xrightarrow{H_2/Pt\,O_2} >CH-CH<_{OH}$$

and it is hard to understand therefore how the same product could arise from mitragynol and rotundifoline.

(d). Again one probably has the addition of the methylene group across a double bond, followed this time by the rupture of the remaining single bond and the rearrangement of the bonds:

XLII

An example in the literature of a similar reaction is the conversion of isatin (XLIII) to a mixture of 2:3-dihydroxyquinoline (XLIV) and (probably) 3:4-dihydroxyisoquinoline (XLV)[73].



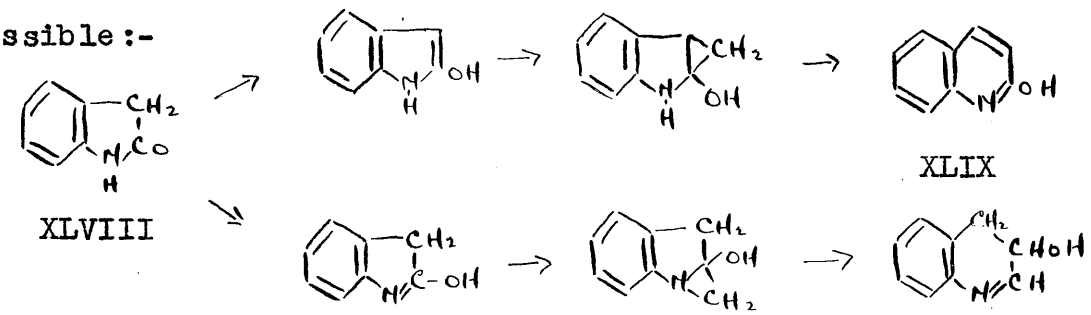
XLIV XLIII XLV

This reaction, however, proceeds in a totally different manner from that of diazomethane with indane 1:2:3-trione XLVI (which may be regarded as isatin with the imino replaced by a keto group) to give a methylene dioxy compound XLVII[74].

XLVI XLVII

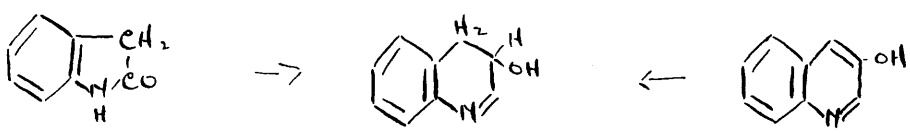
If ring enlargement did take place to form a compound of the type of XLII, the ultra-violet spectrum of the product should be quinolinic in type. Actually it is indolic.

If mitragynol contains a lactam structure (XLVIII), then either of the following series of reactions may be possible:-



XLVIII XLIX L

Against both these reaction mechanisms there are serious objections. As a 2-hydroxyquinoline, XLIX should be phenolic and show a quinolinic ultra-violet spectrum. "A" is a non-phenolic indole, L would explain satisfactorily the loss not only of the phenolic properties of mitragynol, but also of the enolic properties of rotundifoline if the latter were the 3-hydroxyquinoline (LI):-



XLVIII L LI

Again, however, there are several objections. First, in this

system the phenolic group that has been converted into a non-phenolic group would occur possibly in mitragynol but certainly in rotundifoline. Secondly, one would expect the alcoholic group in L to give derivatives. Thirdly, reduction of the conjugated double bond in the 3:4-position of L should have a marked effect on specific rotation. As already seen, there is no difference in this respect between rotundifoline and dihydrorotundifoline. Fourthly, if reduction of this double bond has negligible effect, as it has, on the ultra-violet spectrum, then that should be quinolinic. It is not. While these objections have been concerned with the conversion of rotundifoline to "A", the final objection refers to the conversion of mitragynol. Since the nitrogen of the lactam ring becomes a member of a pyridine ring, one would expect this nitrogen to become basic. Therefore if mitragynol is a mono-acidic base, "A" should be diacidic. Actually both mitragynol and "A" are monoacidic bases.

So far the rôle of acetylating agents in the formation of "A" has not been considered. Kondo & Ikeda[19] have noted that acetylation of iso-rhynchophylline yields, not acetylisorhynchophylline, but a mixture of acetylrhynchophylline and unchanged rhynchophylline. It would seem that isorhynchophylline is first isomerised to rhynchophylline and that this is then acetylated in poor yield to acetylrhynchophylline:-

Isorhynchophylline $\xrightarrow{\text{isomerisation}}$ rhynchophylline

$\xrightarrow{\text{acetylation}}$ acetylrhynchophylline.

This isomerisation is the reverse of that shown in the esterification by diazomethane of rhynchophyllic acid (from rhynchophylline) to isorhynchophylline. Since certain alcohol groups (cf.p.80) are known not to acetylate, one may explain the action of acetylating agents on mitragynol as isomerising it to "A".

The question of the isomerising power of diazomethane is more difficult. That the analyses indicate different formulae for mitragynol and compound "A" is not an insuperable difficulty, for with such complex molecules as these it is extremely difficult to distinguish between two possible empirical formulae differing by a carbon atom or a methylene group. The isomerisation in the reaction series:-

Mitragynine $\xrightarrow{\text{MeOH-KOH}}$ mitragynic acid $\xrightarrow{CH_2N_2}$ isomitragynine

and

Rhynchophylline $\xrightarrow{\text{MeOH-KOH}}$ rhynchophyllic acid $\xrightarrow{CH_2N_2}$

isorhynchophylline

occurs more probably at the hydrolysis than at the esterification stage. Again from the rhynchophylline analogy, one would expect the isomerising influences of acetic anhydride and of diazomethane (if the latter has any influence) to act in opposite directions:-

$$A \underset{Ac_2O}{\overset{CH_2N_2}{\rightleftharpoons}} B$$

With regard to the action on mitragynol of diazomethane, all one can say is that it is known to fail to etherify a phenolic group, and may possibly cause isomerisation. Unlikely as isomerisation may be, it seems at present the explanation most capable of fitting the experimental results.

Rhynchophylline.

Identity of "Mitrinermine" with Rhynchophylline.

As already mentioned, although Raymond-Hamet[30] suggested on pharmacological grounds the identity of "mitrinermine" with rhynchophylline, and although Barger, Dyer & Sargent[3] obtained no depressions of melting point with mixtures of either rhynchophylline or "mitrinermine" with an alkaloid of similar properties isolated from M. rotundifolia, Millat[31] has recently suggested, on arguments from melting points, specific rotations, empirical formulae, and colour reactions, that "mitrinermine" and rhynchophylline are in fact two distinct alkaloids. Although unfortunately no specimen was available of rhynchophylline isolated from Ourouparia rhynchophylla, specimens of the alkaloid from both M.inermis and M.rotundifolia were thoroughly compared. In the discussion following, the specimens from these sources will be designated as "I" and "R" respectively. It will be convenient in this discussion to use as points of identity

the points of differentiation proposed by Millat.

(a) Melting Points. "I" melted at 212-3°; "R" at 213-4° - no depression on mixing. Although Millat[31] cites mitrinermine as melting at 203°, his own original paper to which he refers gives not 203°, but 215-6°[25]. He has since informed me in private conversation: "C'était par mégarde que j'ai cité le chiffre 203°". Although absence of mixed melting point depression is not necessarily proof of identity[67,68], at least with "mitrinermine" and rhynchophylline there is not the discrepancy in melting point suggested by Millat.

(b) Specific Rotations. The apparent difference in specific rotation between mitrinermine, $[\alpha]_D$ = -21 to -23°, and rhynchophylline, $[\alpha]_D$ = -14.5°, has now been shown to be due to concentration. In chloroform, both "I" and "R" had $[\alpha]_D$ = -15° (c = 2.3) and -22° (c = 1.1). Comparison of rotations of "I" and "R" at these concentrations in chloroform over the range λ = 7000 to 5000 Å, in N hydrochloric acid at concentration 1.5 over the range 7000 - 4750 Å showed complete identity of the results of the two specimens. Since at c = 5.3, "R" showed $[\alpha]_D$ = -6°, the variation of $[\alpha]$ with c is linear, c.f. Fig. 3 . The rotations are recorded in detail on pp. 157,161 and are shown graphically in Fig. 2.

Empirical Formula. Although the single analysis of Akamatsu[27] does suggest $C_{21}H_{26}O_4N_2$ as the empirical formula

of rhynchophylline, the numerous results of Kondo et al[19,26] suggest the formula $C_{22}H_{26-28}O_4N_2$, cf. mitrinermine, $C_{22}H_{28}O_4N_2$

(d) Colour Reactions. Although, as Millat points out, rhynchophylline is described as giving a blue colour with Fröhde's reagent whereas "mitrinermine" does not, Raymond-Hamet attributes this colour to an impurity[75]. Rhynchophylline purified by further recrystallisation no longer gave the reaction. "R" likewise gives no colouration.

(e). Further proof of the identity of "mitrinermine" with rhynchophylline is the fact that "I" (as well as "R") gives the chloroplatinate and chloroaurate described by Kondo as formed by rhynchophylline.

(f). A final indication of identity although not as rigid a proof as is the other evidence, is the fact that within the limits of experimental error, the ultra-violet spectrum of "R" is identical with that of "I" as determined by Raymond-Hamet[12]. The significance of this is lessened, of course, by the fact that the spectra of the Mitragyna alkaloids in general are fairly similar.

The experimental evidence for the identity of mitrinermine with rhynchophylline is summarised in Table 14.

Table 14.

	Experimental Values		Literature Values	
	I	R	Mitrinermine	Rhynchophylline
m.p.	212-3°	213.4°	2.5-6°	215-6°
$[\alpha]_D$($CHCl_3$, c=5.3)	-6.0°	-	-	-
$[\alpha]_D$(" c=2.3)	-15.7°	-14.3°	-	-14.5, -14.7°
$[\alpha]_D$(" c=1.1)	-23.0°	-22.0°	-23 to -26°	-
$[\alpha]_D$(HCl, c=1.5)	+2.7°	+3.3°	-	-
Ultra-violet spectrum				
λ max	-	2785Å(log =3.16)	2798Å(log 3.2)	-
	-	2440Å(log =4.24)	2447Å(log 4.2)	-
λ min	-	2780Å(log =3.16)	2786Å(log 3.2)	-
	-	2245Å(log =4.00)	-	-
H_2PtCl_6, dec.pt.	236°	235°	-	238°
$HAuCl_4$, m.pt.	132°	132°	-	134°
dec.pt.	155-7°	155°	-	155°.

It is seen, therefore, that from "I" and "R" all the physical constants listed for both rhynchophylline and "mitrinermine" have been obtained. One must bear in mind that, as discussed for dihydro-rotundifoline, p. 41, two closely related alkaloids may show almost identical properties both of themselves and oftheir derivatives.

Again, direct comparison of "mitrinermine" with a

specimen of rhynchophylline from O.rhynchophylla was impossible Under these circumstances the identity of "mitrinermine" with rhynchophylline may not have been rigidly proved. All evidence for non-identity, however, has been removed.

There remains the question of nomenclature. Although the alkaloid was first discovered in O.rhynchophylla and so the name rhynchophylline is the older, the similarity to the generic names mitragynine, mitragynol, mitraspecine and mitraversine makes the name "mitrinermine" attractive. Probably, however, it is unwise to depart from traditional practice, and so it is proposed to use the name rhynchophylline, a name with the added advantage of showing the relationship of the alkaloid to the isomer isorhynchophylline.

General Chemistry of Rhynchophylline.

Rhynchophylline has been found to contain three active hydrogen atoms at 160°. Colour reactions and the ultra-violet spectrum are similar to those of the other Mitragyna and Ourouparia alkaloids. Rhynchophylline does not react with 2:4-dinitrophenylhydrazine, and on hydrogenation takes up one mole of hydrogen over Adams' catalyst, but none over palladium. With diazomethane, rhynchophylline reacts to give a compound $C_{23}H_{30}O_4N_2$, m.p. 225-7°, $[\alpha]_D$ +26° (c = 1.5 in chloroform). The ultra-violet spectrum of this compound is similar to that of rhynchophylline.

Identity of $C_{10}H_9NO$.

Barger, Dyer & Sargent[3] found that distillation of rhynchophyllic acid in vacuo from calcium oxide caused a degradation more radical than mere decarboxylation, and they isolated, inter alia, a neutral product $C_{10}H_9NO$ (C). Soluble in boiling alkali, "C" gave neither an Ehrlich nor a pine splinter test for indoles. "C" contained neither N-methyl nor methoxyl groups. Distillation of "C" from zinc dust gave an uncharacterised compound "D", which gave an Ehrlich Test. Since carbostyril is known under certain conditions to yield indole[76], Barger, Dyer & Sargent concluded that although "C" was certainly non-phenolic, it might easily be a methyl carbostyril which on zinc dust distillation changed into an indole.

There are several fallacies in this argument. First, as is well known, 2:3-dialkylindoles give neither the pine splinter nor the Ehrlich test for indoles[77]. Secondly, the quinoline-indole change. The two cases which were found in the literature were the conversion of carbostyril and quinolinic acid[78] to indole by alkaline fusion and heating with calcium carbonate respectively. Under these circumstances it seems strange that "C", a quinoline , should survive calcium oxide distillation only to yield to zinc dust. A more feasible suggestion is that "C" is not a methyl carbostyril but a monohydroxy-dimethyl indole. If this

were partially or wholly demethylated by zinc dust distillation, the product "D" would readily give indole tests.

Through the courtesy of Drs. Dyer & Sargent I was able to examine 2 mg. of "C". Determination of the ultraviolet absorption spectrum in ethanol showed λ_{max} 2525 and 2815Å, λ_{min} 2765 and 2310Å. Since the peak at 2815Å is little more than a point of inflexion, the two main peaks are therefore at λ_{max} 2525 and 2200Å.

In Table 15 and Fig.11 are collected the spectral data for a number of quinolines and indoles. Although from the example

Table 15 (vide Fig.11).

Substance	max in Å (log ε in brackets)		min in Å (log ε in brackets)		Ref.
A.	2525 (3.75)	2200 (4.0)	2310 (3.48)		
quinoline I	3110 (3.80)	2750 (3.65)			79
II	3120 (3.27)	2950 (3.21)	3095 (3.08)	2450 (3.08)	80
4-methyl-quinoline	3150 (3.17)	2950 (3.24)	3100 (3.08)	2420 (2.9)	80
2:6-dimethyl quinoline	3220 (3.36)	2840 (3.20)	3130 (3.17)	2520 (3.10)	80
2-hydroxy-quinoline	3270 (3.83)	2690 (3.85)	2950 (3.32)	2550 (3.72)	81
8-hydroxy-quinoline	4400	3625	4100	3200	82
indole	2650 (3.80)	2150 (4.40)	-	-	79
2:3-dimethyl indole	2800 (3.96)	2230 (4.67)	2490 (3.42)		83

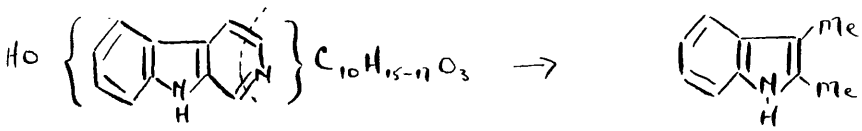
of 2:3-dimethylindole one would expect α : β -methyl groups to displace the indole spectrum towards the red, the spectrum

of "C" is displaced towards the blue.

On the other hand, as is shown by the case of 8-hydroxy quinoline, a hydroxyl group in the benzene ring (and if "C" is a 2:3-dimethyl indole the hydroxyl group must be in the benzene ring) displaces the curve considerably towards the red. Under these circumstances, it would seem that in the positions of the peaks the spectrum of "C" is indolic. Important confirmatory evidence that the spectrum is indolic is to be found in the values of log ε . While for quinolines the value of log ε is approximately the same for both peaks, for indoles there is a difference of 0.5 - 0.6. The spectrum of "C" shows this difference.

It is interesting that 2:3-dimethyl indole has been obtained as a degradation product of quinamine[84].

If, as is quite probable, rhynchophylline is a hydroxy-β-carboline (LII), one can see how a hydroxy-2:3-dimethylindole (LIII) might be formed as a degradation product:



LII LIII

The known properties of the various Mitragyna and Ourouparia alkaloids are summarised in Table 16.

Table 16.

Known Data Concerning the Mitragyna and and Ourouparia Alkaloids.

Alkaloid	Mitraphylline	Mitragynine	Mitraversine	Mitraspecine	Formosanine	Handamine	Isorhyncho-phylline	Gambirine	Rotundifoline	Mitragynol	Rhynchophylline
Formula	$C_{21}H_{26}O_4N_2$	$C_{22}H_{30-32}O_4N_2$ or $C_{23}H_{32-4}O_4N_2$	$C_{21}H_{24-8}O_4N_2$	?	$C_{22}H_{24-6}O_4N_2$	$C_{21}H_{24}O_4N_2$	$C_{22}H_{24-6}O_4N_2$		$C_{22}H_{26}O_5N_2$	? $C_{21}H_{28}O_5N_2$	$C_{22}H_{24-8}O_4N_2$
m.p.	273°	105-115°	237°	244-245°	202-218°	187°			233-4°	ca. 130°	215-6°
Basicity											
1.Salts	1	1	-	-	-	-	-		1	1	1
2.Pot.titrn.	1	1	-	-	-	-	-		-	1	-
$[\alpha]_D$ in $CHCl_3$	0, +7° (c = 2.00)	+49.8° (c = 1.50)	+37.0° (c = 1.33)	59.15° (c = 5.3)	90.0° (c = 2.00)	-	+8.3° (in EtOH)		+123-4° (c = 2)	+0.5° (c = 4.24)	-6.0° (c = 5.3)
	+4.6° (c = 1.00)	+38.7° (c = 0.75)	+38.0° (c = 0.67)	-	90.7° (c = 1.00)	-	-		v.little varn. w. concn.	-3.8° (c = 2.12)	-14.7° (c = 2.3)
	-	-	-	-		-	-			-7.6° (c.1.06)	-22-23° (c.1)
$[\alpha]_D$ in NHCl	+26.7° (c = 2.7	-	+2.7° (c = 1.00)	-	25.3° (c = 1.00)	-	-		+7.0° (c = 2.5)		+3.3° (c = 1.5)
	-	-		-	26.7° (c=0.50						
U.V.Spectrum											
λ max in A°	2820, 2430	3553, 2910	2885, 2400	-	2830, 2443	-	-		2925, 2390	2840, 2400	2785, 2445
	-	2800, 2400	-	-							
λ min in A°	2880, 2305	3030, 2880	2765, 2295	-	2786	-	-		2770, 2390	2740, 2400	2780, 2225
	-	2800, 2400	-	-	-						
m.p.derivs.	Br_2,195-8	pic.217-223°	-	-	-	-	-			HCl, 212-6°	dec.pt.238°
	Pic. 165°	MeI, 211.5°	-	-	-	-	-		H_2 232-3°	-	m.p.134°
	He,152-3°										
	MeI,	Series of salts	-	-	-				Br_2 215°	-	dec.pt.155°
NHMe	Nil	Nil	-	-	-	-	-		Nil	Nil	Nil
NH	Present	Present	-	-	Nil	-	-		Present	Present	Present
OMe distinct from COOMe	Present	Present	-	-		Nil	Present		Present		
OH enolic	Nil	Nil	-	-		-	-		Present	Test masked by phenol	Nil
OH phenolic	Nil	Nil	Present	Nil	Nil	-	Nil		Nil	Present	Nil
OH derivs.	Nil	Nil	-	-		-	-		Nil	Nil	Acetyl m.p.
OCH_2O	Nil	Nil	-	-	Nil	-	-		Nil	Nil	Nil
CO derivs.	Nil	Nil	-	-	Nil	-	-		Nil	-	Nil

Only Pharmacology Described

Table 16 (Contd.

Alkaloid	Mitraphylline	Mitragynine	Mitraversine	Mitraspecine	Formosanine	Handamine	Isorhyncho-phylline	Gamborine	Rotundifoline	Mitragynol	Rhynchophylline
COOMe	Present	Present	Probably	Probably	Probably	Probably	Present		Present	Present	Present
RCOOH											
1. m.p.	173°	155°	-	-	-	-	-			-	
2.Re-esterifcn.	m.p.152°	m.p.124-5°	-	-	-	-	-		m.p. 170°	-	m.p. 150° Isorhynchophyl.
3.Decarboxyln.	-	-	-	-	-	-	-		$C_{20}H_{22}O_3N_2$	-	? an OH indole
No. of Active Hs	3	3	-	-	-	-	-		3	4	3
No. of Hs to 1. Pd	3	-	-	-	1	-	-		1	0	1
2. PtO_2	1	-	-	-	1	-	-		0	0	0
Indole Nucleus											
1.Spectrum	Compatible	?	Compatible	-	Compatible	-	-		Compatible	Compatible	Compatible
2.Colour tests	Positive	Positive	-	-	Positive	-	-		Positive	Positive	Positive
Degradatn. Products	-	? a -carboline	-	-	-	-	-		3:4-Diethyl-pyridine		

General Chemistry.

In this section it is proposed to compare and contrast the various alkaloids under such heads as "Specific Rotation", "Active Hydrogen Atoms", etc., and to discuss in some detail the implications of the results obtained.

Specific Rotation.

The specific rotation of the same substance in the same solvent may vary with (a) temperature, (b) the concentration of optically active substance, (c) wave length of the light source, and (d) (for salts in water) the amount and nature of the acid present. While variation with temperature was not investigated for the Mitragyna alkaloids, the other three types were all encountered.

Variation with concentration was shown by mitraphylline, mitragynine, mitragynol and rhynchophyophylline, but not by mitraversine, formosanine or rotundifoline. This type of variation is quite common with alkaloids, e.g., emetine and its hydrochloride and hydrobromide[85], laudanidine[86,87,88] and peimine[89]. What may seem to be variation with concentration may, when different specimens of alkaloid are used, be due to the presence either of impurity or of the racemic base, e.g., while hyoscine hydrobromide from henbane has a specific rotation of -24 to -25°, the salt of the alkaloid from the Scopolia rhyzome has a rotation of -13.5° - the lower value is due to the presence of the racemic base[90].

All the alkaloids examined showed considerable variation of specific rotation with wave length of the light used. Dispersion ratios 5000/6500Å range from 3.99 for mitraphylline to 1.28 for rhynchophylline - both in chloroform. Biot's Law was in general obeyed. Very few instances are given in the literature of this type of variation. Mention is made, however, of l-hyoscyamine, which, incidentally, does not obey Biot's Law[91], and also of laurepukine[92].

Only mitragynol was examined for variation of the specific rotation of its salts with change of the nature and concentration of the acid used. As is shown by the detailed experimental results on p. 146, there was considerable variation. Solutions of mitragynol in N hydrochloric acid and N potassium hydroxide allowed to stand for 24 hours showed no sign of mutarotation.

The only structural information deducible from rotational data is that the double bond reduced in rotundifoline over Adams' catalyst is unconjugated.

Ultra-Violet Spectra.

The experimental data, and data for certain reference compounds, is collected in Table 17.

Table 17.

Alkaloid	λ_{max} in Å (log ε in brackets)	λ_{min} in Å (log ε in brackets)
Mitraphylline (a)	2820(3.45), 2430 (4.65)	2790(3.44) 2305(4.59)
Mitraphyllic acid (b)	2800(3.63) 2390(4.33)	2780(3.62) 2260(4.18)
Methylmitraphyllate (b)	2840(3.40) 2410(4.00)	2800(3.35) 2345(3.99)
Mitragynine (b)	3550(3.34) 2910(3.00) 2800(3.03)(e) 2400(3.64)(e)	3030(2.70) 2880(2.95) 2800(3.03)(e) 2400 (3.64)(e)
Mitragynine, CH_3COOH (c)	2900(3.77) 2480(4.20	2880(3.75) 2350(4.16)
Mitraversine (a)	2885(3.15) 2400(3.99)	2765(3.10 2295(3.95)
Formosanine (a) (12)	2830(3.15) 2443(4.25)	2786
Rotundifoline (a)	2925(3.47) 2390(4.23)(e)	2770(3.29) 2390(4.23)(e)
" , HCl (d)	2900(3.45) 2470(4.25)	2760(3.38)
Mitragynol	2840(3.81) 2400(4.18)(e)	2740(3.76) 2400(4.18)(e)
" , HCl	2900(3.58) 2460(4.06)	2760(3.39)
" "A"	2915(3.46) 2395(4.14)(e)	2755(3.30) 2395(4.14)(e)
" HCl	2900(3.46) 2450(4.09	2765(3.36) 2355(4.05)
" "B"	2920(3.44) 2395(4.18)(e)	2760(3.29) 2395(4.18)
Rhynchophylline	2785(3.16) 2445(4.24)	2780(3.16) 2225(4.00)
Methylrhynchophylline	2770(3.33)(e) 2395(4.30)	2770(3.33)(e) 2210(4.06
Yohimbine (93)	3000(3.95) 2500(4.4)	2750(3.15)
Coryanthine (94)	2841(3.80)(f) 2461(4.40)	2729(3.75)
Indole (79)	2650(3.80) 2150(4.40)	-
Quinoline (i) (79)	3110(3.80) 2750(3.65)	
" (ii) (80)	3120(3.27) 2950(3.21)	3095(3.08) 2450(3.08)
Isoquinoline (95)	3210(3.5) 2845(3.5)	2810(3.2) 2410(3.4)

(a) in methanol
(b) in ethanol
(c) in water)
(d) in water with a little hydrochloric acid
(e) Point of inflexion
(f) main peak of several peaks very close together.

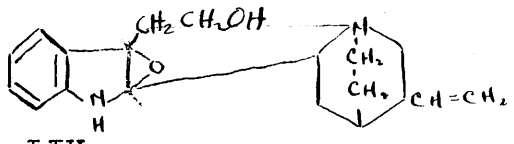
As is seen from the Table, the spectra of the Mitragyna and Ourouparia alkaloids are very similar. In all cases there is a peak at 27-2900Å and a taller one at 23-2500Å. Usually the former is much higher above the neighbouring trough than is the latter above its trough. Indeed, insome cases the former peak and trough are so close as to constitute merely a point of inflexion. In the cases of mitraphylline, methyl mitraphyllate and methyl rhynchophylline the position is reversed and it is the latter which is much nearer its trough in magnitude of log ε . The spectra of such hydrochlorides as were examined were very similar to those of the corresponding free bases, usually, however, the difference in log ε between the peak at 23-2500Å and its neighbouring trough is slightly increased.

The curve for mitragynine is distinctive in two respects. First there are two extra peaks, at 3550 and 2910Å, secondly, for the 2400Å peak, log ε is much lower than is usual for the Mitragyna alkaloids. This irregularity of spectra may be correlated with the appearance of colour in the base. The acetate, which is colourless, shows the normal spectrum of Mitragyna alkaloids.

The curves for the alkaloids are typical of those

described for such alkaloids of proved indolic structure as harmine, harmaline and peganine[96], sempervirine[97], aspidospermine[98], gelsemine[12], yohimbine[95] and strychnine[99], and such indolic alkaloids and degradation products as yobyrine[95] and lysergic acid[83]. The similarity of spectra for these various alkaloids is so striking as to lead Janot & Berton[100] to declare that this type of curve is characteristic of indolic alkaloids.

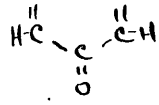
This, however, does not exclude the possibility of alkaloids with this type of curve being α-β-dihydroindole derivatives. The spectrum of quinamine for instance (LIV), is typically indolic. Although one would expect the



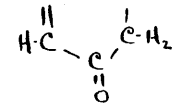
LIV

saturation of the 2:3-double bond, conjugated with the lone pair of electrons on the nitrogen atom to have a marked influence on the absorption spectra, no such influence is shown. Perhaps this is because (although one α-β-double bond has been reduced another still remains) the double bond that had been reduced was part of a crossed conjugated system. In the comparable case of dienones, where the keto group may be compared with the indolic nitrogen atom and its lone pair of electrons, Djerassi & Ryan[101] found it impossible to distinguish the ultra-violet spectra of dienones (LV) from

those of the corresponding monounsaturated compounds (LVI)



LV



LVI

unless recourse was made to the respective 2:4-dinitrophenylhydrazones.

If the alkaloids contain a β-carboline structure one would expect the pyridine ring to influence the spectra. However, in such known β-carboline compounds as yohimbine and coryanthine this influence does not appear; its absence in the Mitragyna alkaloids is no argument against their having β-carboline nuclei. Possibly the explanation is that the β-carboline ring is reduced.

One may sum up the spectral evidence by saying that it points strongly to the presence of indolic and contra-indicates quinolinic or isoquinolinic nuclei.

Fluorescence.

The fluorescence shown by acid solutions of the Mitragyna alkaloids is quite characteristic of β-carboline derivatives. A similar fluorescence is shown in organic solvents, but is not sufficiently intense to warrant examination of the fluorescent spectra.

Colour Reactions.

The colour reactions of the alkaloids, which were

thoroughly examined, are described in detail in the Experimental Section for each alkaloid, and summarised in Table 18. For comparative purposes, carbazole, indole, 2- and 8-hydroxyquinoline, the indolic alkaloids calycanthine and yohimbine, and the non-indolic alkaloid quinine were also examined. Careful control experiments were done also to be sure that the reagents themselves gave the reactions attributed to them, e.g., ferric chloride solution was tested with phenol. Even where a particular colour reaction was described in the literature, it was felt desirable to repeat the reaction in order to have all the results obtained under standard conditions.

Although important conclusions are drawn from the colour reactions, their limitations are appreciated. Four pitfalls are apparent, due to:-

1. Traces of Impurity. Traces of an impurity giving an intense colour with a particular reagent may easily give a false result, especially if the substance being investigated gives no colour with the particular reagent. Cryptopine, present in amounts up to 4% in commercial papaverine doubtless accounts for the colour given by the impure but not by the pure alkaloid [116] The blue colour with Fröhde's reagent given by the impurity associated with rhynchophylline from *O.rhynchophylla* led to the suggestion that rhynchophylline was different from "mitrinermine".

Table 17.

Reagent	Name	Significance	Ref.	Colour Mitraphylline	Reactions Mitragynine	Formosanine	Rotundifoline	Mitragynol	Rhynchophylline	Methyl mitragynol	Yohimbine	Calycanthine	Quinine	Carbazole	Indole	8 Hydroxy quinoline	2 Hydroxy quinoline
H_2SO_4 conc.		Oxidn.		-	+	-	+	-	-	+	-	-	-	+	+	n.d.	n.d.
H_2SO_4 conc.- $K_2Cr_2O_7$		"		+	+	+	+	+	+	+	+	+	+	+	+	n.d.	n.d.
HCl dil. - $K_2Cr_2O_7$		"		-	-	-	-	-	-	-	+	+	+	-	+	n.d.	n.d.
HNO_3 conc.		"		+	+	-	+	+	+	+	+	+	-	+	+	n.d.	n.d.
HNO_3 "(0.5%) in H_2SO_4 conc.	Erdmann	"	102	+	+	+	+	+	+	+	+	+	-	+	+	n.d.	n.d.
Na molybdate in H_2SO_4 conc.	Fröhde	"	102	-	+	-	-	+	-	-	+	+	-	+	+	n.d.	n.d.
Na vanadate in H_2SO_4 conc.	Mandelin	"	102	+	+	+	+	+	+	+	+	+	+	+	+	n.d.	n.d.
Ammon. alum in H_2SO_4 conc.	Kiliani	"	103	+	+	-	+	+	+	-	+	+	-	+	+	n.d.	n.d.
$Ce(SO_4)_2$ " "		"	104	+	+	+	+	+	+	+	+	+	-	+	+	n.d.	n.d.
Pine Splinter		Indole	77	?+	-	-	?+	+	?+	?+	-	+	-	-	+	-	-
p-dimethyl amino-benzaldehyde in HCl dil - MeOH	Ehrlich	"	77	+	+	-	-	+	-	-	-	+	-	+	+	-	-
Ibid + trace $NaNO_2$ heated		"	105 104	+	+	+	+	+	-	+	+	-	-	+	+	-	-
Glyoxylic acid over H_2SO_4 conc.	Hopkins-Cole	"	77	+	+	-	-	+	-	-	-	+	-	+	+	-	-
Vanillin in dil. HCl-MeOH		"	77	+	-	-	-	-	-	-	-	+	-	-	+	-	-
Vanillin in conc. HCl heated		"	77	+	+	+	-	+	-	-	+	-	-	+	+	-	-
Glucose " "	Raspail	"	106	+	+	-	+	+	+	+	+	+	-	+	+	-	-
Chloral in conc. H_2SO_4 heated		"	107	-	+	+	-	+	-	-	+	+	-	+	+	+	-
HCHO in conc. HCl + trace $NaNO_2$	Voisenet	"	108	+	-	-	-	-	-	-	-	+	-	-	+	-	-
HOAc - traces $FeCl_3$ & H_2SO_4		"	109	+	+	+	-	+	+	-	+	+	-	+	+	-	-
HNO_3 conc. + trace $NaNO_2$		"	109	-	+	+	+	+	+	+	+	+	-	+	+	-	-
Br_2 water foll. by NH_4OH	Thalleoquin	Quinine	110	-	-	-	-	-	-	-	-	-	+	-	-	n.d.	n.d.
V.dil.HCl sol. + trace KCl over H_2SO_4 conc.	Rossi	Yohimbine	111	-	-	-	-	-	+	+	+	+	-	-	-	n.d.	n.d.

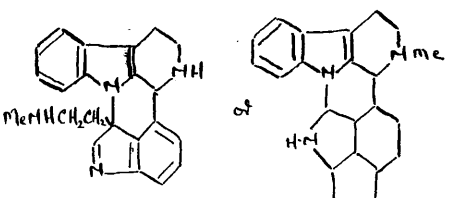
Table 13 (Contd.)

Reagent	Name	Signifi-	Ref.	Mitra-phylline	Mitra gynine	Formos-anine	Rotundi-foline	Mitra-gynol	Rhyncho-phylline	Methyl mitragynol	Yohim-bine	Galynan-thine	Quinine	Carbazole	Indole	8 Hydroxy-quinoline	2 Hydroxy quinoline
Tetranitromethane		Double Bond	113	?+	?+	n.d.	?+	?-	?+	?-	n.d.	+	+	?+	?+	n.d.	n.d.
Br_2 in $CHCl_3$		"		?+	?+	?+	+	?-	?+	+		+	? +	??+	n.d.	n.d.	n.d.
$KMnO_4$ 2% in H_2O	Ipatieff	"	60	+	+	+	+	-	+	-	+	+	+	+	n.d.	n.d.	n.d.
$FeCl_3$ in EtOH		Enol		-	-	-	-	+	-	-	+	n.a.	-	n.a.	n.a.	n.d.	n.d.
$FeCl_3$ in Et_2O		"		-	-	-	+	+	-	-	-	n.a.	-	n.a.	n.a.	n.d.	n.d.
$NaNO_2$ in HOAc		NH		+	+	?+	+	+	+	+	+	n.a.	n.d.	n.a.	n.a.	n.d.	n.d.
Phloroglucinol in H_2SO_4 conc.	Gaebel	OCH_2O	114	-	-	-	-	-	-	-	-	n.a.	-	+	+	n.d.	n.d.
Gallic Acid in MeOH	Labat	OCH_2O	115	-	-	-	-	-	-	-	-	n.a.	-	n.a.	n.a.	n.d.	n.d.
Dinitrobenzene in MeOH/NaOH		-COCH	116	-	-	-	-	-	-	-	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

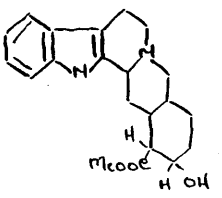
n.a. not applicable

n.d. not determined.

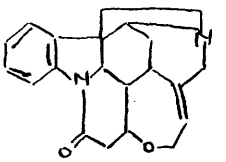
2. Suppression of Reaction. Merely because a certain reaction A is given by a group or nuclear structure B, all substances containing B do not necessarily give A, and failure to give A is not proof of the absence of B. Calycanthine, LVII, and yohimbine, LVIII, both proved to be indolic alkaloids, fail to give certain indole tests, as does carbazole, LIX.



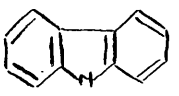
LVII



LVIII



LX

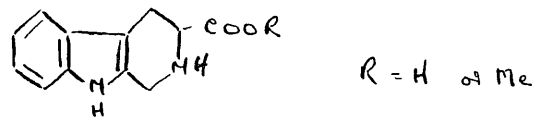


LIX.

Again with the reagent for $>CHCO-$ or $-CH_2CO-$, both strychnine LX and brucine (methyl methoxy strychnine) give only the faintest of colours. An example of particular interest is the failure of α -β-dimethylindole LIII to give either the Ehrlich or the pine splinter test for indoles[77]. As mentioned earlier, failure to appreciate this misled Barger, Dyer & Sargent in the identification of the rhynchophyllic acid degradation product $C_{10}H_9NO$.

3. Lack of Specificity of Reagent. Often a reagent described as specific for A will, in fact, react equally well with B and C. One example of this is the case of tetranitromethane -

discussed later. Another is the alleged colour test for 2:3:4:5-tetrahydro-4-carboxy- or carbomethoxy-β-carboline LXI[117] - a blue colour with concentrated sulphuric acid containing an oxidising agent. As Clemo & Swan point out[118], Dewar & King[119] obtained a similar reaction with the carboxyl and carbomethoxy-free ketone yohimbone.



LXI

4. Colour Given by the Reagent Alone. Often under the conditions of the reaction a colour is given by the reagent itself. One example of this is in the use of fructose heated with concentrated hydrochloric acid as a test for indolic nuclei. Raymond-Hamet[105] describes this test as giving with indoles an orange colour finally turning black. Now fructose heated with even 10% hydrochloric acid gives furfural, and a control experiment showed that fructose heated with hydrochloric acid changed from yellow via orange and brown to black in about a minute.

Individual Reagents.

Several of the reagents or types of reagents used call for comment.

1. Indolic Reagents. As is only to be expected, these often fail with substituted indoles. Positive results are used to

differentiate between α -, and β -indoles[77]. In general, α -indoles give red or orange, and β -, blue colours with vanillin in concentrated hydrochloric acid, or with Ehrlich's reagent.

2. Chloral Hydrate in Concentrated Sulphuric Acid. Since this gave a positive result with 8-hydroxyquinoline, the test is not specific for indoles. Since quinoline itself gave no colour, possibly the only non-indolic substances likely to react are phenols. Until the test is further investigated, however, no reliance may be placed on it.

3. Thalleoquin Reaction. Although this is not given by cinchonine[120] and cinchonidine[121], it is given by most Cinchona alkaloids. Control experiments with ortho and meta aminophenols gave dark brown and yellow-green colours respectively. Under these circumstances the result with mitragynol is meaningless.

4. Rossi Reaction. Difficulty was experienced in obtaining results with this test even for yohimbine itself. Although the aqueous layer was fluorescent, the reddish-pink interface was extremely difficult to see, and the upper layer was only very slightly coloured.

5. Tetranitromethane. Although tetranitromethane is often quoted as a reagent for the determination of double bonds, the test is very unreliable. Even Werner[112], who first drew

attention to the test, noted it was given also by amines and by unconjugated, but not by conjugated fatty acids. Clark, Macbeth & Stewart[122] remarked succinctly that the colour with double bonds is only a specific case of a more general phenomenon, and Macbeth[123] went on to show that these in turn are subject to two types of inhibition:- (a) where the unsaturated molecule contains an electronegative atom; and (b) where conjugation occurs within the molecule. As exceptions to this latter exception, aromatic compounds give colours, and control experiments showed that this applies equally to heterocyclic compounds. Pyridine, indole, carbazole, quinoline and isoquinoline gave yellow, brown, brown, brown and orange-brown colours respectively. Hammick & Young[124] say the colour is given by a variety of compounds having in common the property of anionic activity. They further observed that the colours are very similar to those produced when such polynitro-aromatic substances as trinitrobenzene are mixed in solution with aromatic or unsaturated hydrocarbons or with bases, and further that the intensity of colour with various compounds is parallel to the stability of the picrates.

As Macbeth points out, even solvents may affect the colour. While chloroform hinders, water promotes colour formation.

6. Bromine in chloroform. Decolourisation of a chloroform solution of bromine, unaccompanied by evolution of hydrogen bromide has long been used as a test for unsaturation. With bases, however, there is the danger that decolourisation may possibly be due to perbromide formation. This actually happens with the Mitragyna alkaloids.

Results with Mitragyne and Ourouparia Alkaloids.

These alkaloids gave positive tests for indole nuclei and imino groups and (except for mitragynol) the Ipatieff test for unsaturation, and negative tests for methylene dioxy- and >CHCO or $-CH_2CO-$ groups. Enolic tests were given by rotundifoline and mitragynol, and imino tests by mitraphylline, rotundifoline, mitragynol and rhynchophylline. From the enol test it is evident that the hydroxyl group in rhynchophylline is non-enolic, and therefore not borne by a carbon atom attached to a double bond. Although mitragynol appears to give a thalleoquin reaction, it must be remembered that mitragynol is phenolic and that some phenols at least give this reaction. Among the oxidising reagents ceric sulphate is interesting in that there is a striking resemblance between the red-brown given with the Mitragyna and Ourouparia alkaloids and the carmine given with the curare alkaloids (104).

With regard to indolic reagents, two points call for comment. First, the fact that many of these fail with particular alkaloids is only to be expected from the necessarily

complex nature of these. Secondly, there is a striking parallel between the actions of mitragynol and calycanthine - first observed in the latter case by Barger, Madinaveitia & Streuli[125] - with Ehrlich's reagent in aqueous methanol. In both cases, although the colour developed on heating fades on standing, it is instantly restored on further heating.

Function of the Nitrogen Atoms.

Although all the alkaloids contain two nitrogen atoms, information from potentiometric titration and salt formulae indicates that only one atom is basic. Since the alkaloids are indolic, this monoacidity is in accord with the expected neutrality of the nitrogen atom.

It is noteworthy that in mitraphylline, mitragynine, rotundifoline, mitragynol and rhynchophylline, the only alkaloids analysed for N-methyl groups, none is present. All the alkaloids examined gave an imino group reaction with sodium nitrite in acetic acid.

Nature of the Oxygen Atoms.

With the exception of mitragynol and rotundifoline (each containing five oxygen atoms), all the Mitragyna and Ourouparia alkaloids contain four oxygen atoms. While mitragynol and mitraversine are phenolic, rotundifoline is enolic. The enolic reaction of mitragynol with ferric chloride may be due to the phenolic group. Each alkaloid has an ester group said to be carbomethoxy in the cases where the acids

have been analysed, but no alkaloid examined has a methylene dioxy group. Mitragynine, rotundifoline, mitragynol, rhynchophylline, and isorhynchophylline each contains an additional methoxyl group. Mitraphylline and rotundifoline fail to form acetyl or 3:5-dinitrobenzoyl derivatives and oximes and 2:4-dinitrophenylhydrazones, mitragynine, formosanine and rhynchophylline fail also to give 2:4-dinitrophenylhydrazones. Rhynchophylline is unique in that it forms an acetyl derivative and also methylates with diazomethane. Neither mitraphylline, formosanine, nor rotundifoline reacts with diazomethane. Its action with mitragynol has already been full discussed.

The known functions of the oxygen atoms are summarised in Table 19.

Table 19.

Alkaloid	Mitraphylline	Mitragynine	Mitraversine	Formosanine	Hanadamine	Mitragynol	Rotundifoline	Rhynchophylline	Isorhynchophylline
Total No O's	4	4	4	4	4	5	5	4	4
COOMe	1	1	1	-	1	1	1	1	1
Other OMe	0	1	-	1*	-	1	1	1	1
OH phenol	0	0	1	0	-	1	0	0	0
OH enol	0	-	-	-	1	0	0	1	-
CO	0	0	-	0	-	0	0	0	-
Uncharacterised	2	1	1	3	1	1	2	1	1

*Total No. of methoxyl groups - probably present as the ester.

In cases where no attempt has been made to find certain groups, some or all of the uncharacterised oxygen for that alkaloid may be found in them. In the other cases, however, since a careful search has been made, the uncharacterised oxygen must be either part of a ring or in a hindered group. Of the latter possibility more will be said shortly.

Two features demanding further consideration are the ester groups and the uncharacterised oxygen atoms.

Ester Groups.

The interesting point about the ester groups is that hydrolysis and re-esterification give not the original alkaloids but isomers. Doubtless the overall reaction is

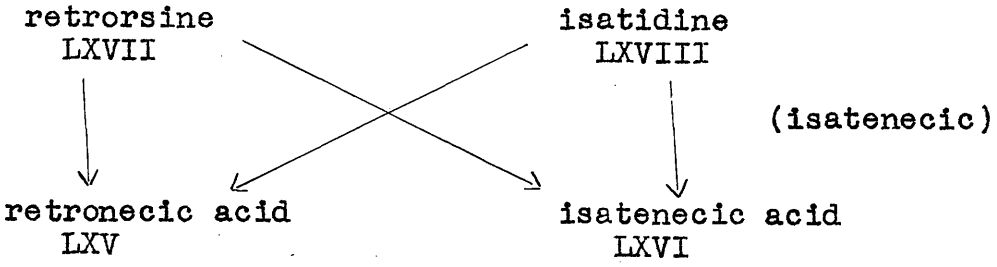
R COOMe $\longrightarrow$ R COOH $\longrightarrow$ isoR COOH $\longrightarrow$ iso RCOOMe

LXII LXIII LXIV LXV

This phenomenon is not peculiar to the Mitragyna alkaloids, but is known also in the Yohimbé alkaloids, e.g.,

corynanthine $\longrightarrow$ corynanthic acid $\longrightarrow$ yohimbine (126)

In some cases there is definite evidence that the isomerisation takes place in the step LXIII to LXIV above. As Fourneau & Benoit(127) point out, alkaline hydrolysis of corynanthine gives varying amounts of the two forms of corynanthic acid. Again, both retronecic LXV and isatinecic LXVI acid are obtained from retrorine LXVII and isatidine LXVIII on alkaline hydrolysis(128).



In some cases isomerisation takes place in alkali without hydrolysis and re-esterification. Examples include:-

1. Yohimbine ⟶ apo-yohimbine ⟶ -isoyohimbine[129]

2. Corynanthine ⟶ yohimbine[126] (standing in alkali)

3. Yohimbine ⟶ yohimbine + neoyohimbine (5 hours reflux) with 0.5 moles potassium hydroxide[129].

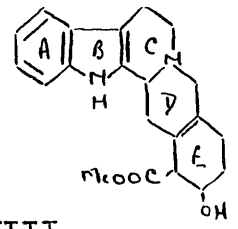
4. Quinamine ⟶ isoquinamine (heated with amyl alcoholic potassium hydroxide[130] - here there is no ester group in the molecule.

It would seem, therefore, that the isomerising agent is the alkali.

In the case of mitragynine an intermediate has been isolated[2] in which a molecule of methyl alcohol has added on to the mitragynine - possibly across a double bond.

With rhynchophylline an anomalous result is obtained. Although rhynchophylline has been found to methylate with diazomethane, rhynchophyllic acid with diazomethane gives an isomer of rhynchophylline. One can only assume that in the isomerisation the methylating ability of the hydroxyl group has been destroyed.

In the case of yohimbine LVII, Winkop[93] has



LXVIII

explained the change as due to cis-trans isomerism about the bond between ring D and E. The suggestion of isomerisation has been advanced by various other workers also, not only for the Yohimbé alkaloids[125], but also for the case of the necic acids[131].

This isomerisation raises several interesting questions which might be investigated in future work on the Mitragyna and Ourouparia alkaloids:-

1. Can different acids be obtained from the same alkaloid under different hydrolysis conditions?

2. Do isomeric alkaloids, e.g., rhynchophylline and isorhynchophylline, formosanine and handamine give the same acids?

3. Can one alkaloid be isomerised into another by treatment with acid or alkali?

4. Can the hydrolysis products be esterified to give isomeric alkaloids?

Investigation of questions 3 and 4 might lead to two important types of results. First, the conversion of mitragynol into compound "A" or of mitraversine into one of the

alkaloids found in M. rotundifolia would illuminate some of the obscurities already discussed with regard to mitraversine and compound "A". Secondly, the conversion of a Mitragyna alkaloid into one of another family of alkaloids of either known or unknown structure. Such a link would be extremely useful in elucidating the structure of the Mitragyna alkaloids.

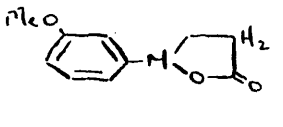
Uncharacterised Oxygen Atoms.

Although, where a search for ester, methoxyl, hydroxyl, keto and methylene dioxy groups has resulted in an oxygen atom or atoms still remaining uncharacterised, it is tempting to say such atom or atoms must be in a ring. Nevertheless such groups may be present although undetected. Three types in particular methoxy, keto and hydroxy groups warrant discussion.

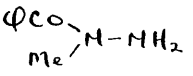
Methoxyl Groups.

Usually, where low methoxyl values have been obtained, the corresponding N-methyl values have been high, and the anomaly has been attributed to isomerisation from O-methyl to N-methyl under the analysis conditions. Examples in the literature include β-methoxy pyridyl betaine LXIX[132], the methiodide of strychnine methyl ether[133].

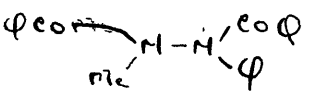
More common, however, are the low values obtained on analysis of N-methyl groups, e.g., in N-methyl-N-benzoyl-, and N-N'-dibenzoyl-N-methyl-N'-phenyl-hydrazines LXX and LXXI[134]



LXIX



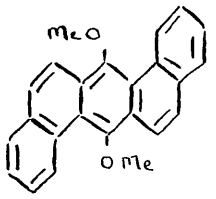
LXX



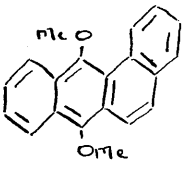
LXXI

Since, as far as has been examined, the Mitragyna alkaloids showed on analysis no N-methyl group, the explanation of methyl migration to a nitrogen atom cannot be invoked to characterise unaccounted oxygen atoms as belonging to methoxyl groups.

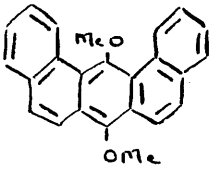
Recently, however, reference has been made to low methoxyl values in several compounds containing no nitrogen. Schoental[136] failed to detect any methoxyl groups in either 9:10-dimethoxy-1:2:5:6-dibenzanthracene (LXXII) or its picrate. Successful analyses, in the same laboratory and



LXXII



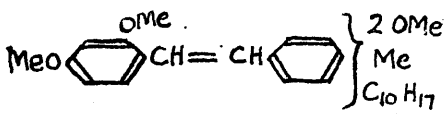
LXXIII



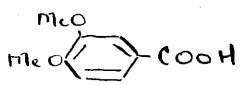
LXXIV

with the same batches of reagents, of 9:10-dimethoxy-1:2-benzanthracene and 9:10-dimethoxy-1:2:7:8-dibenzanthracene (LXXIII and LXXIV) make this failure all the more surprising, and contra-indicate the possibility of its being due to steric hindrance. Although the failure is possibly due to the insolubility of the anthracene in hydriodic acid, according to Pregl[136], as long as the substance is soluble in

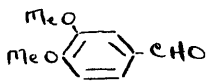
the phenol-acetic anhydride mixture, the analysis should be satisfactory. Again, if insolubility were the explanation, one would expect a low rather than a negative result. Nunn & Rapson[138] have found Zeisel determinations unsatisfactory not only for chlorophorin LXXV and hexahydrochlorophorin ethers, but even for such simple substances as 3:4-dimethoxybenzoic acid LXXVI, and benzaldehyde, LXXVII, despite the fact that in one case at least, the analysis was done at 350°.



LXXV



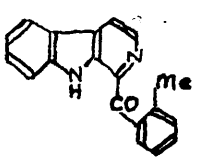
LXXVI



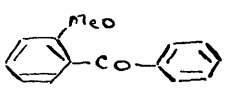
LXXVII

Hydroxyl and Ketonic Groups.

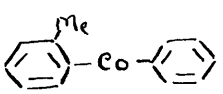
Numerous examples of inert hydroxyl and ketonic groups are given in the literature. Fessler & Shriner discuss the difficulty of inducing tertiary hydroxyl groups to form derivatives[139]. In discussing the inertness of the keto group of yohyrone, LXXVIII, Witkop[129] points out that if



LXXVIII



LXXIX



LXXX

C-CO group is between two tetra-substituted carbon atoms, e.g., hexamethyl acetone, phorone, fenchone, etc., the group is sterically hindered. He further points out ortho-substitution likewise leads to strong hindrance. Thus, while

o-tolyl phenyl ketone (LXXIX) gives an oxime with difficulty, o-o'-xylyl phenyl ketone (LXXX) gives none at all.

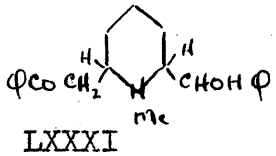
As this thesis is concerned with alkaloid chemistry, I shall deal briefly with various examples of inert oxygen atoms in alkaloids.

Hydroxyl Groups.

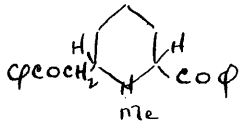
Reference has already been made to the failure both of the hydroxyl group in leucenol to acetylate and of the phenolic group in puketaine to react with diazomethane.

Keto Groups.

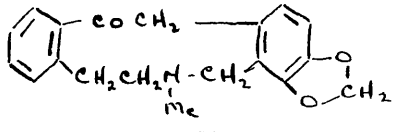
As Henry[139] points out, the carbonyl groups in keto alcohol and diketo bases are not detected by the usual reagents. Virgiline[140], lobeline (LXXXI)[139] and lobelanine (LXXXII)[141] have inert carbonyl groups, and in cryptopine (LXXXIII) the presence of a carbonyl group is doubtful.



LXXXI

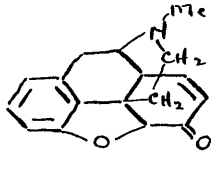


LXXXII

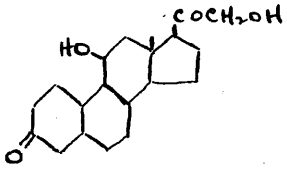


LXXXIII

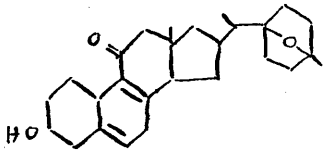
As Fieser & Fieser[142] point out, "the keto group of the 6-keto alkaloids of the morphine series is inert" eg., codeinone (LXXXIV). A case reminiscent of the inertness of cortisone (LXXXV) is



LXXIV



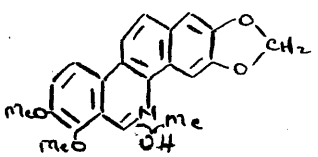
LXXXV

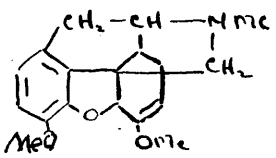


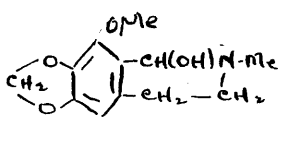
LXXXVI

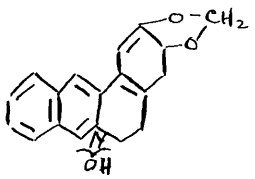
gervine (LXXXVI) with an inert keto group at C_{11}[143]. The example of yobyrone has already been mentioned.

By way of contrast, certain compounds, e.g., chelerythrine (LXXXVII)[144], thebaine (LXXXVIII) and dihydrothebaine[145], cotarnine (LXXXIX), berberine (XC) and


LXXXVII


LXXXVIII


LXXXIX


XC

alkyl dihydroberberines, none of which contains keto groups, all nevertheless form keto derivatives.

It is seen therefore that failure to give analytical results for, or derivatives of a group does not necessarily mean that that group is absent.

Active Hydrogen Atoms.

In the estimation of active hydrogen atoms with methyl magnesium iodide in phenetole, it was found that whole number values were obtained only on gently refluxing the reaction mixture (cf. the examples given in Table 20). Use of n-amyl ether as solvent either for substance and Grignard reagent, or for reagent alone, gave similar results.

Table 20.

Alkaloid	Number of Active Hydrogen Atoms found at Room Temp.	50-60°	100°	160°
Mitragynine	1.89	2.55	2.84	2.97
Rhynchophylline	0.87	1.53	1.81	3.08.

Since the highest temperature mentioned in the literature on the Zerewitinoff determination of active hydrogen is 140°, a series of control experiments were done at the four temperatures mentioned in Table 20 with both substance and reagent dissolved in phenetole and on 38 different acids, bases and substances containing active methylene groups. The results of this investigation (described fully in the supplementary section, pp. 172-204) showed that the effects of heating are threefold. First, as is already well known, increase in temperature promotes the activity of hydrogen in certain types of group, e.g., the second atom in amino groups. Secondly, there is the purely physical effect of liberating the methane absorbed by an insoluble reaction product. Thirdly, insoluble substances may be made more reactive by being brought into solution. At the high temperature used no abnormally high value was encountered.

Concerning the results with the Mitragyna alkaloids, several points are noteworthy.

1. The values found were considerably higher than those in literature, e.g., 3 for rotundifoline and rhynchophylline instead of 1.4 and 0.8 as found by Barger, Dyer & Sargent[3]. This is obviously due to the temperature used, for at room temperature a value of 0.87 was obtained for

rhynchophylline. With calycanthine in pyridine, Barger et al[125] found values of 2 at 22° and 4 at 95°.

2. That mitragynol has one more active hydrogen atom than has rotundifoline or dihydrorotundifoline (the isomeric form of mitragynol), is in accord with the absence of phenolic property in the two latter.

3. Dihydrorotundifoline, although no longer showing the enolic colour given by rotundifoline with ferric chloride, nevertheless contains the same number of active hydrogen atoms. This, however, is compatible with the conversion of an enolic to a secondary alcoholic group.

4. If one assumes one of the active hydrogen atoms comes from the NH group, then in the cases of mitraphylline, rotundifoline, dihydrorotundifoline and rhynchophylline, the number of remaining active hydrogens agrees with the number of uncharacterised oxygen atoms. Although, of course, some or all of the uncharacterised active hydrogen atoms may be present in active methylene groups, it is felt that some of the atoms at least may be present in hydroxyl groups.

Until more is known of the structure of the various alkaloids, however, the nature of most of the active hydrogen atoms must remain conjectural.

Hydrogenation.

In general the alkaloids show one double bond when hydrogenated over Adams' catalyst, and none over palladium. White mitragynol cannot be hydrogenated, mitraphylline shows one double bond with palladium and three over Adams' catalyst. The identical specific rotations of rotundifoline and dihydrorotundifoline show that in this case at least the double bond reduced is unconjugated.

Possible Skeletal Structure of the Alkaloids.

In this section, first it will be shown that the alkaloids are indolic rather than quinolinic or isoquinolinic, and then the various known types of indole alkaloids will be examined to see if the Mitragyna alkaloids might fit any of these skeletons. Finally, the possibility of an unknown structure will be examined. It cannot be too strongly emphasised that this section deals more with hypotheses than with facts capable of rigid proof.

Possible Heterocyclic Nuclei.

Five possible types of nuclei may be discussed:- pyridine, quinoline, isoquinoline, indole and reduced indole.

1. Pyridine.

The occurrence of 3:4-diethylpyridine as a degradation produce of rotundifoline means that rotundifoline and mitragynol, at least, contain a pyridine nucleus. Since

the alkaloids of the genera Mitragyna and Ourouparia are all from plants of closely related families and all show very similar chemical behaviour, it is reasonable to assume that the alkaloids are closely related chemically and that the pyridine nucleus is common to them all.

2. Quinoline and Isoquinoline.

The main evidence against such nuclei is the absence of any sign of quinolinic or isoquinolinic spectrum for any of the alkaloids.

3. Indole.

The evidence for the occurrence of indolic nuclei is fourfold. First the ultra-violet spectra of the alkaloids are similar to those of indoles and indolic alkaloids. Secondly the monoacidity of the Mitragyna and Ourouparia alkaloids is compatible with one of the nitrogen atoms being in an indole ring. Thirdly, the colour reactions are typically indolic, and, finally, indoles are almost certainly among the degradation products of rotundifoline and rhynchophylline[3].

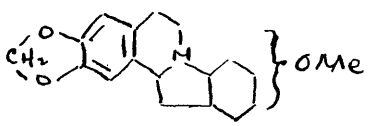
There is no evidence that the pyrrole ring is reduced. As already mentioned, it is thought to be impossible to distinguish between the spectra of indoles and reduced indoles sufficiently to say that an unknown substance belongs to the one class rather than to the other.

It may be that the indole and pyridine nuclei are combined in a β -carboline nucleus. As already mentioned, Ing & Raison suggest that the compound $C_{14}H_{14}ON_2$ obtained by the zinc dust distillation of mitragynine, is a β -carboline and if the compound actually is, then that is reasonable proof of the occurrence of the β -carboline nucleus in mitragynine at least.

Possible Skeletal Structures.

It is proposed here briefly to survey the known types of indole alkaloids and to see how the properties of alkaloids with these structures compare with the properties of the Mitragyna and Ourouparia alkaloids.

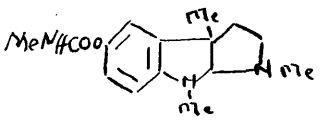
1. Erythrina Alkaloids - typical member erythraline (XCI)[146].



XCI

The evidence against this being the skeleton of the Mitragyna and Ourouparia alkaloids is threefold:- (1) no indolic colour reactions are given; (2) unless there was an ethyl group attached to the pyridine ring in position 3, it would be impossible to obtain 3:4-diethylpyridine as a degradation product; (3) the spectrum is typical of 1:2:3:4-tetrahydroisoquinoline.

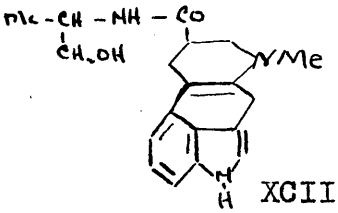
2. Physostigma Alkaloids - typical member physostigmine (XCII)[147].



XCII

The reasons against this skeleton are two:- (i) The Mitragyna and Ourouparia alkaloids fail to show the physiological action on the parasympathetic nervous system - an action so distinctive of the Physostigma alkaloids; (ii) 3:4-diethyl pyridine could not be obtained as a degradation product from such a skeleton.

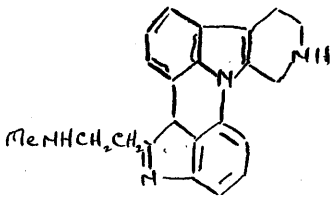
3. Ergot Alkaloids. - typical member ergometrine (XCIII)[148].



XCIII

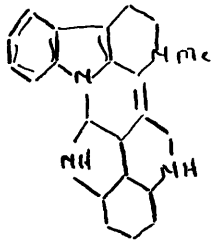
The main evidence against this structure is (i) the difference in ultra-violet spectra - the ergot alkaloids have λ max 3180Å, λ inflexion 2420Å; (ii) the impossibility of obtaining 3:4-diethylpyridine as a degradation product. Less important but corroborative evidence is the blue colours given by the alkaloids with several indolic reagents, and the muta rotation and instability to light shown by the members of the d-series.

4. Calycanthe Alkaloids - typical member calycanthine ((149)



(a)

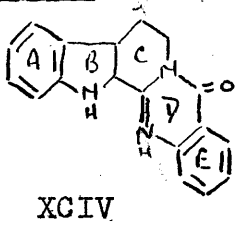
or



(b)

Again the evidence against the formula is twofold: (i) The Mitragyna and Ourouparia alkaloids fail to show the tetanic properties shown by the Calycanthe alkaloids. (Of course replacement of two of the nitrogen atoms might destroy this property). (ii) When one replaces one nitrogen atom in (a) and two in (b) by carbon atoms, there are too many carbon atoms in the skeleton for the Mitragyna and Ourouparia alkaloids to be fitted on to it.

5. Evodia Alkaloids - typical member rutaecarpine (XCIV) (150).

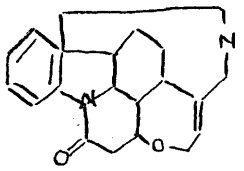


XCIV

If one replaced the nitrogen atom in ring D by carbon, then the skeleton would be very similar to that of Yohimbé alkaloids.

To obtain 3:4-diethylpyridine from the modified Evodia skeleton one would need ethyl substitution at X. There would then be more carbon atoms than in the Mitragyna and Ourouparia alkaloids after deduction of carbon atoms in methoxy and carbomethoxy groups.

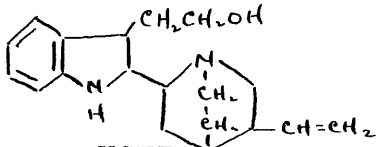
6. Strychnos Alkaloids. - typical member strychnine (XCV)(151).



XCV

The difficulty with this type of formula is the absence in the Mitragyna and Ourouparia alkaloids of not only the strychnic property of causing tetanic convulsions, but also the characteristic play of colours with nitric acid and other oxidising agents.

7. Pseudocinchona Alkaloids - Typical member cinchonamine (95) (XCVI)



XCVI

At first sight there seems to be much evidence that the skeletal structure of the Mitragyna and Ourouparia alkaloids might well be that of the Pseudocinchona alkaloids. First there is the similarity in ultra-violet spectra. Again, while a hydroxy-2:3-dimethylindole has probably been

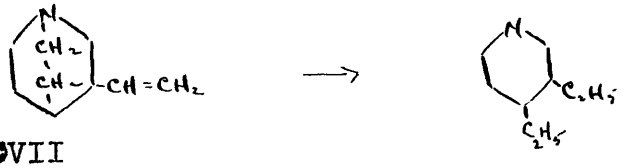
obtained from rhynchophylline, 2:3-dimethylindole itself has been obtained from quinamine by zinc dust distillation[152]. Further 3:4-diethylpyridine could be obtained from the quinuclidine part of the Pseudocinchona alkaloids.

On the other hand, not only is this evidence inconclusive, but there is also evidence to the opposite effect.

1. Since the ultra-violet spectra are due to the indole part of the molecule, and are uninfluenced alike by the extra rings in the Yohimbé or the quinuclidine half of the Pseudocinchona alkaloids, spectroscopic evidence is meaningless.

2. Although the 2:3-dimethylindole skeleton is probably common to both types of alkaloids, it was obtained from quinamine by zinc dust distillation - a method which with mitragynine at least gives not an indole but a compound $C_{14}H_{14}ON_2$.

3. Although the quinuclidine (XCVII) fragment could be split and the vinyl group hydrogenated to give 3:4-diethylpyridine,



XCVII

quinuclidine itself gives 4-ethylpyridine[153].

4. Over Adams' Catalyst quinamine, like formosanine, rotundifoline and rhynchophylline, absorbs one molecular equivalent of hydrogen. In the case of quinamine doubtless

it is the vinyl group which is saturated. All alkaloids having the quinuclidine nucleus have the vinyl substituent also. Since mitragynine and mitragynol absorb no hydrogen, in them at least the substituent must be missing. It must be absent also in rotundifoline, for saturation of a vinyl group could not destroy the enolic nature of an enol group, as is done in the conversion of rotundifoline to dihydro-rotundifoline.

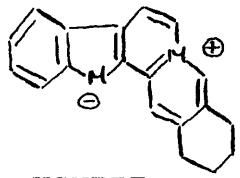
5. As has already been mentioned, in acid solution mitragynol, at least, shows considerable variation in rotation with nature and concentration of the acid. Oudemans[154] found that over a range of 8 different acids present in concentrations ranging from 0.5 to 60 moles, all rotations lay between 108 and 118°.

6. Cinchonamine is described as a strong convulsant[155]. None of the Mitragyna nor Ourouparia alkaloids shows this effect.

7. Finally, there is the fact that, unlike quinamine, the Mitragyna and Ourouparia alkaloids give no reaction with diazotised sulphanilic acid.

Although probably none of these points on its own is sufficient reason for saying that the Mitragyna and Ourouparia alkaloids could not have the cinchonamine-quinamine skeleton, it is felt that the cumulative effect is such as to make the skeleton very improbable.

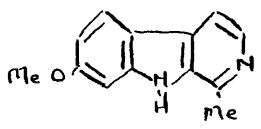
8. Gelsemium Alkaloids - Typical member sempervirine (XCVIII)[158]



XCVIII

Although gelsemine is perhaps more typical of the alkaloids of this group, the only member of which the structure is known is sempervirine. The reasons for the rejection of this formula are the colour of the base and the totally different type of spectrum.

9. Peganumharmala Alkaloids.- Typical member harmine (XCIX)[157]

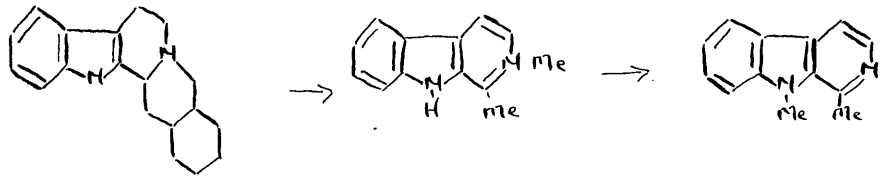


XCIX

The properties of the Mitragyna and Ourouparia alkaloids are quite compatible with their having this β-carboline structure. The 3:4-diethylpyridine might easily be obtained from the rest of the molecule. Since the Rutaceae (cohort of P.harmala) are more primitive than the Rubiaceae, it is understandable that the alkaloids of the genera of the latter will be more complex.

10. Yohimbé Alkaloids - Typical member, yohimbine (LVII) (156).

Only two minor difficulties may be raised against this. Both refer only to mitragynine. First, if mitragynine has the formula $C_{19}H_{26-30}O_4N_2$, then there are not sufficient carbon atoms in the mitragynine skeleton to fit that of yohimbine. However, since, as has been shown on pp. 6 - 9, both the empirical formula and the number of methoxyl groups in mitragynine are uncertain, then this objection is not serious. The second refers to the colour of the base and the nature of the spectrum. Since the base is amorphous, it is possibly impure, and this impurity may account for the colour and the spectrum. It is significant that the colourless crystalline salt gives what may be called a normal spectrum. The third difficulty is that if the product of zinc dust distillation of mitragynine is 1:2-dimethyl-β-carboline, it is difficult to see how this could be formed from a yohimbine skeleton without migration of a methyl group. On the other hand, migration is not impossible.

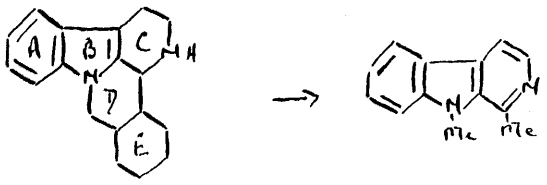


Again, as already pointed out, it is not certain that the product is a β-carboline.

Possible New Type of Skeletal Formula.

To explain more readily the formation of the zinc dust distillation product (2) from mitragynine, if this

product really is formed, the new skeleton (C) is suggested. Degradation could then take place as shown :-



Degradation to 3:4-diethylpyridine and to x:hydroxy-2:3-dimethylindole would present no difficulties.

On the other hand, however, new structural types are not lightly to be invoked, and, as already pointed out, the evidence requiring this new type of formula is very scanty.

Possible Future Work.

It is obvious that, although certain additions have been made to our knowledge of the chemistry of the Mitragyna and Ourouparia alkaloids, much still remains to be done before this is complete. There seem to be several directions in which future work may develop:-

A. More complete characterisation of individual alkaloids and fuller examination of the functions of their oxygen atoms. As is shown by Table 16, much fundamental information about certain alkaloids is still lacking.

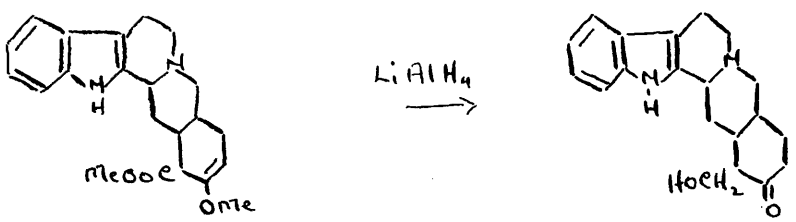
B. Such classical degradations as oxidation, Hoffmann degradation (and the Emde modification), zinc dust distillation, dehydrogenation and alkaline fusion.

C. Certain special reactions.

As mentioned earlier, hydrolysis of the alkaloid and re-esterification merit special investigation.

Lithium aluminium hydride reduction might well give several types of important results. The classical use of the reagent is, of course, to reduce the ester groups to primary alcohols. Because in such reactions as the Hoffmann degradation the ester group of a Mitragyna probably hydrolyse and, by giving an amphoteric product make it more difficult to isolate, prior conversion of the ester to an alcohol would be advantageous. An alternative to this would be hydrolysis and decarboxylation, with the possible danger of decomposition beyond the decarboxylation stage. This happens in rhynchophylline. Two unusual reactions of alkaloids with lithium aluminium hydride may well prove very useful.

(i). Chatterjee & Karrer[169] have recently shown that lithium aluminium hydride attacks the C_{17} methoxy group of corynantheine (CI) to form the keto derivative (CII). Similar



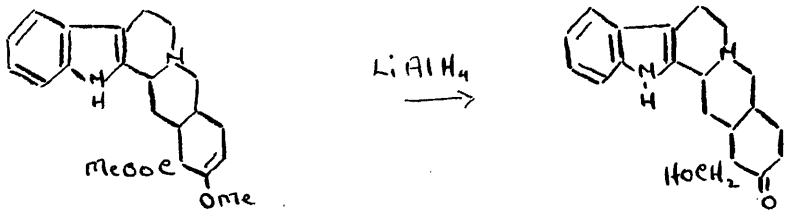
CI CII

ketone formation by Mitragyna and Ourouparia alkaloids would show a further similarity of these with those of the Yohimbé.

As mentioned earlier, hydrolysis of the alkaloid and re-esterification merit special investigation.

Lithium aluminium hydride reduction might well give several types of important results. The classical use of the reagent is, of course, to reduce the ester groups to primary alcohols. Because in such reactions as the Hoffmann degradation the ester group of a Mitragyna probably hydrolyse and, by giving an amphoteric product make it more difficult to isolate, prior conversion of the ester to an alcohol would be advantageous. An alternative to this would be hydrolysis and decarboxylation, with the possible danger of decomposition beyond the decarboxylation stage. This happens in rhynchophylline. Two unusual reactions of alkaloids with lithium aluminium hydride may well prove very useful.

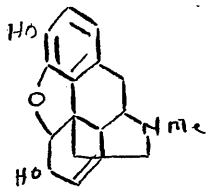
(i). Chatterjee & Karrer[169] have recently shown that lithium aluminium hydride attacks the C_{17} methoxy group of corynantheine (CI) to form the keto derivative (CII). Similar



CI CII

ketone formation by Mitragyna and Ourouparia alkaloids would show a further similarity of these with those of the Yohimbé.

(ii). Recently Briggs & Locker[160] have observed that with solanidine lithium aluminium hydride opens a ring at an oxygen bridge. Such a rupture, if it did occur, would prove the occurrence of ring oxygen and so account for uncharacterised oxygen atoms. In this connection the classical reduction with sodium and amyl alcohol used to open the oxygen ring of morphine (CIII)[161] might also be examined.



CIII

D. Much useful information might also be gained from X-ray crystal structure studies. In the alkaloid field this method has been applied to calycanthine . Although a complete X-ray crystal structure study of a compound is a lengthy process, the method might well be used to determine to which if any of the various groups of indole alkaloids the Mitragyna and Ourouparia alkaloids belong.

E. Infra-red spectroscopy might well give much valuable information, especially as to the nature of substituent groups and of double bonds.

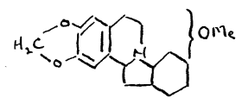
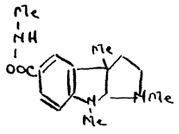
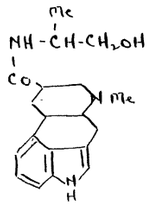
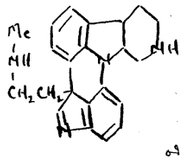
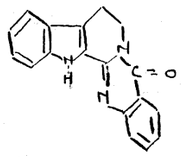
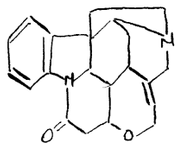
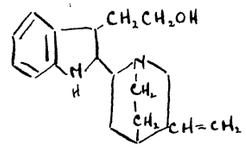
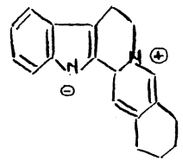
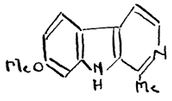
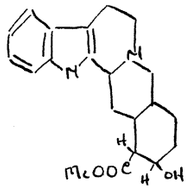
This list is of possible approaches to the problem of the structure of the alkaloids. It is intended not to be exhaustive, but rather to suggest a few of the directions in which work might proceed.

Conclusion.

In this investigation an attempt has been made to classify the known facts about the Mitragyna and Ourouparia alkaloids, and then to fill in some of the worst gaps in our knowledge. Since it has been shown that the alkaloids are indolic, an attempt has been made to compare the properties of the known types of indole alkaloids with those of the Mitragyna alkaloids to see if the latter might belong to one of the known types. It would seem that, although the Pseudocinchona type is possibly that of the Mitragyna's, the most likely structure is that of the Yohimbés. Finally, some suggestions have been made as to possible future work in this field. Although much still remains to be done, it is felt that in the present investigation some contribution has been made to our knowledge of the chemistry of the Mitragyna and Ourouparia alkaloids.

Table 20

Salient Properties of Indole Alkaloids compared with Properties of Ourouperia and Mitragyna Alkaloids.

Type	Erythrina	Rhysostigma	Ergot	Calycanthe	Evodia	Strychnos	Pseudocinchona	Gelsemium	harmala	Yohimbé	Mitragyna
Bot.order	Leguminosae	Leguminosae	Claviceps	Calycanthaceae	Rutaceae	Loganiaceae	Rubiaceae	Loganiaseae	Rutaceae	Rubiaceae	Rubiaceae
Simplest member	Erythraline	Physostigmine	Ergometrine	Calycanthine	Rutecarpine	Strychnine	Cinchonamine	Sempervirine	Harmine	Yohimbine	
				 A or B							
	$C_{18}H_{19}O_3N$	$C_{15}H_{21}O_2N_3$	$C_{19}H_{23}O_2N_3$	$C_{22}H_{26}N_4$		$C_{21}H_{22}O_2N$	$C_{19}H_{24}ON_2$	$C_{19}H_{16}N_2$	$C_{13}H_{12}ON_2$	$C_{21}H_{26}O_3N_2$	
U.V. spectra compared with those of Mitragyna's	Dissimilar	-	Dissimilar	Similar but displaced towards red	-	Similar	Similar		Similar	Similar	
Colour reactions	-	-		Indolic	-	Indolic	Indolic		Indolic	Indolic	Indolic
3:4 Et_2 Pyridane*	Yes	No	No	No	No	Yes	No		No	Yes	Yes
OH:2:3:Me_2 Indole**	?No	?No	No	Yes	Yes	Yes	Yes		Yes	Yes	Yes
Pharmacology	-	Parasympathetic nervous action	Hypertensive Uterine contrcn.	Cardiac depressant Strychninic tetany	Increases arterial pressure	Hypertensive Yetanic convulsions	Blood Hypotensive pressure lowered		Cardiac weakener Uterine contraction	Sympathetdepressant Vasodilator	Hypoten-
Enough C's for this skeletal formula?	Yes	Yes	Yes	Yes, for A	Yes	Yes	Yes	Yes	Yes	Yes	Yes

* Could 3:4-diethylpyridine be obtained as a degradation product from such a nuclear structure?

** If the degradation product from the rhynchophyllic acid is a hydroxy-2:3-dimethyl indole, could this be obtained as a degradation product from such a nuclear structure? One would need to assume, if necessary, the presence of an extra hydroxyl group, as one assumes any necessary extra carbomethoxy group.

Experimental.

Extraction and Purification of Products.

Quinovic Acid.

Extraction from M.inermis Bark.

The 20 kg. of bark from which the alkaloid had been extracted was first let stand for 24 hours with 20 gals. 10% sodium carbonate solution, and then percolated with a further 20 gals. The bark was pressed manually, and the combined extracts, after treatment with supercel, were acidified with 15% hydrochloric acid. After filtration the precipitated acid was redissolved in sodium carbonate solution and re-precipitated. Recrystallisation of the crude acid from ethanol (with charcoal treatment) gave a yield of 50 gms., i.e., 0 = 25% crystalline quinovic acid, m.p. 298° $[\alpha]_D^{20}$ = 99° (c = 2.48 in pyridine, l = 1), = 86° (c = 1.70 as acid in potassium carbonate solution) - lit., m.p. 298°; $[\alpha]_D$ = 87° (as acid in potassium carbonate)

Found: C, 73.8, 74.1; H, 9.3; 9.4%, (equivalent by titration (240, 248, 249).

Calc. for $C_{30}H_{46}O_5$ C, 74.0; H, 9.5% (equivalent by titration 243).

The acid took up no hydrogen over Adams' catalyst in acetic acid. The catalyst was prepared in the usual way.

Methyl Quinovate.

The methyl ester was prepared by the action of excess diazomethane in ether on 0.50 gm. quinovic acid. Recrystallisation of the product from 60-80 petroleum either gave

colourless crystals of methyl quinovate, m.p. 172-3° not depressed on mixing with authentic methyl quinovate; $[\alpha]_D^{21}$ = 117.4° (c = 2.1 in chloroform, l = 1) - lit., m.p. 173-4° $[\alpha]_D$ = 115.2 or 116.8° (in chloroform). A Rast determination of molecular weight gave a value of 517 - calculated for $C_{32}H_{50}O_5$, 514.

Acetyl Quinovic Acid.

After 0.50 gms. of quinovic acid had been refluxed for 4 hours with 2 ml. acetic anhydride (c. 18 moles) and 1 gm. anhydrous sodium acetate (c. 9 moles), the mixture was then poured into water. Triacetyl quinovic acid crystallised out, m.p. 177° - lit. m.p. 180°. Recrystallisation from methanol gave colourless needles of monoacetyl quinovic acid, m.p. 281-282° - lit. 284° (no depression on mixing with authentic mono-acetyl quinovic acid.)

Found: C, 73.0, 72.8; H, 9.05, 9.2%

Calc. for $C_{32}H_{48}O_6$, C, 72.6; H, 9.15%

Isolation of Quinovic Acid from other Species of Mitragyna.

Specimens of bark and leaves of M.ciliata and M.rubrostipulacea were separately let stand for 24 hours in 10% sodium carbonate, and the extracts worked up as already described. In each case the mone-acetyl derivative and the methyl ester were also prepared. Rotations of the specimens of the acid were measured in potassium carbonate solution at 20° (l = 1, c = 1.5 - 2.0). Although mixed melting points

with authentic specimens were determined in all cases, no depression was noted. The physical constants obtained are shown in Table 21.

Table 21.

Source Part of Plant	M.inermis bark	M.ciliata bark	M.ciliata leaves	M.rubrostipulacea bark	M.rubrostipulacea leaves	Literature for Quinovic Acid
acid m.p.	298°	300°	298°	303°	301°	298°
$[\alpha]_D$	+86°	87°	87°	43°, 44°	86°	87°
Monoacetyl Deriv.	281-2°	280-1°	283°	284°	281-1.5°	283-4°
Ester	172-3°	172-3°	171-3°	171-3°	172-4°	173-4°

β-Sitosterol.

M.Raymond-Hamet was kind enough to make available a specimen (thought to be a sterol) of a non-alkaloidal substance extracted from the residues left after removal of alkaloid from the benzene extract of M.inermis bark. The crude material, m.p. 125°, on recrystallisation from methanol, gave colourless needles, m.p. 134-5°; $[\alpha]_D^{20}$ = -35.6° (c = 1.50 in chloroform, l = 1), no depression of m.p. on mixing with authentic β-sitosterol - lit., m.p. 137.5-138.5°; $[\alpha]_D$ = -34.0°. The substance immediately decolourised a 2% solution of potassium permanganate (Ipatieff test for unsaturation) and also decolourised bromine water to give a white precipitate.

Sterol colour reactions[162] were given as follows:-

(a). When a chloroform solution was carefully poured on to

an equal volume of concentrated sulphuric acid, a red-brown interface was formed, and, when the two layers were shaken together, a deeply coloured chloroform and a paler acid layer resulted.

(b). When a chloroform solution containing a few drops of acetone was carefully poured on to an equal volume of concentrated sulphuric acid, a green chloroform and a pale acid layer resulted.

(c). When a methanolic solution was carefully poured on to concentrated sulphuric acid, a brown interface was formed.

(d). No colour was given with a methanolic solution of trichloracetic acid, even after the addition of a few drops of water.

Sterol Acetate.

0.20 gm. sterol were let stand overnight with 2.0 ml. acetic anhydride (c, 35 moles) and 10 ml. freshly dried pyridine. The mixture was then poured into water and the resulting precipitate recrystallised from methanol, m.p. 128° - no depression on mixing with authentic β-sitosterol acetate, $[\alpha]_D^{21} = -38.5^\circ$ (c = 1.20 in chloroform, l = 1) - lit., m.p. 126.5-127.5°; $[\alpha]_D = 34.7^\circ$.

Sterol 3:5-Dinitrobenzoate.

To 1.00 gm. 3:5-dinitrobenzoic acid and 0.80 gm. freshly dried pyridine in 2.0 ml. anhydrous ether was added 0.51 gm. thionyl chloride (1 mole) and the solution

refluxed for 10 mins. (cf. Jackman, Macbeth & Mills[163]). To this 0.20 gms. sterol (1 mole) was added and the mixture first refluxed for one hour and then let stand overnight. Recrystallisation from methanol of the resulting product showed it to be β-sitosterol 3:5-dinitrobenzoate, m.p. 203-204° - no depression on mixing with authentic β-sitosterol 3:5-dinitrobenzoate - $[\alpha]_D^{21} = -20.7°$ (c = 1.20 in chloroform, l = 1) - lit., m.p. 208-209°, $[\alpha]_D = -21.7°$ (in chloroform).

The physical constants of the sterol and its derivatives are compared in Table 22 with those of β-sitosterol.

Table 22.

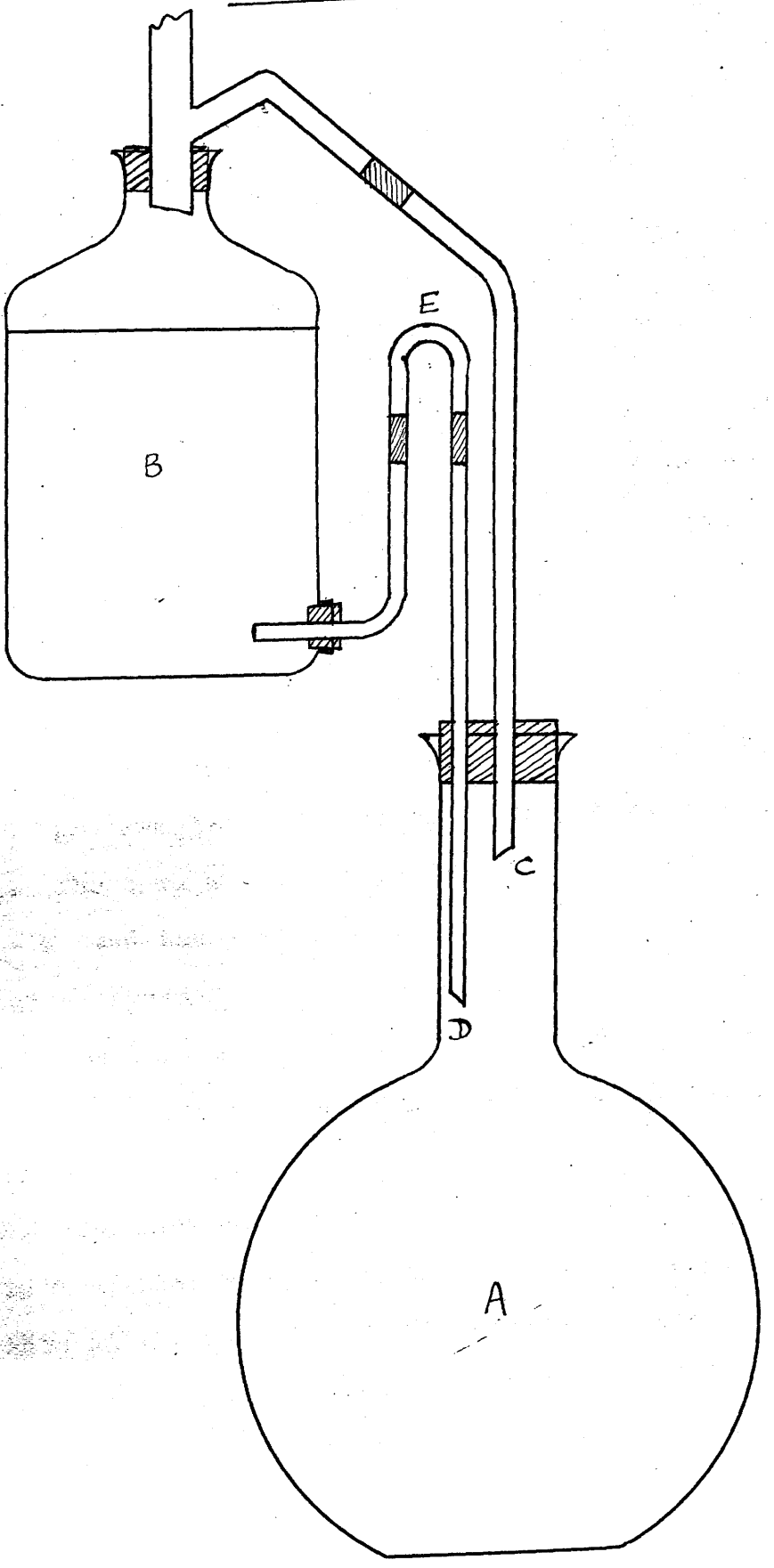
	Sterol from M.inermis	β-sitosterol
Sterol m.p.	134-5°	137.5-138.5°
$[\alpha]_D$	-35.6°	-34.0°
Acetate m.p.	128°	126.5-127.5°
$[\alpha]_D$	-38.5°	-34.7°
3:5-Dinitrobenzoate m.p.	203-4°	208-9°
$[\alpha]_D$	-20.7°	-21.7°

Alkaloids.

Extraction Apparatus.

Plant material was extracted in a modified Soxhlet apparatus adapted from that of Wester[49]. The modified apparatus, illustrated in Fig.1, differed from that of Wester in the following respects:-

FIGURE I



(i). The flask A was placed almost directly under the aspirator B instead of to one side.

(ii). The exit tube C leading from A to the condenser was made considerably wider than the siphon tube D, and did not extend nearly as far into A as did D.

(iii). D was not made from one continuous piece of tubing, but had a separate top piece E connected by rubber tubing to the two limbs of the siphon. If the extractant was slow to start siphoning, this was induced by bending E over and so suddenly increasing the head of liquid.

Trial Extractions.

The following methods of extraction were tried on 500 gm. samples of M.inermis bark ground in a coffee mill:-

1. The bark was let stand for 24 hours in 2% hydrochloric acid, and the alkaloid precipitated from the filtered extract and recrystallised from methanol.

2. The bark was let stand for 24 hours in glacial acetic acid and the filtered extract concentrated in vacuo and worked up as before.

3. The bark was extracted for 12 hours with absolute alcohol in a Soxhlet apparatus, and the concentrated extract poured into dilute ammonia. After being filtered off, the precipitate was extracted with successive quantities of 2% hydrochloric acid until this extract gave a negative test with Mayer's Reagent. The extract was then worked up as in the

first method.

4. The bark was moistened with 10% sodium carbonate solution, air-dried, and then Soxhlet-extracted for 12 hours with benzene. After concentration in vacuo, the concentrate was worked up with 2% hydrochloric acid as was the precipitate in Method 3.

5. The bark was mixed with 20% by weight of magnesium oxide and the mixture extracted as in Method 4.

6. Chloroform was substituted for benzene in Method 4.

7. Calcium oxide was substituted for magnesium oxide in Method 6.

The yields from all seven methods were similar.

Extraction of Alkaloid from Various Plant Materials.

M.inermis. 33 lbs. of finely ground bark were mixed with 11 lbs. calcium oxide, moistened, and air-dried at 40°C. The mixture was Soxhlet-extracted with chloroform until a sample of extractant contained no alkaloid as shown by shaking with dilute hydrochloric acid and then adding Mayer's reagent to the acid. The paste left after removal of the chloroform was extracted with 2% hydrochloric acid until a sample of extractant gave a negative test for alkaloid with Mayer's reagent. Rhynchophylline was precipitated from the acid solution by adding concentrated ammonia.

The following methods were tried for purifying the crude alkaloid:-

1. Chromatography from chloroform. The alumina was freshly heated for 2 hours at 400°, and the chloroform dried over

lime and freshly distilled. No purification was achieved.

2. Chromatography from chlorobenzene. Although the adsorption was much stronger, the method again was unsatisfactory. All attempts to elute the alkaloid with either chlorobenzene or acetone resulted in elution of impurity also.

3. Recrystallisation from methanol. Repeated recrystallisation from methanol (with treatment with animal charcoal) gave 2.0 gms. small colourless prisms, m.p. 212-3° - not depressed on mixing with authentic rhynchophylline, $[\alpha]_D^{19} = -15.7^\circ$ (c = 2.26 in chloroform, l = 1) and 23.0 (c = 1.13) - lit., m.p. 213-5°, $[\alpha]_D = -14.7^\circ$ and -21-23° respectively.

Separation of Rhynchophylline and Rotundifoline.

Attempted solution in chlorobenzene of a mixture of rhynchophylline and rotundifoline extracted from the leaves of M.rotundifolia and supplied by Dr. W. Klyne showed that only rhynchophylline was soluble. The separation was thus easily carried out. On recrystallisation, the rhynchophylline melted at 211-2° and the rotundifoline, at 233-5° - no depressions on mixing with the authentic alkaloids - lit. for rotundifoline, m.p. 233°.

3. M. rubrostipulacea leaves:(i) 100 gms. ground leaves were mixed with 25 gms. magnesium oxide, moistened, air-dried, and let stand 24 hours in chloroform. Working up in the usual way gave a yield of 0.25 gm. mitraphylline. Extraction for

a further 10 hours gave an additional 0.10 gm., i.e. combined yield 0.35%. (ii). 300 gm. ground leaves were mixed with 130 gms. calcium hydroxide, moistened, air-dried and Soxhlet extracted for 10 hours. Working up in the usual way gave a yield of 2.5 gm. crude alkaloid, i.e., 0.50%. Recrystallisation from methanol (with animal charcoal treatment gave pure l-mitraphylline) m.p. 262 - no depression on mixing with either mitraphylline from the bark of M.rubrostipulacea or with an authentic specimen: $[\alpha]_D^{20} = -7.4^\circ$ (c = 1.22 in chloroform, +1 = 1) - lit., m.p. 258-270°, $[\alpha]_D = -9.8^\circ, -7.7^\circ, 0^\circ$.

The picrate prepared from the leaf alkaloid melted at 165° - no depression on mixing with a specimen of the picrate from the bark alkaloid (lit., m.p. 165°).

4. M.rubrostipulacea bark. (i) 135 gms. ground bark were mixed with 35 gm. magnesium oxide, moistened, air-dried, and let stand with chloroform for 24 hours. Yield of alkaloid on working up:- 0.165 gms., i.e., 0.14%.

(ii). 500 gm. ground bark were mixed with 125 gm. calcium oxide, moistened, air-dried, and Soxhlet-extracted for 24 hours with chloroform. Yield of crude alkaloid - 2.5 gms., i.e., 0.50%. Recrystallisation from methanol (with animal charcoal) gave pure d-mitraphylline, m.p. 262° - no depression on mixing with either authentic mitraphylline or the d-mitraphylline obtained from the leaves of M.rubrostipulaeea; $[\alpha]_D^{21} = +7.1^\circ$ (c = 2.00 in chloroform, l = 2).

The picrate prepared from the bark alkaloid melted at 165° - no depression on mixing with the picrate from the leaf alkaloid.

Bulk Extraction of M.rubrostipulacea Leaves and Bark.

20 kg. each of leaves and bark were Soxhlet-extracted as already described. Unfortunately the commercial firm which did the chloroform extraction mixed the extracts.

Hydrochloric acid extraction of the chloroform extract was unexpectedly difficult. After the chloroform had been removed the following methods were tried for extracting the alkaloid from the residual paste:-

(a) A chloroform solution of the paste was shaken with 5% hydrochloric acid. This gave an emulsion which could not be broken either by the addition of water, chloroform or sodium chloride, or by filtration.

(b) A methanolic solution was poured into dilute hydrochloric acid, the precipitate filtered off, and the filtrate basified with ammonia. The difficulties here were the large amount of methanol required, and the partial solubility of mitraphylline in the dilute alcohol.

(c) An acetic acid solution was poured into water. The procedure, but also, unfortunately, the difficulties were the same as in Method (b).

(d) A chloroform solution was chromatographed. The chlorophyll was only partly adsorbed.

(e) The dry extract was let stand for 24 hours in 2% hydrochloric acid. Unfortunately the material had become so hard as to prevent thorough extraction.

(f) The extract was made into a thick paste with chloroform, mixed with sand which had previously been well washed with acid to remove its fairly high iron content, and ground in a mortar with successive quantities of dilute hydrochloric acid. The crude alkaloid precipitated with ammonia from the acid solution was purified in the usual way - yield 14 gm., i.e., 0.035%.

Although the aqueous liquors left after precipitation of alkaloid were extracted with benzene in a continuous liquid extraction for 24 hours, very little further alkaloid was obtained.

5. M.ciliata Leaves. 500 gm. ground leaves were mixed with 100 gm. calcium oxide, moistened, air-dried, and Soxhlet-extracted with chloroform for 16 hours - yield of crude alkaloid on working up:- 0.80 gms., i.e., 0.16%. Recrystallisation from methanol (with charcoal treatment) gave white prisms of rotundifoline, m.p. 233° - no depression on mixing with authentic rotundifoline; $[\alpha]_D^{21} = 123.5°$ (c = 2.1 in chloroform, l = 1). The alkaloid gave a garnet-red colour with ethereal, but no colour with alcoholic ferric chloride. With antimony trichloride a flocculent precipitate was formed.

On micro-hydrogenation in acetic acid over Adams' catalyst, there was an uptake of hydrogen equivalent to one double bond. These tests served to distinguish the alkaloid from dihydro-rotundifoline, which has a similar specific rotation and melting point, and which gives no depression on mixed melting point determination with rotundifoline.

6. M.ciliata Bark. 500 gms. ground bark were mixed with 100 gms. calcium oxide, moistened, air-dried, and Soxhlet-extracted for 16 hours with chloroform - yield of crude alkaloid:- 0.10 gm., i.e., 0.02%. The extraction was repeated with 1700 gm. bark and 300 gm. calcium oxide. Recrystallisation of the crude alkaloid from methanol (with charcoal treatment) gave colourless prisms, m.p. 211° - no depression on mixing with authentic rhynchophylline. While the chloroaurate melted at 133° and decomposed at 155-7° (lit. 134° and 155°), the chloroplatinate decomposed at 236° (lit. 238°). Mixture with specimens of chloroaurate and chloroplatinate prepared from authentic rhynchophylline gave no depressions of melting or decomposition points.

Chemistry of Individual Alkaloids.

Mitraphylline.

Analysis

Found: C, 68.3; H, 7.0; N, 7.3; OMe, 8.5; OMe and NMe, 10.2%

Calc. for $C_{21}H_{26}O_4N_2$: C, 68.0; H, 7.0; N, 7.6; OMe, 8.4.

Active hydrogen atoms were determined at 160° with methyl magnesium iodide in phenetole as described in the separate account of "The Determination of Active Hydrogen Atoms". No. of active hydrogen atoms:- 4.05, 4.1, 4.2, 3.95 and 4.1. (In the last determination the Grignard reagent was dissolved in n-amyl ether and the mitraphylline, in phenetole).

A potentiometric titration was done by adding 0.055 N sodium hydroxide (carbonate free) to 0.100 gm. mitraphylline in 5.0 ml. 0.050 N hydrochloric acid made up to 40 ml. The measurements of p_H were made with a glass electrode at 18°. The results were calculated by the standard method(164). K_B was found to be 9.66. There was only one neutralisation point.

Specific Rotation.

Determinations were made as follows:-

(a) mitraphylline from M.rubrostipulacea leaves: $[\alpha]_D^{20}$ = -7.4 (c = 1.22 in chloroform, l = 1).

(b) Mitraphylline from M.rubrostipulacea bark (in chloroform l = 2, t = 21°).

	Specific Rotation at									
	7000Å	6500	5900	5500	5250	5000	4750	5000/6500	5500/5900	Concn.
[α]	14.3	5.4	7.1	9.2	11.7	14.8	19.1	3.41	1.30	1.96
[α]	2.5	3.5	4.6	5.6	7.7	10.2	13.3	3.99	1.31	0.98
[α]	-	-	7.4	-	-	-	-	-	-	1.62

(c) Mitraphylline supplied by M. Raymond-Hamet, $[\alpha]_D^{21} = 0$ (c = 2.00 in chloroform, l = 1).

(d) Mitraphylline from the mixed extract of bark and leaves of M.rubrostipulacea $[\alpha]_D^{21} = 0$ (c = 1.00 in chloroform, l = 1).

(e) -Mitraphylline in 2N hydrochloric acid (calculated as free mitraphylline, l = 1; t = 21°).

	Specific Rotation at									
	7000Å	6500	5900	5500	5250	5000	4750	λ5000 / λ6500	λ5500 / λ5900	Concn.
[α]	+18.6	22.6	26.7	30.4	34.1	37.8	41.9	1.67	1.14	2.70
[α]	14.8	17.8	21.5	28.9	32.6	36.3	40.8	1.83	1.34	1.35

Ultra-Violet Spectrum.

The ultra-violet spectrum was determined in methanol on a Unicam Spectraphotometer. The methanol was purified by refluxing over iodine for 12 hours and then distilling

λ max 2820, (log ε = 3.45) 2430 (log ε = 4.65)

λ min 2790 (log ε = 3.44) 2305 (log ε = 4.59)

For comparative purposes, the spectrum of calycanthine was determined in ethanol

λ max 2755 (log ε = 3.85) 2490 (log ε = 4.45)

λ min 2745 (log ε = 3.56) 2245 (log ε = 4.07)

These results are shown graphically in Fig.5.

The colour reactions of mitraphylline are given in Table 23.

Table 23.

Reagent	Reaction
H_2SO_4 conc.	Slight white fluorescence
" " - $K_2Cr_2O_7$	Orange-red
HCl dil. - "	Yellow precip. sol. on heat.
HNO_3 conc.	Pale yellow
Erdmann	Very pale yellow-green on heat.
Fröhde	White fluorescence
Mandelin	Brown turning reddish-brown, intense fluorescence.
Kiliani	Orange-pink deepens on standing
H_2SO_4 conc. - $Ce(SO_4)_2$	Pinkish orange fades on standing.
Pine splinter	Pale yellow
Ehrlich	Pinkish-violet on standing
ibid - trace $NaNO_2$ & heat	Yellowish brown
Hopkins-Cole	Brown top layer on standing
HCl dil - MeOH-vanillin	Nil
HCl conc.-vanillin & heat	Orange-red deepening on standing
ibid - glucose & heat	Orange-yellow
H_2SO_4 -conc.-chloral & heat	Colour no deeper than that of "blank".
Voisenet	Nil
HOAc-traces $FeCl_3$ & H_2SO_4 conc.	Cherry-red on heating fading somewhat on standing
HNO_3 conc. + trace $NaNO_2$	Nil.
Thalleoquin	Nil.
Rossi	Nil.
Tetranitromethane	Yellow.

Table 23 (Contd.)

Reagent	Reaction
Br_2-$CHCl_3$	Decolourised.
Ipatieff	Decolourised.
$FeCl_3$-EtOH	Nil.
$FeCl_3$-Et_2O	Nil.
HOAc-$NaNO_2$	Yellow.
Gaebel	Nil.
Labat	Nil.
m-dinitrobenzene -NaOH-MeOH	Nil.

Control experiments done were of two types:-

(a) To assure that the reagents were properly prepared, each was tested against a known substance containing the group sought.

(b) To examine the reactions of the various reagents with compounds possibly related to the Mitragyna and Ourouparia alkaloids, and with non-indolic alkaloids, the further tests listed in Tables 24/-28 were carried out.

Table 24

Yohimbine.

Reagent	Reaction
H_2SO_4 conc.	Very pale greenish-yellow; fluorescent.
ibid - $K_2Cr_2O_7$	Orange; purplish streak on side of tube.
HCl dil.- "	Orange.
HNO_3 conc.	Greenish-yellow.
Erdmann	Greenish-yellow turning orange-brown.
Fröhde	Blue-violet turning violet

Table 24 (Contd.)

Reagent	Reaction
Mandelin	Orange-brown
Kiliani	Purplish-blue turning green
H_2SO_4 conc.-$Ce(SO_4)_2$	Green turning first fairly pale purplish blue and later yellow
Pine splinter	Nil
Ehrlich	Nil
Ibid-trace $NaNO_2$ & heat	Orange-brown on standing
Hopkins-Cole	Nil
HCl dil.-MeOH-vanillin	Nil
HCl conc.-vanillin & heat	Cherry-red
ibid-glucose & heat	Pinkish-purple on standing
H_2SO_4 conc.-chloral & heat	Blackish-purple even in cold
Voisenet	Nil
HOAc-traces $FeCl_3$ & H_2SO_4	Purple on standing
HNO_3 conc.-trace $NaNO_2$	Yellow
Thalleoquin	Nil
Rossi	Very pale pink interface, slight pink tinge in upper zone which is fluorescent
Br_2 - $CHCl_3$	Decolourised
Ipatieff	Decolourised
EtOH - $FeCl_3$	Deep yellow
Et_2O - $FeCl_3$	Nil
HOAc - $NaNO_2$	Yellow
Gaebel	Nil

Table 24 (Contd.)

Reagent	Reaction
Labat	Nil
m-dinitrobenzene-MeOH-NaOH	Nil.

Table 25

Calycanthine

H_2SO_4 conc.	Nil
H_2SO_4 conc. - $K_2Cr_2O_7$	Dark red-brown
HCl dil.- "	Yellow precip. sol. on heat & reprecipd. on cooling
HNO_3 conc.	Green-brown
Erdmann	Brown turning green
Fröhde	Yellow turning yellowish-pink
Mandelin	Scarlet
Kiliani	Brown-green
H_2SO_4 conc. - $Ce(SO_4)_2$	Orange-brown
Pine splinter	Yellow on heating
Ehrlich	Crimson on heating, fades somewhat on standing, regenerated on further heating. After standing several hours has purplish tinge.
ibid-trace $NaNO_2$ & heat	Nil
Hopkins-Cole	Immediate violet interface, then green upper layer later turning violet.
HCl dil. - MeOH-vanillin	Nil
HCl conc.- vanillin & heat	Deep red-brown
ibid - glucose & heat	Red turning purple and fading to yellow
H_2SO_4 conc. - chloral & heat	Violet-black

Table 25 (Contd.)

Reagent	Reaction
Voisenet	Blue on standing
HOAc-traces $FeCl_3$ & H_2SO_4	Pale brown turning purple
HNO_3 conc. - trace $NaNO_2$	Greenish-brown turning brown
Rossi	Both zones yellow
Br_2 -$CHCl_3$	Decolourised. White precipitate
Ipatieff	Decolourised
HOAc - $NaNO_2$	Pale yellow.

Table 26

Quinine.

H_2SO_4 conc.	Nil
ibid - $K_2Cr_2O_7$	Green
HCl dil. - "	Yellow precipitate slowly formed
HNO_3 conc.	Nil
Erdmann	Nil
Fröhde	Nil
Mandelin	Orange-red
Kiliani	Nil
H_2SO_4 conc.-$Ce(SO_4)_2$	Nil
Pine Splinter	Nil
Ehrlich	Nil
ibid-trace $NaNO_2$ & heat	Nil
Hopkins-Cole	Nil
HCl dil.-MeOH-Vanillin	Nil
HCl conc.-Vanillin & heat	Nil

Table 26 (Contd.)

Reagent	Reaction
HCl conc. - glucose & heat	Nil
H_2SO_4 conc. - Chloral & heat	Nil
HOAc - traces $FeCl_3$ & H_2SO_4	Nil
HNO_3 conc. - trace $NaNO_2$	Nil
Rossi	Nil.

Table 27

Carbazole.

H_2SO_4 conc.	Yellow quickly fading; yellow fluorescence
ibid - $K_2Cr_2O_7$	Deep blue-green
HCl dil.- "	Nil
HNO_3 conc.	Green changing via yellow-green to yellow
Erdmann	Greenish-blue
Fröhde	Immediate green-blue rapidly intensifying.
Mandelin	Dark green
Kiliani	Green
H_2SO_4 conc. - $Ce(SO_4)_2$	Green
Pine splinter	Green
Ehrlich	Very pale pinkish-blue on heat
ibid - $NaNO_2$ & heat	Nil
Hopkins-Cole	Pale interface fading on standing. Shaking gives blue bottom layer
HCl dil.-MeOH-Vanillin	Nil
HCl conc.-Vanillin & heat	Pinkish-blue rapidly going violet. Fades somewhat on standing

Table 27 (Contd.)

Reagent	Reaction
HCl conc.-glucose & heat	Very pale pink turning pinkish blue
H_2SO_4 conc.-chloral & heat	Yellow in cold. On heat. rapidly deepens via purple to deep blue
Voisenet	Nil
HOAc - traces $FeCl_3$ & H_2SO_4	Nil
HNO_3 conc. - trace $NaNO_2$	Greenish yellow
Thalleoquin	Nil
Br_2 - $CHCl_3$	Decolourised
Ipatieff	Decolourised
NaHOAc - $NaNO_2$	Yellow-green

Table 28

Indole

H_2SO_4 conc.	Yellow fluorescence
ibid - $K_2Cr_2O_7$	Purplish, turning dirty green
HCl dil.- "	Orange
HNO_3 conc.	Dark brown
Erdmann	Yellow turning brown
Fröhde	Greenish-yellow turning green
Mandelin	Dark brown
Kiliani	Deep blue burning yellow
H_2SO_4 conc. - $Ce(SO_4)_2$	Cherry-purple turning orange-yellow
Pine Splinter	Immediate pink
Ehrlich	Violet
ibid-trace $NaNO_2$ & heat	Orange
Hopkins-Cole	Top layer cherry; bottom, brown

Table 28 (Contd.)

Reagent	Reaction
HCl dil. - MeOH-Vanillin	Pinkish-orange
HCl conc. - Vanillin	Cherry turning deep blood red on heating
ibid - Glucose	Slight pink in cold; orange on heating turning brown
H_2SO_4 conc. - Chloral	Yellow deepening to reddish-brown
Voisenet	Nil
HOAc - traces $FeCl_3$ & H_2SO_4	Reddish orange
HNO_3 conc. - trace $NaNO_2$	Red-brown
Thalleoquin	Nil
Rossi	Cherry-purple interface; salmon lower layer; upper layer slowly becomes green
Tetranitromethane	Dark reddish-brown
Br_2-$CHCl_3$	Decolourises
HOAc - $NaNO_2$	Cherry.

8-Hydroxyquinoline gave a yellow solution in acids. With Fröhde's reagent it gave a greenish-yellow, with Mandelin's a brown colour, and with chloral hydrate in concentrated sulphuric acid on heating a bluish green with a violet fluorescence. No reaction was given with any of the other oxidation or indole reagents.

2-Hydroxyquinoline gave no reaction with any of the indolic reagents.

Attempts to Acetylate Mitraphylline.

(a). 0.20 gm. mitraphylline was let stand for four days

with 2.0 ml. (4 moles) acetic anhydride in 15 ml. freshly dried pyridine and the reaction mixture then poured into water. Addition of concentrated ammonia gave a precipitate which on recrystallisation from ethanol proved to be unchanged mitraphylline, m.p. 263° - no depression on mixing with authentic mitraphylline.

(b). 0.20 gm. mitraphylline was let stand overnight with 1 ml. acetyl chloride (5 moles) in 10 ml. freshly dried pyridine, and the reaction mixture then poured into water. Addition of concentrated ammonia gave a precipitate which on recrystallisation proved to be unchanged mitraphylline, m.p. 262° - no depression on mixing with authentic mitraphylline.

(c). 0.20 gm. mitraphylline was refluxed 6 hours with 1 ml. acetic anhydride (2 moles) in 10 ml. freshly dried pyridine. On being worked up in the usual way the product was found to be unchanged mitraphylline, m.p. 264° - no depression on mixing with authentic mitraphylline.

(d). 0.20 gm. mitraphylline and 0.50 gm. freshly fused sodium acetate (15 moles) were refluxed with 1.0 ml. acetic anhydride in 10 ml. freshly dried pyridine, and then let stand overnight. On being worked up in the usual way the product was found to be unchanged mitraphylline, m.p. 264° - no depression on mixing with authentic mitraphylline.

(e) 0.20 gm. mitraphylline was let stand one hour with 7 ml. acetic anhydride and 0.7 gm. magnesium in freshly dried

chloroform. After filtration the solution was shaken with ammonia. The chloroform layer was then separated off and evaporated to dryness on the steam bath. Recrystallisation of the product from methanol showed it to be unchanged mitraphylline, m.p. 264° - no depression on mixing with authentic mitraphylline.

Action of 3:5-Dinitrobenzoyl Chloride.

0.26 gm. thionyl chloride was added to 0.50 gm. 3:5-dinitrobenzoic acid (0.5 mole) and 0.4 ml. freshly dried pyridine in 5.0 ml. anhydrous ether and refluxed for 10 minutes. After 0.10 gm. (0.5 mole) mitraphylline had been added the mixture was refluxed for a further hour and then let stand overnight. Pouring the ethereal solution into ammonia and allowing the ether to evaporate led to the formation of a precipitate which on recrystallisation from methanol proved to be unchanged mitraphylline, m.p. 261° no depression on mixing with authentic mitraphylline.

Action of 2:4-Dinitrophenyl Hydrazine.

0.20 gm. mitraphylline and 0.11 gm. 2:4-dinitrophenylhydrazine (1.0 mole) and 0.5 ml. concentrated sulphuric acid in 10 ml. ethanol were let stand 40 hours, and then heated 30 minutes on the steam bath. Since no precipitate appeared, the solution was evaporated on the steam bath to small bulk and then basified with ammonia. Recrystallisation from ethanol of the product proved it to be unchanged

mitraphylline, m.p. 262° - no depression on mixing with authentic mitraphylline.

Action of Hydroxylamine Hydrochloride.

(a) 0.10 gm. mitraphylline, 1.0 gm. hydroxylamine hydrochloride (5 moles) and 0.20 gm. sodium acetate (12 moles) were refluxed for 3 hours on the steam bath in 2 ml. water and 7 ml. ethanol. Since no precipitate appeared, the solution was made alkaline with ammonia. On recrystallisation from ethanol the resulting precipitate proved to be unchanged mitraphylline; m.p. 262° - no depression on mixing with authentic mitraphylline.

(b) 0.05 gm. each of hydroxylamine hydrochloride and mitraphylline in 5 ml. anhydrous pyridine were heated for 4 hours on the steam bath. Since no precipitate appeared on standing overnight, concentrated ammonia was added. Unchanged mitraphylline crystallised out, m.p. 263° - no depression on mixing with authentic mitraphylline.

Action of Diazomethane.

0.10 gm. mitraphylline in 5 ml. ether and 2 drops methanol were stood for 3 days with 0.15 gm. diazomethane (13 moles) (prepared in the usual way from Nenitrosomethylurea). The product left after removal of the ether and diazomethane proved, on recrystallisation from methanol, to be unchanged mitraphylline, m.p. 265° - no depression on mixing with authentic mitraphylline.

Action of Benzaldehyde.

0.05 gms. mitraphylline and 1.0 ml. freshly distilled benzaldehyde (c 70 moles) were refluxed for 3 hours in 5 ml. methanol. No precipitate nor change of colour resulted. After evaporation on the steam bath almost to dryness, the residue was washed with dilute acid. Addition of ammonia to the acid extract gave a precipitate. Although the absence of colour suggested the mitraphylline had remained unchanged, insufficient was recovered for recrystallisation.

Mitraphylline Picrate.

To 0.20 gm. mitraphylline in a minimum amount of methanol was added 0.07 gm. picric acid (1 mole) in 10% ethanol. The mitraphylline picrate, which immediately precipitated out, was recrystallised from methanol.

Found: C, 51.2, 51.2; H, 5.0, 5.0; N, 11.0, 10.8%.

$C_{21}H_{26}O_4N_2, C_6H_3O_7N_3, 2H_2O$ requires: C, 51.0; H, 5.2; N, 10.8%

(lit., m.p. 165°, no analyses given).

Hydrogenation.

Microhydrogenation of mitraphylline in glacial acetic acid over Adams' catalyst gave an absorption of 3 Moles of hydrogen - the first in 18 minutes, and the other two each in 40 minutes. After the solution had been filtered it was cautiously neutralised with concentrated ammonia. On

recrystallisation, the resulting precipitate gave colourless crystals, m.p. 252-3°.

Found: C, 67.6; H, 7.7; OMe, 8.5%

$C_{21}H_{32}O_4N_2$ requires: C, 68.0; H, 8.5; OMe, 8.4%.

Attempts to prepare the picrate, styphnate picrolonate and trinitrobenzene derivative of hexahydromitraphylline by mixing 0.05 gm. of the base with the equivalent amount of reagent in methanol were unsuccessful.

Action of Bromine.

To 0.50 gm. mitraphylline in chloroform a chloroform solution of bromine was added until a faint colour persisted. Removal of the chloroform on the seam bath left a residue only partially soluble in dilute acid. Refluxing the insoluble residue for 7 hours with acetone led to the formation of lachrimatory fumes (probably of bromacetone). The residue left on removal of the acetone was now soluble in dilute acid. This acid solution was now combined with the previous acid extract, and the base precipitated with concentrated ammonia. Since recrystallisation from methanol, ethanol, and acetone proved impracticable, purification was effected as follows. A methanolic solution was decolourised with animal charcoal. The methanol was removed on the steam bath and the compound purified to constant melting point by repeated solution in dilute acid, and reprecipitation with

ammonia. The substance melted at 195-8°. Addition of chloroplatinic acid to a solution of the substance in concentrated sulphuric acid gave a chloroplatinate. After recrystallisation from 50% methanol this melted with decomposition at 230°.

Action with Diazotised Sulphanilic Acid.

Diazotised sulphanilic acid was prepared in the usual way by adding 3.5 gm. sodium nitrite (1 mole) to an ice-cooled solution of 10 gm. sulphanilic acid in sufficient N sodium carbonate to dissolve the acid. To destroy any excess nitrous acid, 0.5 gm. urea was added. A few drops of this solution added to 0.01 gm. mitraphylline in 1 ml. N hydrochloric acid gave a yellow colouration. No change of colour, nor formation of precipitate resulted on letting the solution stand for 12 hours or on making alkaline with 2 N sodium hydroxide. Although similar results were given by aniline, yohimbine and calycanthine, dimethyl- and diethyl/aniline immediately gave precipitates of methyl orange and its homologue.

Hydrolysis.

(a). 1.0 gm. mitraphylline was refluxed for three hours with 35 ml. 4N methanolic caustic potash and then let stand overnight. After neutralisation to about p_H 7 (to universal indicator) the solution was concentrated to small bulk, and since careful titration over the range p_H 3 to 9 failed to

give any precipitate, the p_H was adjusted to 7 and the solution extracted with chloroform. Removal of the chloroform left 0.75 gm. crude acid extremely soluble in methanol, ethanol and chloroform, but insoluble in ether, benzene and chlorobenzene. After unsuccessful attempts at recrystallisation from methanol, ethanol and chloroform and mixtures of these with ether and with one another, chromatography was tried. Treatment of chloroform and chloroform-chlorobenzene solutions on silica columns was unsuccessful. The purest specimen of acid, m.p. 173°, was obtained ultimately from a chloroform-benzene solution. This was soluble in both dilute acid and sodium carbonate. No colour was given with alcoholic or ethereal-alcoholic ferric chloride. The ultra-violet spectrum was determined in methanol.

λ max 2800 (log ε = 3.63), 2390 (log ε = 4.33)

λ min 2780 (log ε = 3.62), 2260 (log ε = 4.18).

These results are shown graphically in Fig. 5.

(b) 0.20 gms. mitraphylline was refluxed for 2 hours with 10 ml. 50% hydrochloric acid. Again, after the approximately neutralised solution had been concentrated to small volume, titration over the p_H range 3 to 9 failed to give a precipitate, the reaction mixture was neutralised to p_H 7 (to universal indicator) and then extracted first with ether and then with chloroform. Removal on the water bath of the solvents from the combined extracts left no visible residue. The flask,

however, had a peculiar phenolic or indolic odour.

Methyl Mitraphyllate.

To 0.20 gms. mitraphyllic acid in 2 ml. methanol was added 0.30 gms. of diazomethane (12 moles) in 10 ml. ether. Although nitrogen was immediately evolved, the reaction mixture was let stand for 3 days before removal of the solvents and excess diazomethane. The resulting solid was extremely soluble in acetone, benzene, methanol, ethanol and dilute acids, but insoluble in alkali. After fruitless attempts at purification by recrystallisation, the purification was finally achieved by repeated solution in dilute acid and reprecipitation with ammonia. The ultra-violet spectrum was determined in ethanol.

λ max. 2840 ($\log \varepsilon = 3.40$), 2410 ($\log \varepsilon = 4.00$)

λ min. 2800 ($\log \varepsilon = 3.35$), 2345 ($\log \varepsilon = 3.99$).

These results are shown graphically in Fig.5.

Action of Methyl Iodide.

1.0 gm. mitraphylline was refluxed for 6 hours with 1.0 gm. methyl iodide (2.5 moles) in 30 ml. acetone. Removal of the acetone on the water bath left a yellowish brown methiodide extremely soluble in acetone, methanol and ethanol and insoluble in benzene, chloroform and petroleum ether. The crude amorphous material melted at 85-7°. All attempts to crystallise it failed, c.f. mitragynine methiodide[2].

Potash Fusion.

1.0 gm. mitraphylline was fused with 2.0 gm. potassium hydroxide (14 moles) for 90 minutes at 260-280°. During the fusion, which was accompanied by considerable charring, an unpleasant indolic odour was noticed. Ether and chloroform failed to extract any organic material from the cooled fusion mixture. It was then made acid with concentrated hydrochloric acid and again extracted as before without result. Finally, the now acid solution was made alkaline with sodium carbonate and re-neutralised with acetic acid. Chloroform and ether extraction were again fruitless.

Mitragynine.

Through the courtesy of Mrs. E. Stedman of Edinburgh University, I was able to examine specimens of mitragynine acetate and picrate.

Isolation of Base.

Mitragynine was precipitated by the addition of concentrated ammonia to solutions of the picrate in acetone and the acetate in dilute hydrochloric acid. The crude base was redissolved in dilute hydrochloric acid and re-precipitated with concentrated ammonia.

Analysis

Found (for picrate) : N, 10.65%.

Calc. for $C_{22}H_{32}O_4N_2, C_6H_3O_7N_3$, N, 11.3%
$C_{22}H_{31}O_5N_1, C_6H_3O_7N_3$, N, 9.1%
$C_{23}H_{34}O_4N_2, C_6H_3O_7N_3$, N, 11.1%.

Active hydrogen. Found 3.1 active hydrogen atoms at 160°.

Specific Rotations

Rotations were determined in chloroform as follows (t = 14°, l = 0.75)

Specific Rotation at								
7000Å	6500	5900	5500	5250	50000	$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	Concn.
[α] -	-44.4°	49.8	57.8	63.1	68.4	1.54	1.16	1.50
[α] -32.6	46.2	58.7	69.6	77.6	-	1.68	1.19	0.75

Ultra-Violet Spectra.

The ultra-violet spectrum of mitragynine acetate was determined in distilled water

λ max. 2900Å (log ε = 3.77), 2480 (log ε = 4.20)

λ min. 2880Å (log ε = 3.75), 2250 (log ε = 4.16)

The ultra-violet spectrum of mitragynine was determined in ethanol.

λ max. 3555Å (log ε = 3.34), 2910 (log ε = 3.00)

λ min. 3030Å (log ε = 2.70), 2880 (log ε = 2.95)

λ inflexn. 2800Å (log ε = 3.03), 2400 (log ε = 3.64).

These results are shown graphically in Fig. 6.

Colour Reactions.

These are shown in Table 29.

Table 29.

Reagent	Reaction
H_2SO_4 conc.	Orange-red
ibid - $K_2Cr_2O_7$	Dark brown
HCl dil- "	Nil

Table 29 (Contd.)

Reagent	Reaction
HNO_3 conc.	Yellow rapidly becoming brown
Erdmann	Green rapidly deepening & ultimately burning dark brown
Fröhde	Red rapidly passing to deep green
Mandelin	Red-brown rapidly darkening
Kiliani	Greyish blue-green
H_2SO_4 conc. -$Ce(SO_4)_2$	Red turning black
Pine Splinter	Nil
Ehrlich	Pale green turning purple
ibid-trace $NaNO_2$ & heat	Orange-brown
Hopkins-Cole	Brownish bottom layer; purple interface
HCl dil.-MeOH-Vanillin	Nil
HCl conc.-Vanillin	Violet
ibid-Glucose & heat	Transient cherry turning purple
H_2SO_4 conc.-Chloral & heat	Transient red-brown turning black
Voisenet	Nil
HOAc-trace $FeCl_3$ & H_2SO_4	Green
HNO_3 conc.-trace $NaNO_2$	Dark brown
Thalleoquin	Nil
Rossi	Nil
Tetra-nitromethane	Orange-brown
Br_2-$CHCl_3$	Decolourised
Ipatieff	Decolourised
EtOH-$FeCl_3$	Nil

Table 29 (Contd.)

Reagent	Reaction
Et_2O-$FeCl_3$	Nil
HOAc-$NaNO_2$	Brown
Gaebel	Nil
Gallic Acid	Nil
n-Dinitrobenzene-MeOH-NaOH	Nil

Action of 2:4-Dinitrophenylhydrazine.

0.02 gm. mitragynine was heated for 5 minutes on the water bath with 1 ml. freshly prepared Brady's reagent (1.0 gm. 2:4-dinitrophenylhydrazine in 4 ml. concentrated sulphuric acid and 30 ml. methanol) and the mixture was then let stand overnight. No precipitate was formed and no colour change observed.

Action of Diazomethane.

To 0.20 gm. mitragynine was added 0.30 gm. (13 moles) diazomethane with 1 ml.methanol in 10 ml. ether, and the reaction mixture let stand 3 days. Removal of solvents and excess diazomethane left a brownish paste. Addition of a methanolic solution of this to a solution of picric acid in 10% ethanol gave a voluminous precipitate, which on recrystallisation from methanol proved to be mitraphylline picrate, m.p. 113-5° - no depression on mixing with authentic mitraphylline picrate (lit., m.p. 116-123°).

Action of Diazotised Sulphanilic Acid.

Addition of a few drops of a solution of diazotised sulphanilic acid to a solution of 0.01 gm. mitraphylline in 1.0 ml. N hydrochloric acid gave a yellow colouration. No change of colour nor formation of precipitate resulted either on letting the solution stand for 12 hours, or on making alkaline with 2N sodium hydroxide solution.

Potash Fusion.

1.0 gm. mitragynine acetate was added to 1.0 gm. potassium hydroxide dissolved in a minimum quantity of water. The mixture was heated in a metal bath first for 30 minutes at 260-280°, and then at 280-300° for one hour. An inflammable gas accompanied by an unpleasant odour was given off. Extraction of the cooled mixture with methanol gave only potassium hydroxide. Evaporation on the water bath of a chloroform extract gave no residue. The mixture was next acidified with concentrated hydrochloric acid, and extracted unsuccessfully with ether and chloroform. Finally the now-acid solution was made alkaline with sodium carbonate and the neutralised with acetic acid. Again ether and chloroform failed to extract any organic material.

Selenium Dehydrogenation.

0.30 gm. mitragynine mixed with 0.25 (4 moles) selenium was heated in sealed tube for 24 hours at 300°. The product was extracted first with dilute hydrochloric

acid and then with chloroform but no fusion product was isolated in sufficient quantity to permit identification.

Mitraversine.

Mrs. E. Stedman was kind enough to give me about 0.3 gm. of mitraversine. Mitraversine was found to be soluble in sodium hydroxide but not in sodium carbonate. Mixed melting point determinations with both rotundifoline and dihydrorotundifoline showed definite depressions.

Specific Rotations.

Rotations of the free base in chloroform (t = 14°, l = 0.75) were as follows:-

	Specific Rotation at								
	7000Å	6500	5900	5500	5250	5000	4750	$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	Conc.
[α]	+31°	29	37	47	52	57	63	1.96	1.27	1.33
[α]	30°	28	38	47	48	54	59	1.93	1.24	0.67

The rotations of hydrochloride (calculated as free base) in 2N hydrochloric acid (t = 14°, l = 0.75) were:-

	Specific Rotation at						
	7000Å	6500	5900	5500	5250	5000	$\frac{\lambda 5500}{\lambda 5900}$	Concn.
[α]	-13.3°	-6.7	+2.7	13.3	17.3	24.0	4.93	1.00

Ultra-Violet Spectrum.

The ultra-violet spectrum was determined in methanol

λ max. 2885Å (log ε = 3.15) 2400 (log ε = 3.99)

λ min. 2765Å (log ε = 3.10) 2295 (log ε = 3.98)

These results are shown graphically in Fig.6.

Formosanine.

M.Raymond-Hamet was kind enough to give me about 0.2 gm. formosanine, m.p. 211-4° (lit. 202-8°).

Specific Rotations.

The rotations of the base in chloroform (t = 18°, l = 0.75) were:-

	Specific Rotation at								
	7000Å	6500	5900	5500	5250	5000	4700	$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	Concn.
[α]	+77.3°	83.3	90	102	114.7	128.7	142.7	1.54	1.13	2.00
[α]	76	82.7	90.7	102	114.7	128	142.7	1.54	1.13	1.00

The rotations of the hydrochloride (calculated as free base) in 2N hydrochloric acid (t = 18°, l = 0.75) were:-

	Specific Rotation at								
	7000Å	6500	5900	5500	5250	5000	4750	$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	Concn.
[α]	+20°	22.7	25.3	30.7	38.7	45.3	52	2.00	1.21	1.00
[α]	18.7	23.3	26.7	32	37.3	45.3	50.7	1.95	1.20	0.50

Colour Reactions:

These are shown in Table 30.

Table 30.

Colour Reactions

Reagent	Reaction
H_2SO_4 conc.	Nil
ibid-$K_2Cr_2O_7$	Brownish green
HCl dil.- "	Nil
HNO_3 conc.	Nil
Erdmann	Very pale green on standing
Fröhde	Nil

Table 30 (Contd.)

Reagent	Reaction
Mandelin	Reddish orange quickly turning orange brown; green fluorescence
Kiliani	Nil
H_2SO_4 conc.-$Ce(SO_4)_2$	Reddish brown turning brown on standing
Pine Splinter	Nil
Ehrlich	Nil
Ibid-trace $NaNO_2$ & heat	Yellow - more intense than "blank"
Hopkins-Cole	Nil
HCl.dil-MeOH-Vanillin	Nil
HCl conc.-Vanillin & heat	Very pale pink on standing
ibid-Glucose & heat	Nil
H_2SO_4 conc.-Chloral " "	Yellow-brown
Voisenet	Nil
HOAc-trace $FeCl_3$ & H_2SO_4	Nil
Conc. HNO_3-trace $NaNO_2$	Nil
Thalleoquin	Nil
Rossi	Nil
Br_2-$CHCl_3$	Decolourised
Ipatieff	Decolourised
EtOH-$FeCl_3$	Nil
Et_2O-$FeCl_3$	Nil
HOAc-$NaNO_2$	Pale yellow
Gaebel	Nil
Gallic Acid	Nil
m-Dinitrobenzene-MeOH-NaOH	Nil

Action of 2:4-Dinitrophenylhydrazine.

0.02 gm. formosanine was heated 5 minutes on the water bath with 1 ml. freshly prepared Brady's reagent, and then let stand overnight. No formation of precipitate nor change of colour was observed.

Action of Diazomethane.

To 0.02 gm. formosanine dissolved in 0.5 ml. methanol and 5.0 ml. ether was added 0.03 gm. diazomethane (13 moles) in 5 ml. ether. After the mixture had stood for 3 days the solvents and excess diazomethane were allowed to evaporate off, and the residue recrystallised from methanol. Only unchanged formosanine was obtained, m.p. 205° - no depression on mixing with authentic formosanine.

Action of Diazotised Sulphanilic Acid.

Addition of a few drops of a solutionof diazotised sulphanilic acid to a solution of 0.01 gm. formosanine in 1.0 ml. N hydrochloric acid gave a yellow colouration. No change of colour nor formation of precipitate resulted either on letting the solution stand for 12 hours or on making it alkaline with 2N sodium hydroxide.

Hydrogenation.

On microhydrogenation over Adams' catalyst in glacial acetic acid, formosanine took up 0.9 molecular equivalents of hydrogen in 30 minutes. The filtered acetic acid solution of dihydroformosanine was neutralised with concentrated

ammonia and the reduced base filtered off. Its spectrum was determined in ethanol.

λmax. 2415 (log ε = 4.21)

λmin. 2235 (log ε = 4.06)

These results are shown graphically in Fig.7.

Rotundifoline.

Analysis

Found: OMe, 16.2%

Calc. for $C_{22}H_{26}O_5N_2$ OMe, 17.8%.

Active hydrogen at 160° - 3.2, 3.05, 3.1 (latter value with rotundifoline dissolved in phenetole and Grignard reagent in amyl ether).

Specific Rotations.

Rotations in chloroform (t = 21°, l = 1 , R = alkaloid from M.rotundifolia, C = alkaloid from M.Ciliata) were:-

7000Å	6500	5900	5500	5250	5000	4750	$\frac{5000}{6500}$	$\frac{5500}{5900}$	Con.	Alkaloid
[α] +85.7	98.6	123.3	145.2	163.9	184.8	217.6	1.87	1.18	2.10	C
[α] 84.8	97.6	122.4	143.8	162.4	184.3	214.3	1.89	1.18	2.10	R
[α] 88.6	102.8	123.8	148.6	168.6	191.4	221.0	1.86	1.20	1.05	R
[α] 89.1	102.8	127.6	154.3	171.4	194.3	228.6	1.89	1.12	0.525	R

These rotations are shown graphically in Fig.4.

Rotations of the hydrochloride (calculated as free base) in 2N hydrochloric acid (t = 21°, l = 1) were:-

Specific Rotations at

7000Å	6500	5900	5500	5250	5000	4750	$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	Concn.
$[\alpha]$ +44.8°	48.4	60.9	70.6	79.0	90.7	109.6	1.87	1.16	2.48

Ultra-Violet Spectra.

The ultra-violet spectrum was determined in methanol.

λ max. 2925Å (log ε = 3.47)

λ min. 2770Å (log ε =3.29)

λ inflexn. 2390Å (log ε =4.23).

These results are shown graphically in Fig.8.

The ultra-violet spectrum was also determined in very dilute hydrochloric acid.

λ max. 2900Å (log ε = 3.54); 2470 (log ε = 4.25)

λ min. 2760Å (log ε = 3.38).

These results are shown graphically in Fig.9.

Colour Reactions.

These are shown in Table 31.

Table 31.

Reagent	Reaction
H_2SO_4 conc.	Very pale yellow on standing; white fluorescence
ibid-$K_2Cr_2O_7$	Orange brown
HCl dil.- "	Nil
HNO_3 conc.	Pale yellow; slight green fluorescence
Erdmann	Very pale yellow burning blue-green on standing.
Fröhde	White fluorescence
Mandelin	Deep red-brown; yellow fluorescence

Table 31 (Contd.)

Reagent	Reaction
Kiliani	Orange-pink deepening on standing
H_2SO_4 conc.-$Ce(SO_4)_2$	Pinkish orange fading on standing
Pine Splinter	Yellow
Ehrlich	Nil
ibid-trace $NaNO_2$ & heat	Orange-yellow turning brown
Hopkins-Cole	Nil
HCl dil.-MeOH-Vanillin	Nil
HCl conc.-Vanillin & heat	Nil
ibid-Glucose & heat	Pinkish yellow turning orange
H_2SO_4 conc.-Chloral " "	Nil
Voisenet	Nil
HOAc-traces $FeCl_3$ & H_2SO_4	Nil even on heating
HNO_3 conc.-trace $NaNO_2$	Yellow on heating
Thalleoquin	Nil
Rossi	Nil
Tetra-nitromethane	Orange
Br_2-$CHCl_3$	Decolourised
Ipatieff	Decolourised
EtOH-$FeCl_3$	Nil
Et_2O-$FeCl_3$	Garnet red
HOAc-$NaNO_2$	Orange
Gaebel	Nil
Gallic Acid	Nil
m-Dinitrobenzene-MeOH-NaOH	Nil

Action with Antimony Trichloride.

With rotundifoline antimony trichloride in dry chloroform gave a flocculent precipitate.

Rotundifoline Chloroplatinate.

Addition of chloroplatinic acid in 50% hydrochloric acid to a concentrated hydrochloric acid solution of rotundifoline gave orange-yellow needles which on recrystallisation from water decomposed at 242-4°.

Found: C, 43.4; H, 5.05%

Calculated for $C_{44}H_{54}O_{10}N_4PtCl_6$ C, 43.7; H, 4.6%.

Action with Acetic Anhydride.

0.20 gm. rotundifoline, 1.0 ml. acetic anhydride (20 moles) ~~acetic-anhydride~~, and 0.50 gm. freshly fused sodium acetate (16 moles) were refluxed for 6 hours in 10 ml. freshly dried pyridine. The mixture was then poured into ammonia. Recrystallisation from 60-80 petroleum ether of the resulting precipitate showed it to be unchanged rotundifoline, m.p. 235° - no depression on mixing with authentic rotundifoline.

Action with 3:5-Dinitrobenzoyl Chloride.

0.26 gm. thionyl chloride was added to 0.50 gm. 3:5-dinitrobenzoic acid in 5 ml. freshly dried anhydrous ether, and the whole refluxed for 10 minutes. After the addition of 0.10 gm. rotundifoline, the mixture was refluxed for a further 30 minutes and let stand overnight. The

solution was then poured into dilute ammonia. Recrystallisation from methanol showed that the resulting precipitate was unchanged rotundifoline, m.p. 235° - no depression on mixing with authentic rotundifoline.

Action with 2:4-Dinitrophenylhydrazine.

0.10 gm. rotundifoline was refluxed 30 minutes with 2.0 ml. freshly prepared Brady's reagent and the mixture let stand overnight. Since no formation of precipitate nor change of colour was observed, the mixture was then poured into dilute ammonia. Recrystallisation from methanol showed the resulting precipitate to be unchanged rotundifoline, m.p. 234° - no depression on mixing with authentic rotundifoline.

Action with Hydroxylamine Hydrochloride.

(i) 0.10 gm. each of rotundifoline, hydroxylamine hydrochloride (5.5 mole) and fused sodium acetate (6.5 mole) were refluxed 3 hours in 2 ml. water and 7 ml. ethanol. Since no precipitate appeared the solution was made alkaline with dilute ammonia. Recrystallisation from methanol showed the resulting precipitate to be unchanged rotundifoline, m.p. 233-234° - no depression on mixing with authentic rotundifoline.

(ii) 0.10 gm. each of rotundifoline and hydroxylamine hydrochloride were refluxed for 4 hours in 10 ml. freshly dried pyridine. Since no precipitate appeared on standing

overnight, concentrated ammonia was then added to the solution. Rotundifoline slowly crystallised, m.p. 233° - no depression on mixing with authentic rotundifoline.

Action with Diazomethane.

0.20 gms rotundifoline were let stand for 3 days with 0.22 gm. diazomethane (10 mole) in 8 ml. ether and 2 drops of methanol. After the solvents and excess diazomethane had been allowed to evaporate off, the residue was recrystallised from methanol and proved to be unchanged rotundifoline, m.p. 233° - no depression on mixing with authentic rotundifoline.

Action with Benzaldehyde.

0.10 gm. rotundifoline was refluxed for 6 hours with 0.5 ml. (1.5 mole) freshly distilled benzaldehyde in 3 ml. acetic anhydride. After standing overnight, the reaction mixture was poured into 5.0 ml. concentrated ammonia. The resulting precipitate was filtered off and dissolved in dilute hydrochloric acid, and this acid extract washed with chloroform. The base was again precipitated with concentrated ammonia and on recrystallisation from methanol was found to be unchanged rotundifoline, m.p. 230-2° - no depression on mixing with authentic rotundifoline.

Action with Diazotised Sulphanilic Acid.

Addition of a few drops of a solution of diazotised

sulphanilic acid to a solution of 0.01 gm. rotundifoline in 1 ml. N hydrochloric acid gave a yellow colouration. No change of colour nor formation of precipitate resulted either on letting the solution stand for 12 hours or on making it alkaline with 2N sodium hydroxide.

Hydrogenation.

On microhydrogenation in acetic acid over Adams' catalyst rotundifoline absorbed hydrogen equivalent to one double bond. Over palladium there was no uptake of hydrogen.

0.203 gm. rotundifoline in 10 ml. glacial acetic acid over 0.100 gm. platinum oxide at 18° and 765 mm. absorbed 11.3 ml. hydrogen. The solution was filtered and then added to 5 ml. concentrated ammonia. After recrystallisation from methanol, the resulting precipitated melted at 233° - no depression on mixed melting point with either rotundifoline or the product formed by the action of diazomethane on mitragynol (q.v.).

Found: C, 65.6; H, 7.3; N, 7.0%

$C_{22}H_{28}O_5N_2$ requires C, 66.0; H, 7.05; N, 7.3%

Active hydrogen - 2.8 at 160° in phenetole.

On microhydrogenation over some of the same batch of Adams' catalyst as was used for the microhydrogenation of rotundifoline, dihydrorotundifoline failed to absorb any hydrogen. Dihydrorotundifoline gave no colour with either

ethanolic or ethereal ferric chloride, and with antimony trichloride in dry chloroform only a faint haziness resulted. Dihydrorotundifoline discharged the colour of a solution of bromine in chloroform. The residue after removal of solvent was dissolved in dilute hydrochloric acid and reprecipitated with concentrated ammonia. A sodium fusion test on the material purified in this way was negative for bromine. The specific rotations of dihydrorotundifoline are described on p.151 in the section on the product formed by the action of diazo methane on mitragynol. Addition of a solution of chloroplatinic acid in concentrated hydrochloric acid to one of dihydrorotundifoline gave a chloroplatinate. On recrystallisation from methanol this decomposed at 224-6° - no depression on mixing with either rotundifoline chloroplatinate or the chloroplatinate from the product of the action of diazomethane with mitragynol.

Rotundifoline Dibromide.

To a solution of 0.50 gm. rotundifoline in chloroform was added a chloroform solution of bromine until a faint colour persisted. The residue, partially insolbule in dilute acids, left after removal of solvent, was refluxed 7 hours with 10 ml. acetone. Lachrimatory fumes (? bromacetone) resoluted, and evaporation of the acetone left a solid completely soluble in dilute acids. Since attempted crystallisations from methanol and ethanol were unsuccessful, the

substance was purified by repeated solution in methanol (with animal charcoal treatment) and reprecipitation by dilution with ammonia The resulting amorphous powder melted at 215°.

Found : C, 47.2; H, 4.8; Br, 28.8%

$C_{22}H_{26}O_5N\ Br_2$ requires C, 47.3; H, 4.7; Br, 28.6%

3:4-Diethylpyridine Picrate.

Dr. W. Klyne was good enough to make available a specimen of the picrate, m.p. 131-3°, of the base $C_9H_{13}N$ obtained by Barger, Dyer & Sargent[3] from the selenium dehydrogenation of rotundifoline. A mixed melting point determination on this picrate with an authentic specimen of 3:4-diethylpyridine picrate showed no depression. The literature value given for the melting point of 3:4-diethylpyridine picrate is 134-6°[165].

Mitragynol

Analysis

Found OMe, 7.4; NMe, 0%

Calc. for $C_{27}H_{26}O_5N_2$, OMe, 16.0%

Active hydrogen at 160° - 4.2, 4.05, 4.0, 4.05.

Potentiometric titration:- 0.095 gm. mitragynol was titrated with 0.050 N hydrochloric acid and 0.055N sodium hydroxide (carbonate free) at 18°.

P_{K_A} was found to be 7.25, and P_{K_B} of the order 11 to 12.

Specific Rotations

Rotations in chloroform (t = 21°, l = 1) were as follows:

	Specific Rotation at						λ5000/λ6500	λ5500/λ5900	
	7000Å°	6500	5900	5500	5250	5000	4750			Concn.
[α]	+2.8°	1.4	0.5	-0.5	0.7	2.1	3.8	-	-	4.24
[α]	-2.8	3.8	4.7	5.2	6.6	8.0	-	2.10	1.13	2.12
[α]	-5.7	7.6	8.7	10.4	12.3	13.2	-	1.74	1.20	1.06

Rotations of the salts (t = 21°) were as follows (i and ii hydrochloride in water calculated as free base; iii base in N hydrochloric acid; iv in N sulphuric acid; v and vi in N potassium hydroxide):-

	Specific Rotation at						λ5000/λ6500	λ5500/λ5900	
	7000Å°	6500	5900	5500	5250	5000	4750			Concn.
i [α]	-	-34.8	44.7	54.5	61.1	70.8	80.7	2.03	1.22	0.93
ii [α]	-28.3	31.9	39.3	47.1	52.3	58.9	65.4	1.85	1.20	3.43
iii [α]	-	-14.0	23.3	44.2	51.3	58.1	-	4.15	1.90	1.15
iv [α]	-	-39.1	45.6	69.6	78.1	85.8	-	2.76	1.53	1.23
v [α]	-	-54.4	72.2	91.1	120.0	-	-	2.21*	1.26	2.40
vi [α]	-	-55.5	71.1	86.6	111.1	140.0	-	2.52	1.22	1.20

* $\frac{\lambda 5250}{\lambda 6500}$

Ultra-Violet Spectra.

The spectrum of mitragynol was determined in methanol.

λ max 2840Å° (log ε = 3.81)

λ min 2740Å° (log ε = 3.76)

λ inflex. 2400Å° (log ε = 4.18).

These results are shown graphically in Fig. 8.

The spectrum of mitragynol hydrochloride was determined in distilled water.

λ max 2900Å ($\log \varepsilon$ = 3.58); 2460 ($\log \varepsilon$ = 4.06)

λ min 2760Å ($\log \varepsilon$ = 3.39)

These results are shown graphically in Fig. 9.

Colour Reactions.

Colour reactions are shown in Table 31.

Table 31.

Reagent	Reaction
H_2SO_4 conc.	Nil
ibid - $K_2Cr_2O_7$	Brown on standing; intense green fluorescence
HCl dil. - $K_2Cr_2O_7$	Nil
Erdmann	Orange turning brown & then almost black
Fröhde	Faint blue-purple tinge on standing; intense blue fluorescence
Mandelin	Pink turning via greenish yellow to pale brown; intense blue fluorescence
Kiliani	Orange-pink deepening on standing
H_2SO_4 conc - $Ce(SO_4)_2$	Orange-pink fading on standing
Pine Splinter	Pink
Ehrlich	Very pale pink on standing: orange-red on heat, fading on standing but re-generated by heat.
Ibid-trace $NaNO_2$ & heat	Yellow with slight greenish tinge
Hopkins-Cole	Blue interface on standing; lower layer turns blue overnight.
HCl dil-MeOH-Vanillin	Nil
HCl conc.-Vanillin & heat	Pink

Table 31 (Contd.)

Reagent	Reaction
HCl conc-Glucose & heat	Pink
H_2SO_4 conc.-Chloral & heat	Green
Voisenet	Nil
HOAc-Traces $FeCl_3$ & H_2SO_4	Cherry on heating, fading somewhat on standing
HNO_3 conc.-trace $NaNO_2$	Orange fading somewhat on standing but regenerated by heat
Thalleoquin	Nil
Rossi	Nil
Tetra-nitromethane	Orange
$BrCHCl_3$-Br_2	Decolourised
Ipatieff	Nil
EtOH-$FeCl_3$	Red-tan precipitate
Et_2O-$FeCl_3$	Brown
HOAc-$NaNO_2$	Yellow
Gaebel	Nil
Gallic Acid	Nil
m-Dinitrobenzene-MeOH-NaOH	Nil

Phenolic Reactions.

(i). With Millon's Reagent. A test-tube containing 0.05 gm. mitragynol in 1 ml. Millon's Reagent (mercurous nitrate in dilute nitric acid) was placed in a beaker of water which was brought to the boil. A pale yellow colour developed.

(ii). To 0.05 gm. mitragynol in 1 ml. concentrated sulphuric

acid a crystal of sodium nitrite was added. When this was warmed gently and shaken, there developed a vivid red colour tinged with purple. In ultra-violet light there was an intense bluish white fluorescence. Pouring the mixture into water destroyed the colour.

(iii). 0.10 gm. each of mitragynol, phthalic anhydride (3 moles) and freshly fused zinc chloride were fused for one minute and then cooled. Addition of 1% sodium hydroxide gave a yellow colour unchanged by the addition of excess alkali.

(iv). 3.3 gm. p-toluidine in 10 ml. concentrated hydrochloric acid and 20 ml. water were diazotised with 2.5 gm. sodium nitrite (1.15 moles) and excess nitrous acid destroyed by the addition of 0.5 gm. urea. Addition of 0.10 gm. mitragynol (0.3 mole) in 2 ml. 5N sodium hydroxide to 3 ml. of the above solution gave a brown precipitate. This was insoluble in both acid and alkali, but except for one small fraction, extremely soluble in organic solvents. Change of p_H of an aqueous suspension of the substance gave little change of colour.

(v). Under similar conditions diazotised aniline gave an orange precipitate insoluble also in alkali. The precipitate was brown in alkali.

(vi). Addition of a few drops of a solution of diazotised

sulphanilic acid to a solution of 0.01 gm. mitragynol in 2.0 ml. N hydrochloric acid gave an orange colouration. This on addition of sodium hydroxide solution deepened to an orange-red. No precipitate was formed from acid or alkaline solution.

Action of Antimony Trichloride.

Antimony trichloride gave a haziness which cleared on standing.

Hydrogenation.

In acetic acid over Adams' catalyst mitragynol showed no uptake of hydrogen.

Action of Diazomethane.

0.60 gm. mitragynol was let stand for 3 days with 0.50 gm. diazomethane (7 moles) in 15 ml. ether and 2 ml. methanol. Evaporation to dryness gave a compound "A" which on recrystallisation from methanol melted at 231-2° - no depression on mixing with either rotundifoline or dihydro-rotundifoline.

Analysis: Found: C, 65.8; H, 6.9; OMe, 15.4%

Calc. for $C_{22}H_{28}O_5N_2$, C, 66.0; H, 7.05; 2 x OMe, 15.6%.

Active hydrogen at 160°. In amyl ether, 3.00; in phenetole, 2.95.

Specific Rotations. Specific rotations in chloroform ($t = 21°$, $l = 1$) were:

	Specific rotation at						λ5000/λ6500	λ5500/λ5900	
	7000Å	6500	5900	5500	5250	5000	4750			Concn.
[α]	+86.6°	101.1	122.6	144.5	163.4	186.9	218.3	1.85	1.18	3.28
[α]	92.7	109.7	126.6	151.2	166.9	194.5	223.8	1.77	1.20	1.64
[α]	88.0	109.7	128.0	154.9	178.0	202.4	225.5	1.84	1.22	0.82
[α]	91.7	103.7	123.7	147.7	163.5	189.6	220.3	1.83	1.19	2.41*

*Values for dihydrorotundifoline.

Selected rotations are shown graphically in Fig.4

~~Representative curves appear in Fig. 4.~~

Ultra-Violet Spectra. The ultra-violet spectrum of mitragynol was determined in methanol.

λ max. 2915Å (log ε = 3.46)

λ min. 2755Å (log ε = 3.30)

λ inflexn. 2395Å (log ε = 4.14).

These results are shown graphically in Fig.8.

The spectrum was determined also in distilled water to which a few drops of hydrochloric acid had been added.

λ max. 2900Å (log ε = 3.46); 2450 (log ε = 4.09)

λ min. 2765Å (log ε = 3.36); 2355 (log ε = 4.05).

These results are shown graphically in Fig.9.

Colour Reactions

These are given in Table 33.

Table 33.

Reagent	Reaction
H_2SO_4 conc.	Very pale yellow; white fluorescence
ibid - $K_2Cr_2O_7$	Brown, intensified on heat.

Table 33 (Contd.)

Reagent	Reaction
HCl dil.- $K_2Cr_2O_7$	Nil
HNO_3 conc.	Pinkish orange fading to orange on standing: no fluorescence
Erdmann	Yellow turning quickly to dark green
Fröhde	Brown on heat: slight fluorescence
Mandelin	Orange-brown: slight fluorescence
Kiliani	Nil
H_2SO_4 conc.-$Ce(SO_4)_2$	Nil
Pine Splinter	Yellow
Ehrlich	Nil
ibid-trace $NaNO_2$ & heat	Orange
Hopkins-Cole	Nil
HCl dil.-MeOH-Vanillin	Nil
HCl conc.-Vanillin	Orange: fades on standing but regenerated by heat
ibid-Glucose & heat	Pinkish orange
H_2SO_4 conc.-Chloral & heat	Orange-brown
Voisenet	Nil
HOAc-trace $FeCl_3$ & H_2SO_4	Pale brown
HNO_3 conc.-trace $NaNO_2$	Nil
Tetranitromethane	Orange
$CHCl_3$-Br_2	Decolourised
Ipatieff	Nil
EtOH-$FeCl_3$	Nil
Et_2O-$FeCl_3$	Nil
HOAc-$NaNO_2$	Nil

Table 33 (Contd.)

Reagent	Reaction
Gaebel	Nil
Gallic Acid	Nil
m-Dinitrobenzene -MeOH-NaOH	Nil

Antimony Trichloride in dry chloroform gave with "A" a slight haziness that cleared on standing.

Microhydrogenation. In acetic acid over Adams' catalyst "A" showed no uptake of hydrogen.

Action of Bromine. To a solution of 0.02 gm. "A" in chloroform a chloroform solution of bromine was added until a slight colour persisted in the solution. The solution was then evaporated to dryness on the water bath and the residue refluxed in acetone for 7 hours. The acetone was then removed on the water bath. A sodium fusion test of the residue was negative for bromine.

"A" chloroplatinate. Mixture of solutions of chloroplatinic acid and of "A" in concentrated hydrochloric acid gave a precipitate of "A" chloroplatinate. This on recrystallisation from ethanol gave orange-yellow prisms, m.p. 224-6° (dec.) - no depression on mixing with the chloroplatinate from either rotundifoline or dihydrorotundifoline.

Found: C, 43.6; H, 5.1; Pt, 15.7%

Calc. for $(C_{22}H_{28}O_5N_2)_2H_2PtCl_6$; C, 43.6; H, 4.8; Pt, 16.1%

Attempted Acetylation of Mitragynol.

(i). 0.20 gm. mitragynol and 0.5 gm. freshly fused sodium acetate were refluxed for 6 hours with 1.0 ml. (c. 20 moles) acetic anhydride in 10 ml. freshly dried pyridine. After the mixture had been poured into water, the addition of sodium bicarbonate gave precipitate. On recrystallisation this melted at 235.5° - no depression on mixing with compound "A". This acetylation product will be called "B".

(ii). 0.15 gm. mitragynol was let stand overnight with 1.0 ml. acetic anhydride (c. 25 moles) in 5 ml. dry pyridine and the mixture was then poured into water. Addition of sodium bicarbonate gave a precipitate which on recrystallisation from methanol melted at 232-3° - no depression on mixing with "B".

(iii). 0.15 gm. mitragynol was let stand overnight with 1.0 ml. acetyl chloride (c. 30 moles) in 10 ml. dry pyridine. On being worked up in the usual way the product melted at 233° - no depression on mixing with "B".

(iv). Preparation (i) was repeated twice with the same result - m.ps. of products 233°, 233-4° - no depressions on mixing with the original specimen of "B".

(v). 0.15 gm. mitragynol was refluxed for 4 hours in 10 ml. pyridine with 0.50 ml. (c. 10 moles) propionic anhydride. After the mixture had been let stand overnight it was poured into water. Addition of sodium bicarbonate gave a precipitate

which on being worked up in the usual way melted at 232-4° - no depression on mixing with "B".

Properties of "B".

Analysis. Found: C, 66.3; H, 6.7%

Calc. for $C_{22}H_{28}O_5N_2$, C, 66.0; H, 7.05%.

Active hydrogen at 160° in amyl ether, 2.85; in phenetole at 160°, 2.90.

Specific Rotations. These were determined in chloroform (t = 14°, l = 0.75).

	Specific rotation at						$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	
7000Å	6500	5900	5500	5250	5000	4750			Concn.
$[\alpha]$ +87°	99	120	141	162	185	210	2.13	1.18	0.54

~~These are shown graphically in Fig. 4.~~

Ultra-Violet Spectrum. This was determined in ethanol.

λ max. 2920Å (log ε = 3.44)

λ min. 2760Å (log ε = 3.28)

λ inflexn. 2395Å (log ε = 4.18).

These are shown graphically in Fig.8.

Colour Reactions. With concentrated sulphuric acid, and with Erdmann's, Fröhde's and Mandelin's reagent "B" gave exactly the same colour reactions as did "A".

Action of Formic Acid on Mitragynol.

0.15 gm. mitragynol was refluxed for 30 minutes with 5.0 ml. 90% formic acid (c. 25 moles), cf. Ruzicka & Stoll[166].

Owing to a typographical mistake, the following page ought to have been numbered p.156, not p.157.

The pagination of the Table of Contents is not affected.

After removal in vacuo of most of the formic acid, 5 ml. of water were added and the solution neutralised with sodium carbonate. Recrystallisation from methanol showed the resulting precipitate to be unchanged mitragynol, m.p. 125° - no depression on mixing with authentic mitragynol (lit., m.p. 130°).

Rhynchophylline.

A. From M.ciliata.

This has already been described on p.109.

B. From M.inermis

Analysis

Active Hydrogen. In phenetole at 160°, 3.1.

Specific Rotations.

Specific rotations were determined in chloroform (t = 19°, l = 1):

	Specific Rotations at					$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	
	7000Å	6500	5900	5500	5000			Concn.
[α]	-12.6	14.6	15.7	17.5	18.7	1.28	1.11	2.26
[α]	-17.7	20.4	23.0	28.3	37.2	1.82	1.39	1.13

Literature - 14.5°, -14.7° (c = 2.5), -23.0° (c = 1.45), -23.1° (c = 1.51), -26.4° (c = 1.45), -26.5° (C = 1.19).

Specific rotations were also determined in 2N hydrochloric acid (t = 19°, l = 1, calculated as free acid):-

	Specific Rotations at						$\frac{\lambda 5000}{\lambda 6500}$	$\frac{\lambda 5500}{\lambda 5900}$	
	7000Å	6500	5900	5500	5250	5000			Concn.
[α]	1.0	1.7	3.3	4.7	6.7	8.3	4.88	1.42	1.50

Colour Reactions.

Colour Reactions are shown in Table 34.

Table 34.

Reagent	Reaction
H_2SO_4 conc.	Nil
ibid-$K_2Cr_2O_7$	Red-brown turning yellow-green on heat
HCl dil. - "	Nil
HNO_3 conc.	Very pale yellow on heat
Erdmann	Pale brown turning reddish. On standing becomes carmine to transmitted and blue to reflected light.
Fröhde	Yellow fluorescence
Mandelin	Scarlet turns brick red, and later green. Green fluorescence
Kiliani	Orange-pink deepening on standing
Conc.H_2SO_4-$Ce(SO_4)_2$	Scarlet fading on standing
Pine Splinter	Yellow
Ehrlich	Nil
ibid-trace $NaNO_2$ & heat	Nil
Hopkins-Cole	Nil
HCl dil-MeOH-Vanillin	Nil
HCl conc.-Vanillin & heat	Nil
ibid - Glucose & heat	Pale yellow-brown
H_2SO_4 conc.-Chloral & heat	Nil
Voisenet	Nil
HOAc-traces $NaNO_2$ & H_2SO_4	Pale orange-brown on heating
HNO_3 conc. - trace $NaNO_2$	Pale yellow on standing intensified on heat

Table 34 (Contd.)

Reagent	Reaction
Thalleoquin	Nil
Rossi	Nil
Tetranitromethane	Orange
$CHCl_3$-Br_2	Decolourised
Ipatieff	Decolourised
EtOH-$FeCl_3$	Nil
Et_2O-$FeCl_3$	Nil
HOAc-$NaNO_2$	Oily derivative
Gaebel	Nil
Gallic Acid	Nil
meDinitrobenzene -MeOH-NaOH	Nil

Rhynchophylline Chloroplatinate.

Mixture of concentrated hydrochloric acid solutions of chloroplatinic acid and of the alkaloid gave a precipitate. On recrystallisation from methanol this gave orange prisms, dec.pt. 235° after darkening - no depression on mixing with the chloroplatinate of the alkaloid from M.rotundifolia (lit. m.p. 238°)

Rhynchophylline Chloroaurate.

Mixture of concentrated hydrochloric acid solutions of chloroauric acid and of the alkaloid gave a precipitate. On recrystallisation from methanol this gave yellow prisms,

m.p. 133°, dec.pt. 155° - no depression on mixing with the chloroaurate of the alkaloid from M.rotundifolia (lit., m.p. 134°, dec.pt. 155°).

Action of 2:4-Dinitrophenylhydrazine.

0.02 gm. of rhynchophylline heated for 5 minutes at 100° with 1 ml. freshly prepared Brady's reagent showed neither change of colour nor formation of precipitate.

Action of Diazomethane.

0.20 gm. rhynchophylline and 0.22 gm. diazomethane (9 moles) were let stand for 3 days in 8 ml. ether and 2 ml. methanol, and the solvents and excess diazomethane removed. Recrystallisation of the residue from methanol gave colourless needles, m.p. 225-7° - definite depression on mixing with authentic rhynchophylline.

Found: C, 66.7; H, 7.1; N, 6.9%

Calc. for $C_{23}H_{28}O_4N_2$,CH_3OH: C, 67.1; H, 7.5; N, 6.5%

Specific Rotations of the product in chloroform (t = 18°, l = 0.75) were:-

	Specific Rotations at						
	7000Å 6500	5900	5500	5250	5000	λ5000/λ6500	λ5500/λ5900	Concn.
[α]	-18.7° 22.0	26.0	32.0	36.0	41.3	1.88	1.23	
[α]	-20.0 22.7	28.0	34.7	37.3	41.3	1.82	1.24	

Ultra-Violet Spectrum - This was determined in ethanol.

λ max. 2395Å (log ε = 4.30)
λ min. 2210Å (log ε = 4.06)
λ inflexn. 2770Å (log ε = 3.33)

These results are shown in Fig.10.

C. From M.rotundifolia.

Specific Rotations.

Specific rotations in chloroform (t = 19°, l = 1) were

	Specific Rotations at							
	7000Å	6500	5900	5500	5000	λ5000/λ6500	λ5500/λ5900	Concn.
[α]	-	-	-6.0°	-	-	-	-	5.30
[α]	-	-12.6	14.3	15.3	16.6	1.32	1.07	2.35
[α]	-16.0	18.0	22.0	28.0	36.0	2.00	1.27	1.00

Specific rotations (calculated as free base) in 2N hydrochloric acid (t = 19°, l = 1) were:-

	Specific Rotations at								
	7000Å	6500	5900	5500	5250	5000	λ5000/λ6500	λ5500/λ5900	Conc.
[α]	+1.0	1.7	2.7	4.0	6.0	8.0	4.71	1.48	1.50

These rotations are compared graphically in Fig.2 with those of the alkaloid from M.inermis.

Ultra-Violet Spectrum.

The ultra-violet spectrum was determined in ethanol.

λ max. 2785Å (log ε = 3.76); 2440 (log ε = 4.14)

λ min. 2780Å (log ε = 3.86); 2445 (log ε = 4.00).

In Fig.10 this spectrum is compared graphically with that of rhynchophylline from M.inermis.

Rhynchophylline Chloroplatinate.

Mixture of solutions of chloroplatinic acid and rhynchophylline in concentrated hydrochloric acid gave a

precipitate. On recrystallisation from methanol this gave orange-yellow prisms, m.p. 235° - no depression on mixing with the chloroplatinates from the rhynchophylline of either M.ciliata or M.inermis.

Rhynchophylline Chloroaurate.

Mixture of solutions of chloroauric acid and rhynchophylline in concentrated hydrochloric acid gave a precipitate which, on recrystallisation from methanol gave yellow prisms, m.p. 132°, dec.pt. 155° - no depression on mixing with the chloroaurates from the rhynchophylline of either M.ciliata or M.rotundifolia.

Hydrogenation.

On microhydrogenation of rhynchophylline in acetic acid over Adams' catalyst hydrogen equivalent to one double bond was adsorbed. Over palladium black in acetic acid there was no uptake.

Action of Bromine.

To 0.50 gm. rhynchophylline in chloroform sufficient of a solution of bromine in chloroform was added to give a permanent colouration. Evaporation of the solvent on the steam bath left a residue only partially soluble in dilute acids. Refluxing this residue in acetone gave lachrimatory fumes (probably of bromacetone). The residue left after removal of acetone was completely soluble in dilute acids. Since attempts at recrystallisation from methanol, ethanol

and acetone were all fruitless, purification was achieved by repeatedly dissolving the substance in methanol and (after charcoal treatment of the first solution) reprecipitation with concentrated ammonia. The resulting amorphous compound melted at 325°. Unfortunately insufficient was obtained for analysis.

Action of Diazotised Sulphanilic Acid.

Addition of a few drops of a solution of diazotised sulphanilic acid to a solution of 0.01 gm. rhynchophylline in 2.0 ml. N hydrochloric acid gave a yellow colouration. No change of colour nor formation of precipitate resulted either on standing the solution for 12 hours or on making it alkaline by the addition of sodium hydroxide solution.

Decarboxylation Product of Rhynchophyllic Acid.

Through the courtesy of Drs. E. Dyer & L.J. Sargent I was able to examine about 3 mgm. of the neutral substance $C_{10}H_9NO$ formed when rhynchophylline acid is distilled from calcium oxide. The ultra-violet spectrum was determined in ethanol.

λ max. 2525Å ($\log \epsilon = 3.75$); 2200 ($\log \epsilon = 4.2$)

λ min. 2310Å ($\log \epsilon = 3.48$).

These results are shown graphically in Fig.11.

FIGURE 2

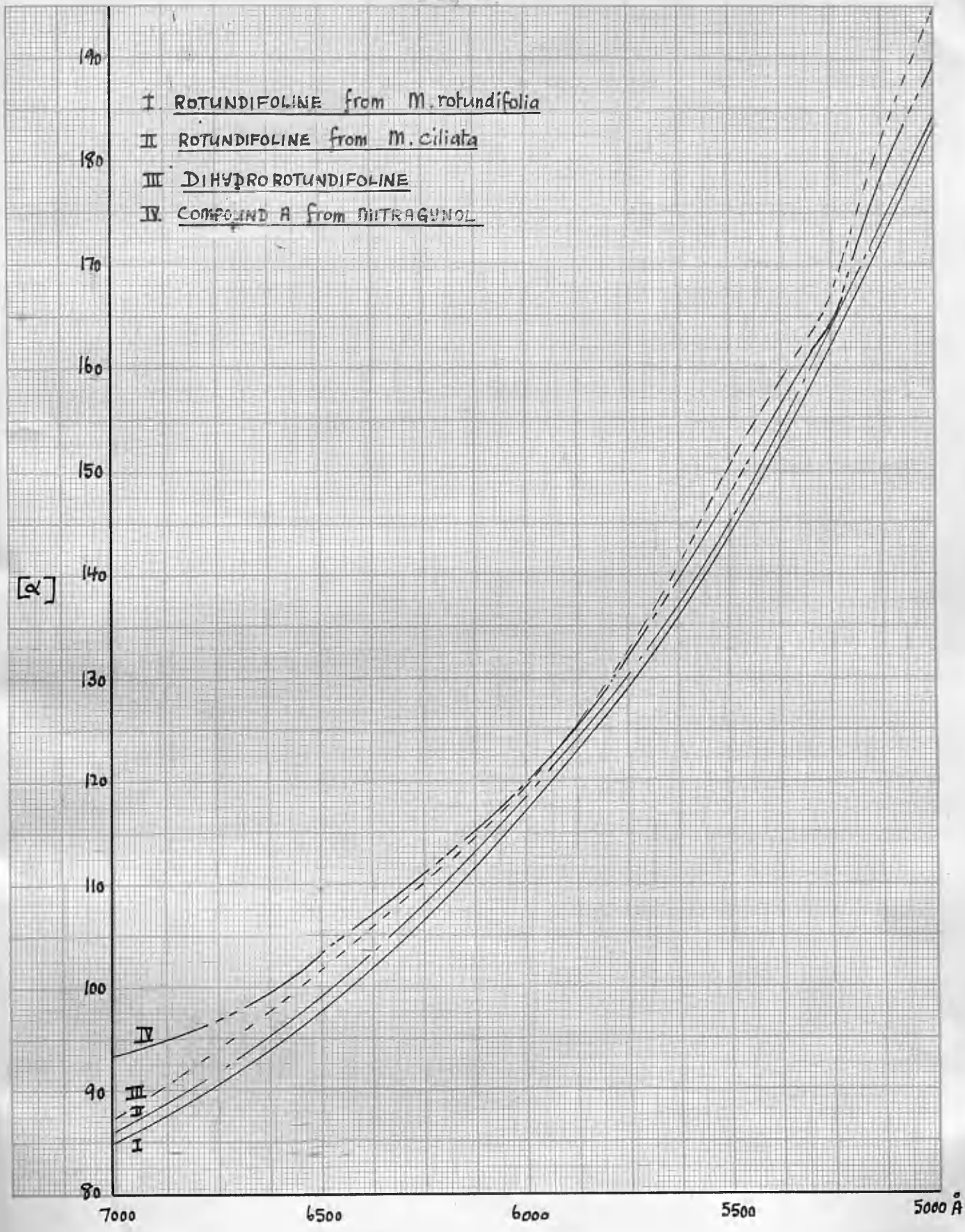

I ROTUNDIFOLINE from M. rotundifolia
II ROTUNDIFOLINE from M. ciliata
III DIHYDRO ROTUNDIFOLINE
IV COMPOUND A from NITRAGYNOL
[α]
190
180
170
160
150
140
130
120
110
100
90
80
IV
III
II
I
7000
6500
6000
5500
5000 Å

FIGURE 3

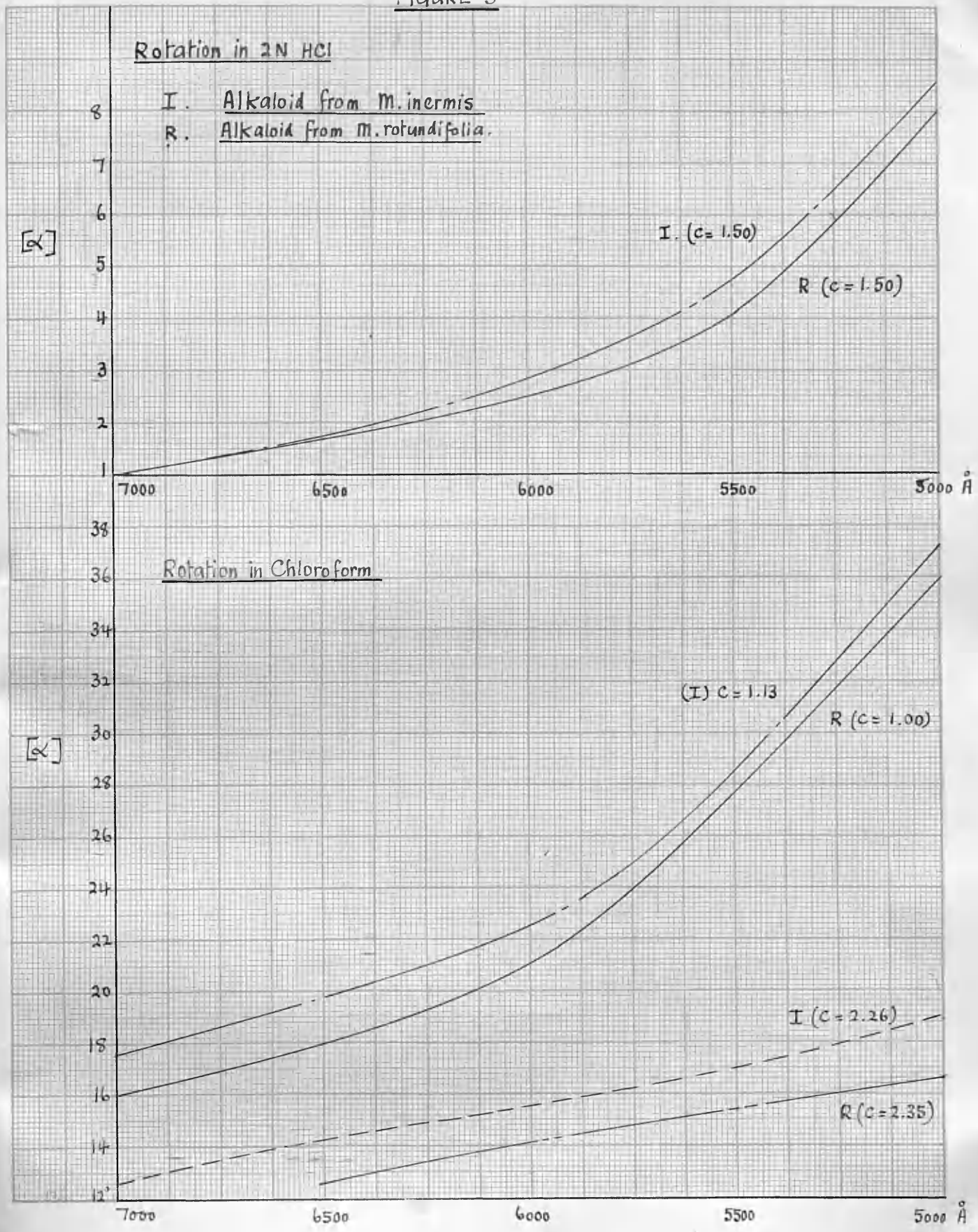

Rotation in 2N HCl
I. Alkaloid from M. inermis
R. Alkaloid from M. rotundifolia.
[α]
8
7
6
5
4
3
2
1
I. (c= 1.50)
R (c= 1.50)
7000
6500
6000
5500
5000 Å
Rotation in Chloroform
[α]
38
36
34
32
30
28
26
24
22
20
18
16
14
12
(I) C = 1.13
R (C = 1.00)
I (C = 2.26)
R (C = 2.35)
7000
6500
6000
5500
5000 Å

FIGURE 4

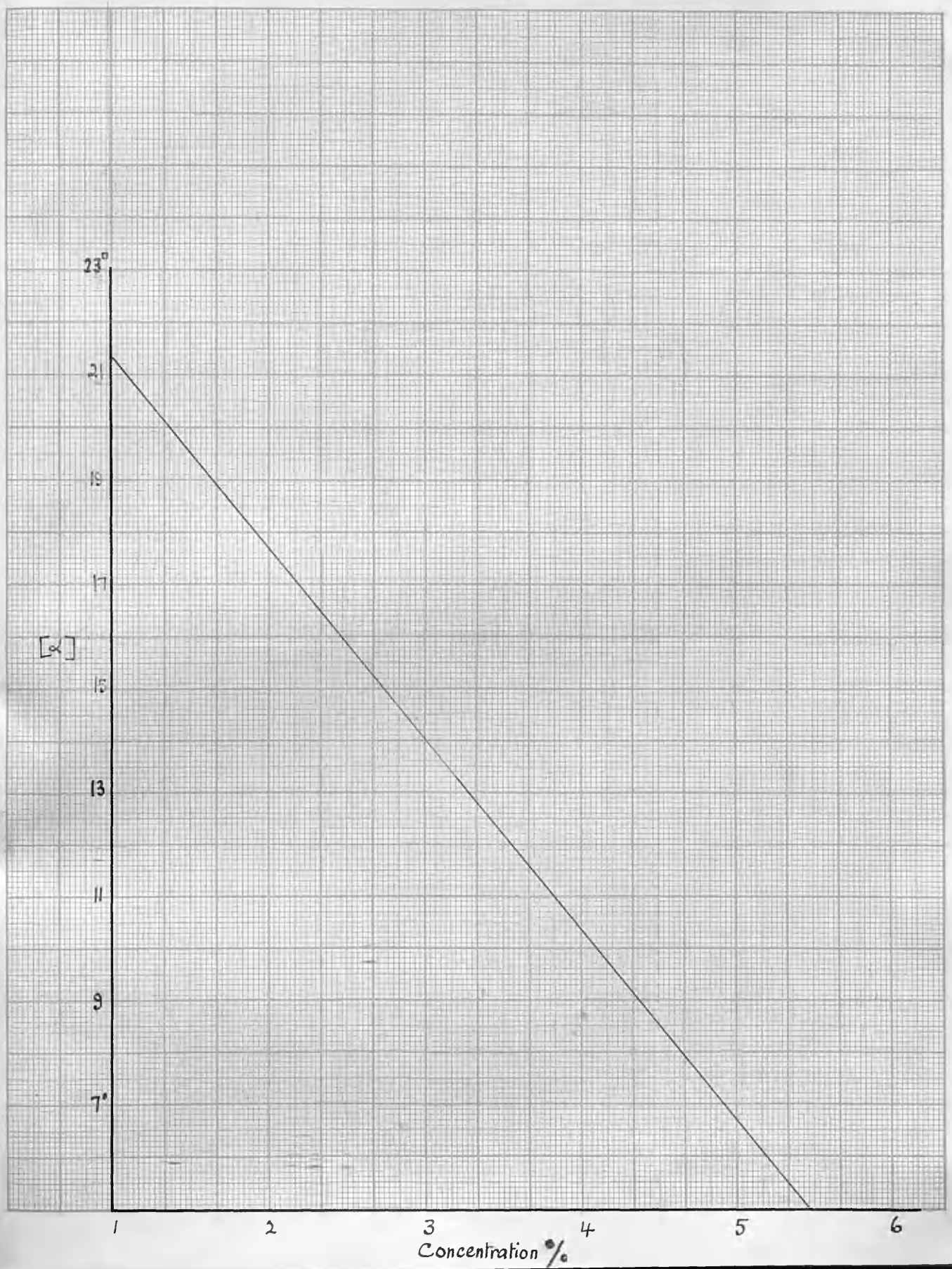


FIGURE 5

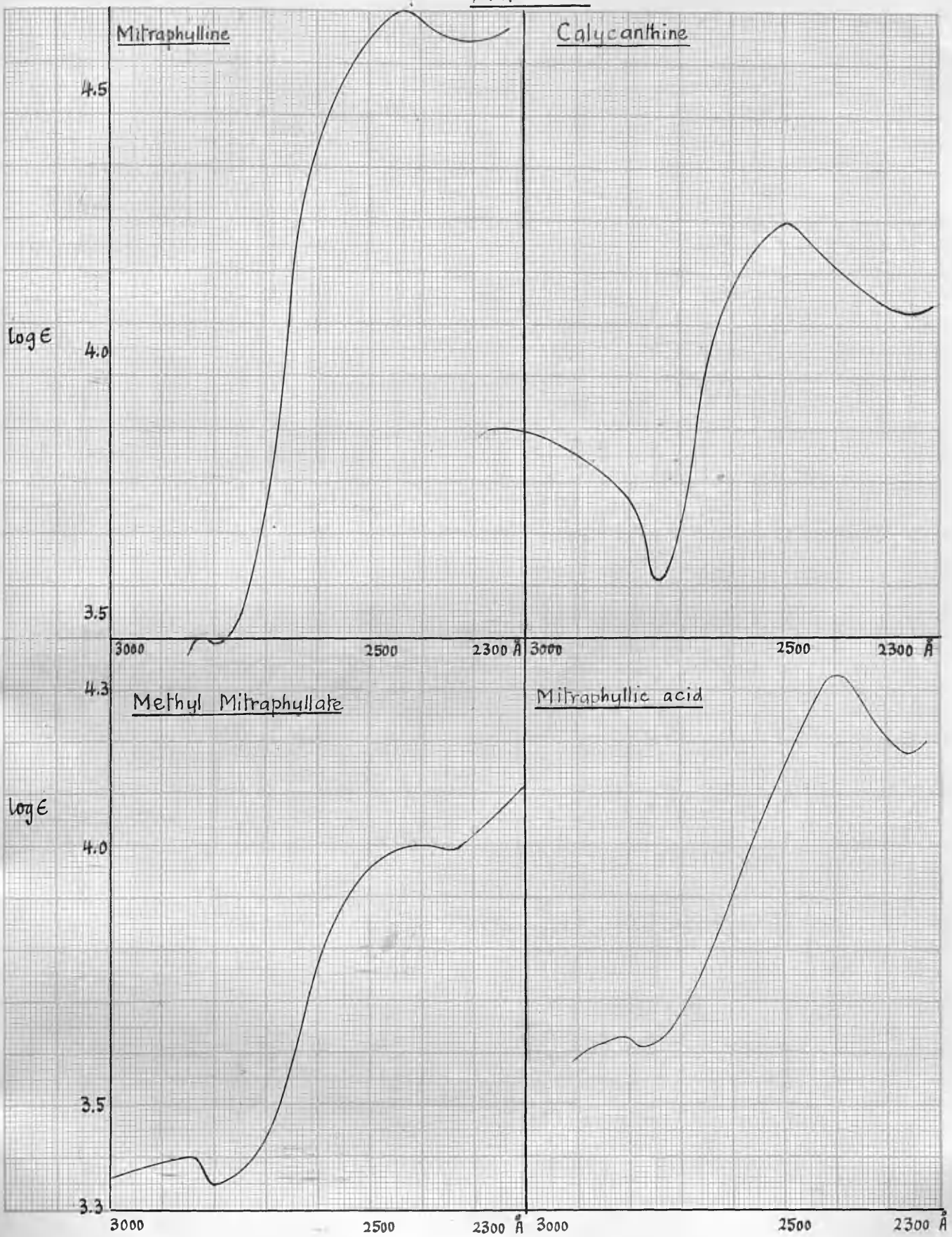

Mitraphylline
Calycanthine
Methyl Mitraphyllate
Mitraphyllic acid
log ε
4.5
4.0
3.5
4.3
3.3
3000
2500
2300 Å

FIGURE 6

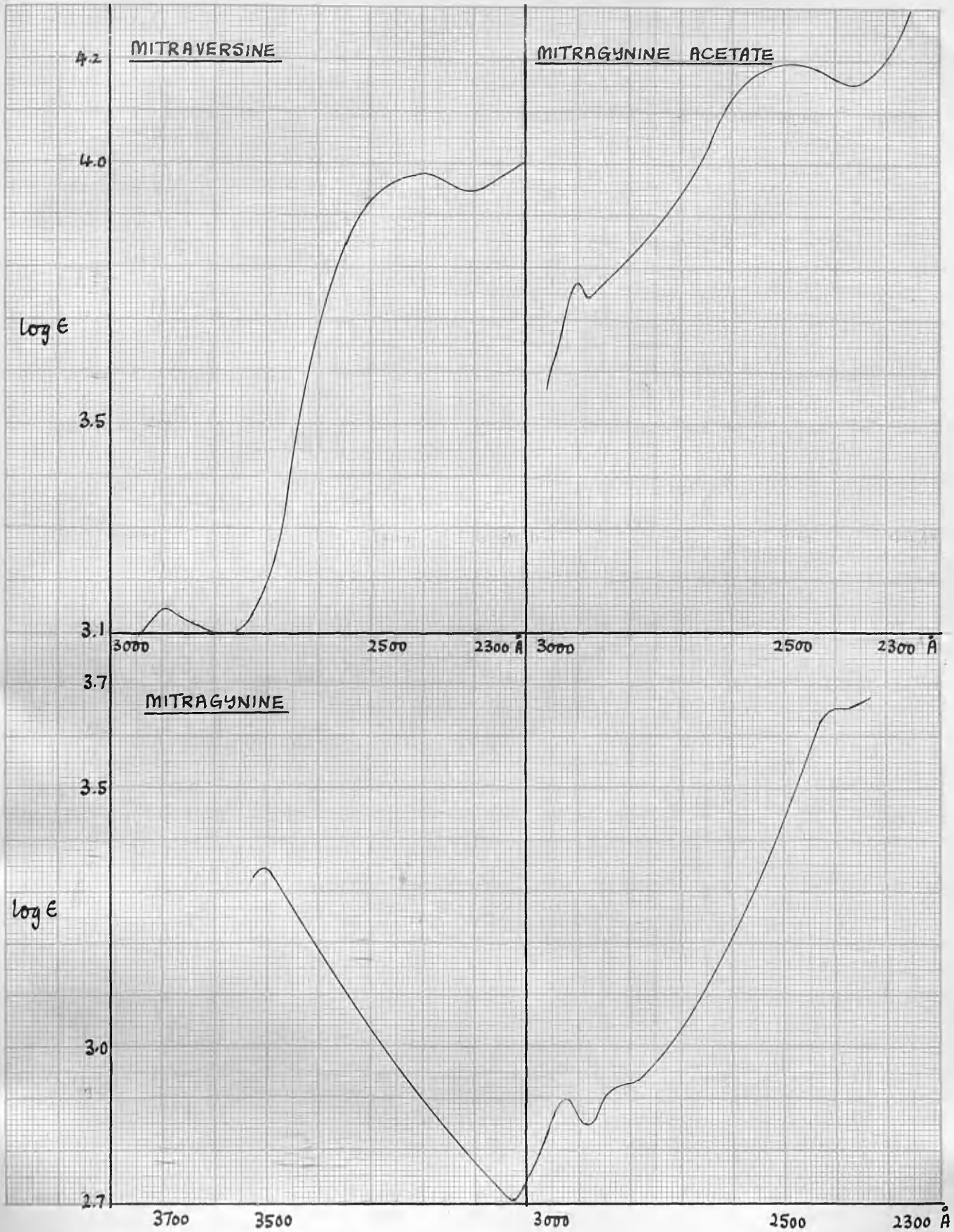

MITRAVERSINE
4.2
4.0
log ε
3.5
3.1
3000
2500
2300 Å
MITRAGYNINE ACETATE
3000
2500
2300 Å
3.7
MITRAGYNINE
3.5
log ε
3.0
2.7
3700
3500
3000
2500
2300 Å

FIGURE 7

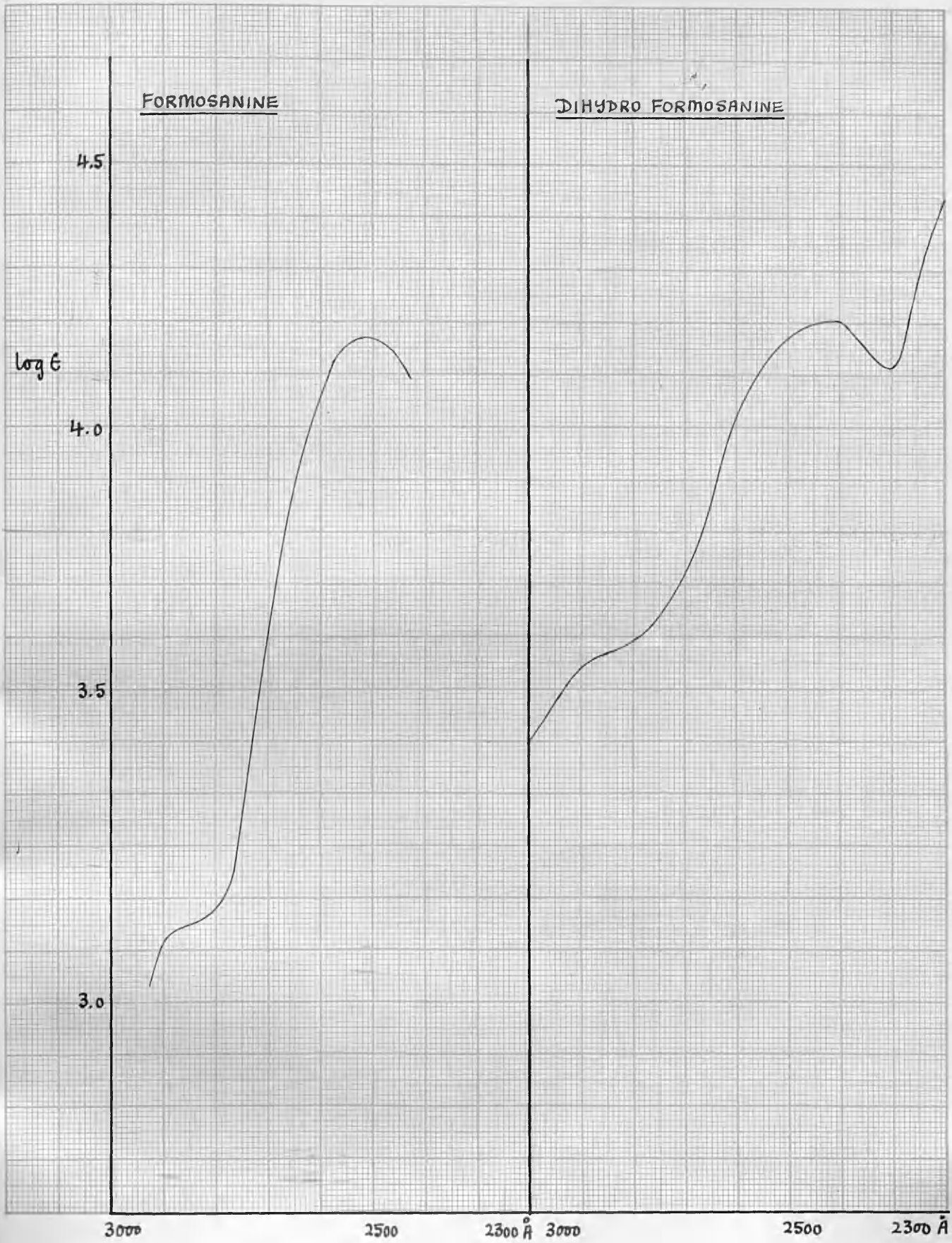

FORMOSANINE
DIHYDRO FORMOSANINE
4.5
log ε
4.0
3.5
3.0
3000
2500
2300 Å
3000
2500
2300 Å

FIGURE 8

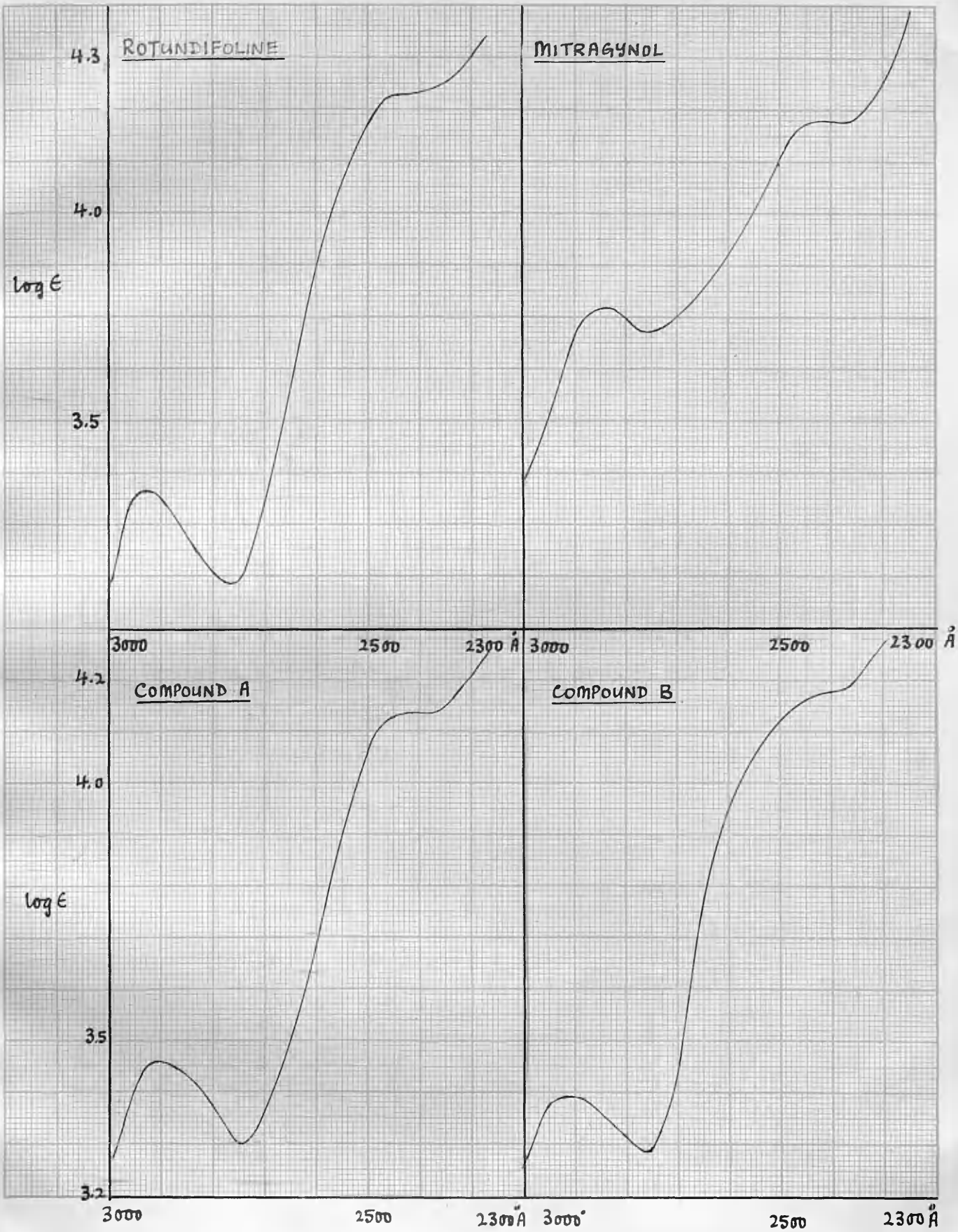

ROTUNDIFOLINE
MITRAGYNOL
COMPOUND A
COMPOUND B
log ε
4.3
4.0
3.5
4.2
3.2
3000
2500
2300 Å

FIGURE 9

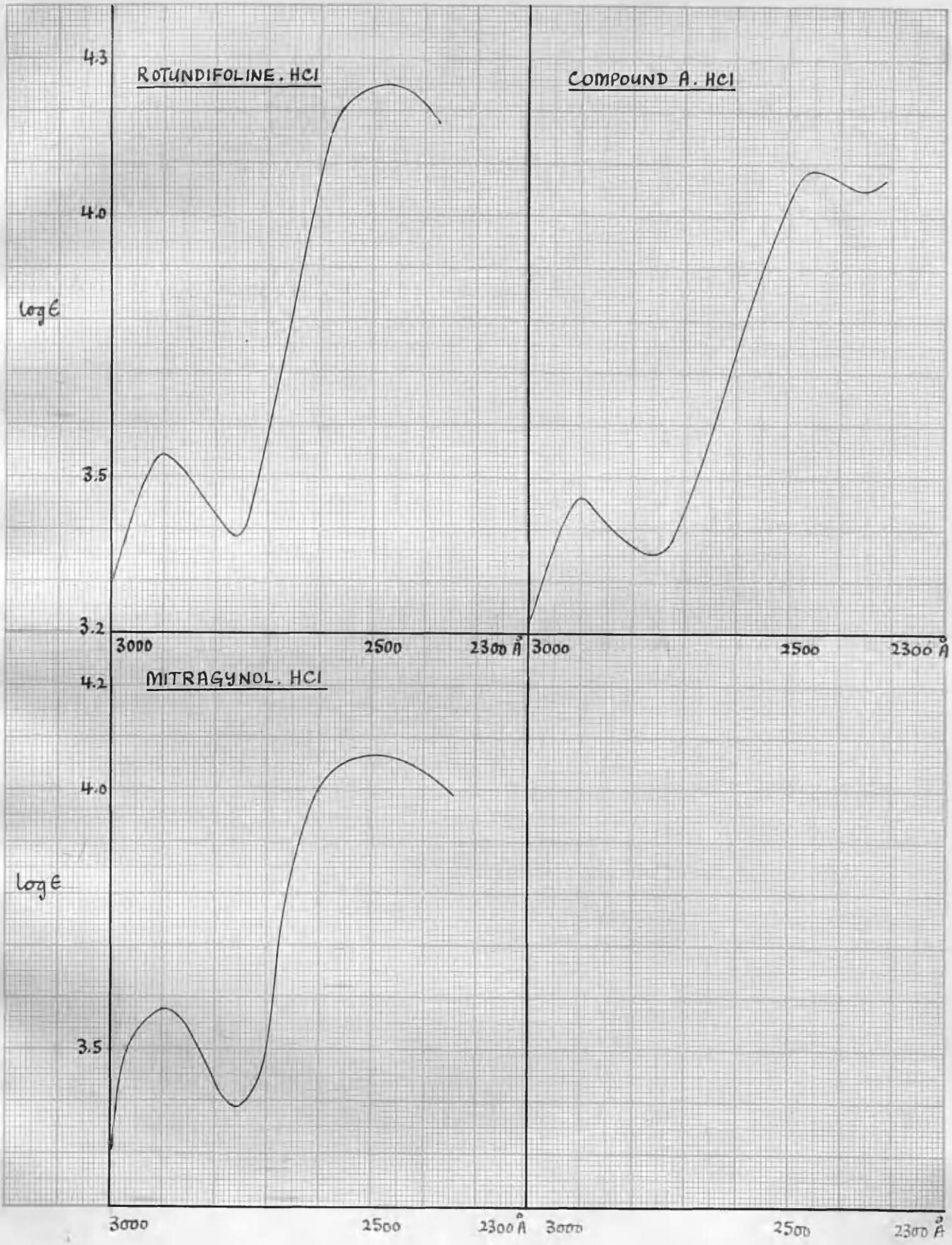

ROTUNDIFOLINE. HCl
COMPOUND A. HCl
MITRAGYNOL. HCl
log ε
4.3
4.0
3.5
3.2
4.2
3000
2500
2300 Å

FIGURE 10

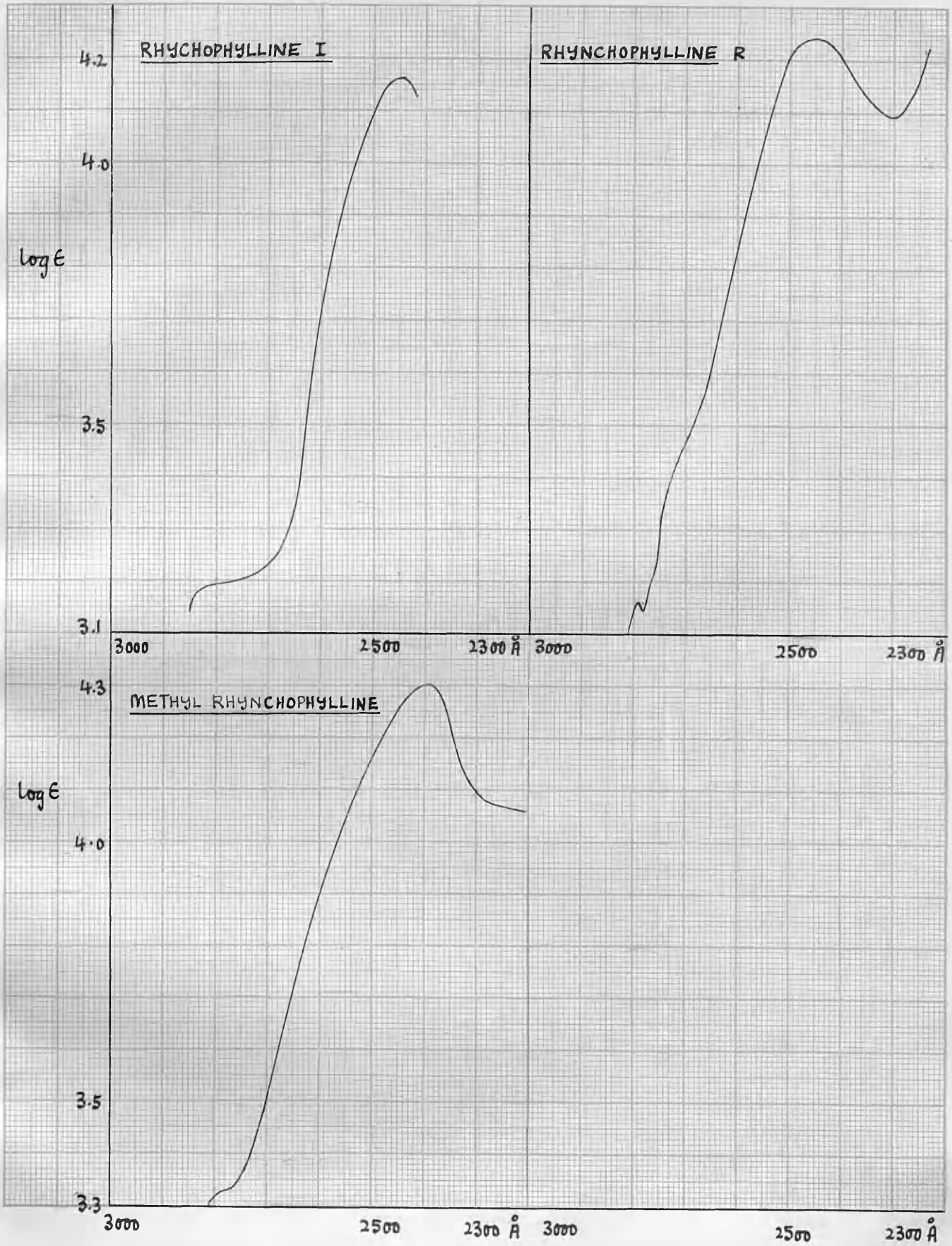
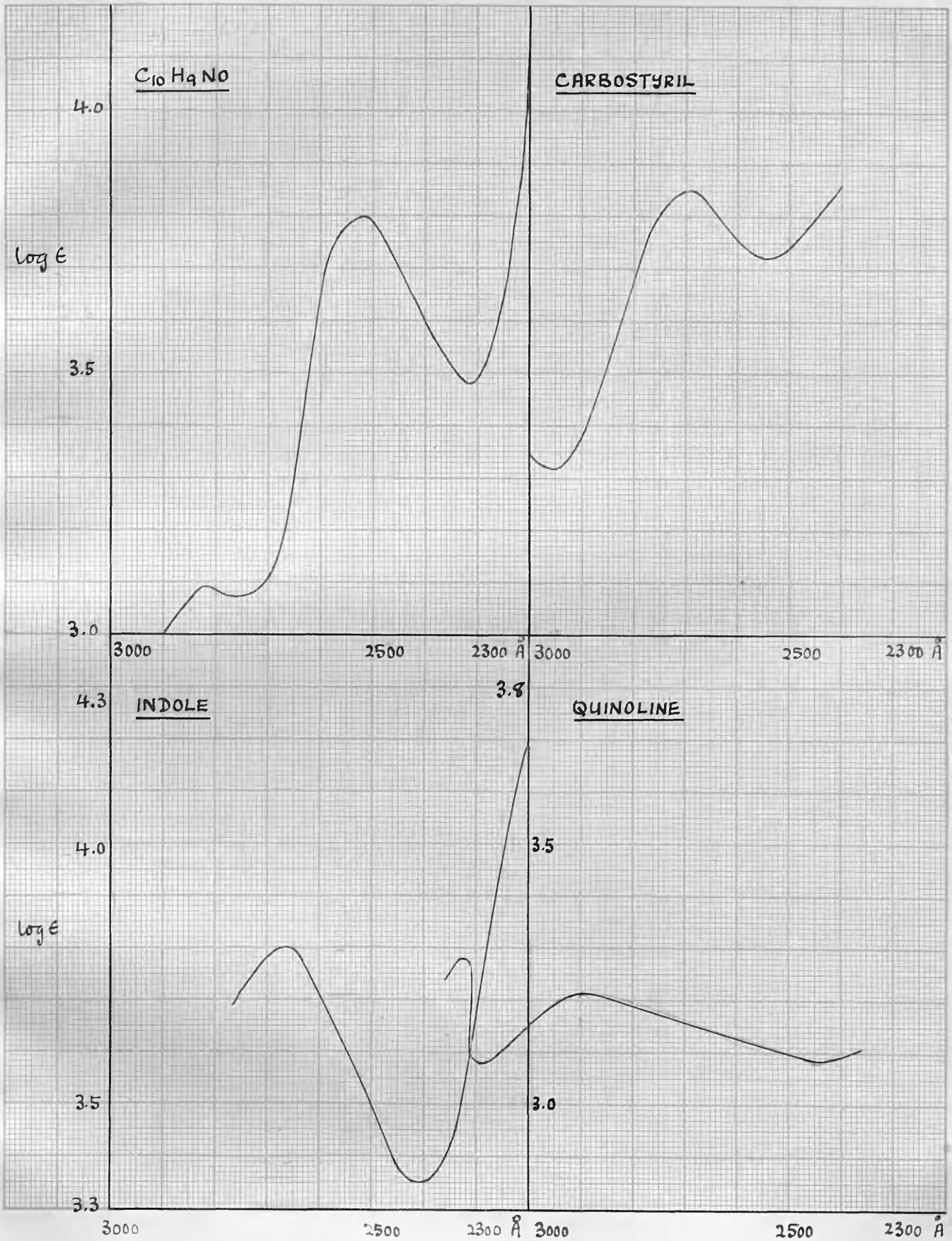

RHYCHOPHYLLINE I
4·2
4·0
log ε
3·5
3·1
3000
2500
2300 Å
RHYNCHOPHYLLINE R
3000
2500
2300 Å
METHYL RHYNCHOPHYLLINE
4·3
log ε
4·0
3·5
3·3
3000
2500
2300 Å
3000
2500
2300 Å

FIGURE II


C10 H9 NO
CARBOSTYRIL
4.0
Log ε
3.5
3.0
3000
2500
2300 Å
3000
2500
2300 Å
3.8
4.3
INDOLE
QUINOLINE
4.0
3.5
Log ε
3.5
3.0
3.3
3000
2500
2300 Å
3000
2500
2300 Å

References.

1. Field, J.Chem.Soc., 1921, 119, 887.
2. Ing & Raison, ibid., 1939, 986.
3. Barger, Dyer & Sargent, J.Org.Chem., 1939, 4, 418.
4. Denis, J.Pharm.Belg., 1927, 9, 22; Am.Chem.Abstr., 1928, 22, 301.
5. Michiels, ibid., 1931, 13, 719; ibid., 1932, 26, 3070.
6. Raymond-Hamet & Millat, Bull.sci.pharmacol., 1935, 42, 602.
7. Michiels & Leroux, Bull.acad.roy.med.Belg., 1925, 5, 403
8. Michiels, J.pharm.Belg., 1935, 17, 1049; Am.Chem.Abstr., 1936, 30, 7780.
9. Denis, Bull.classe sci.acad.roy.Belg., 1937, 23, 174.
10. Ibid, ibid, 1939, 25, 177.
11. Raymond-Hamet, Bull.sci.pharmacol., 1939, 46, 327.
12. Ibid., Compt.rend., 1950, 230, 1405.
13. McCloskey, private communication.
14. Armit & Robinson, J.Chem.Soc., 1925, 127, 1604.
15. Denis, Bull.classe.sci. acad.roy.Belg., 1938, 24, 653.
16. Raymond-Hamet, Compt.rend., 1936, 203, 1383.
17. Ibid., Bull.soc.chim., 1943, 10, 129.
18. Kondo & Oshima, J.Pharm.Soc.Japan, 1932, 52, 528 (in German, 63); Am.Chem.Abstr., 1933, 27, 1345.
19. Kondo & Ikeda, ibid., 1937, 57, 881 (ibid., 237); ibid., 1938, 32, 1272.
20. Raymond-Hamet, Bull.acad.méd., 1934, 112, 513; Am.Chem.Abstr., 1935, 29, 7493.
21. Rothlin & Raymond-Hamet, Compt.rend.soc.biol., 1935, 119, 37; Am.Chem.Abstr., 1935, 29, 4830.

22. Roebuck, Pharm.J., 1936, 136, 68; Am.Chem.Abstr., 1937, 31, 7603.

23. Badger, Cook & Ongley, J.Chem.Soc., 1950, 867.

24. Raymond-Hamet & Millat, Compt.rend., 1934, 199, 587.

25. Ibid., J.pharm.Chim., 1934, 20, 577; Am.Chem.Abstr., 1935, 29, 4133.

26. Kondo, Fakuda & Tomita, J.pharm.Soc.Japan, 1928, 48, 321; Am.Chem.Abstr., 1928, 22, 3166.

27. Akamatsu, Nagasaki Igakkai Zasshi, 1928, 6, 333; quoted by Millat (31).

28. Raymond-Hamet, Bull.sci.pharmacol., 1940, 47, 194.

29. Blaise, cited by Henry, The Plant Alkaloids, London, 1949, 756.

30. Raymond-Hamet, Compt.rend.soc.biol., 1938, 128, 777; Am.Chem.Abstr., 1938, 32, 7570.

30a. Ibid., Arch.intern.pharmacodynamic, 1941, 66, 330; Am.Chem.Abstr., 1942, 36, 2917.

31. Millat, Ann.pharm.franç., 1946, 4, 27.

32. Oudemans, Rec.trav.chim., 1883, 2, 160.

33. Soliman, J.Chem.Soc., 1939, 1760.

34. Schmitt & Wieland, Ann., 1945, 557, 1.

35. Ruzicka & Anner, Helv.Chim.Acta., 1943, 26, 129.

36. Noller, Ann.Rev.Biochem., 1945, 14, 383.

37. Wallis & Chakravorty, J.Org.Chem., 1937, 2, 335.

38. Cook & Paige, J.Chem.Soc., 1944, 336.

39. Anderson & Shriner, J.Am.Chem.Soc., 1926, 48, 2976.

40. Anderson, Shriner & Burr, ibid., 2987.

41. Sandquist & Bengtsson, Ber., 1931, 64, 2167.

42. Heilbron, Jones, Roberts & Wilkinson, J.Chem.Soc., 1941, 344.

43. Gloyer & Schuette, J.Am.Chem.Soc., 1939, 61, 1901.

44. Liebermann, Ber., 1884, 17, 868; 1885, 18, 1803.

45. Hesse, Ann., 1885, 228, 288.

46. Dirscherl & Nahm, ibid., 1943, 555, 57.

47. Windaus & Deppe, Ber., 1933, 66, 1689.

48. Dirscherl, Z.physiol.Chem., 1938, 257, 239.

49. Wester, quoted by Rosenthaler, The Chem.Investigation of Plants, London, 1930, 190.

50. White, private communication.

51. Ibid., N.Z.J.Sci. & Tech., 1944, 25, 100.

52. Henry, op.cit., XI.

53. Aston, J.Chem.Soc., 1910, 97, 1381.

54. White, N.Z.J.Sci. & Tech., 1944, 25, 143.

55. Ibid., ibid, 148.

56. Ibid., ibid, 103.

57. Ibid., ibid, 109

58. Ibid., ibid, 137.

59. Pruckner & Witkop, Ann., 1943, 554, 127.

60. Ipatieff, Thomson & Pines, J.Am.Chem.Soc., 1948, 70, 1658.

61. Landolt, Optical Rotation of Organic Substances (Edited by Long), Easton Pa., 1902, 667 et seq.

62. Henry, op.cit., 2.

63. Barger & Girardet, Helv.Chim.Acta., 1931, 14, 481.

64. Cheronis & Entrikin, Semimicro Qualitative Organic Analysis, New York, 1947, 129.

65. King, J.C.S., 1937, 1472.

66. Cheronis & Entrikin, op.cit., 128.

67. Henry, op.cit., 669.

68. Ibid., 271.

69. Stewart, Stereochemistry, London, 1919, 81.

70. Kotake, Mor & Mitsuwa, Sci.Papers Inst.Chem & Phys. Res., Tokio, 1937, 31, 334; Am.Chem.Abstr., 1937, 31, 5805.

71. Goutarel, Janot, Prelog & Taylor, Helv.Chim.Acta, 1950, 33, 150.

72. Sivadijian, J.pharm.chim., 1932, 15, 352; Am.Chem. Abstr., 1932, 26, 4133.

73. Heller, Ber., 1926, 59, 704.

74. Moubasher, Awad, Ibrahim & Othman, J.Chem.Soc., 1950, 1999.

75. Raymond-Hamet, private communication.

76. Morgan, Chem.News, 1877, 36, 269.

77. Perkin & Robinson, J.Chem.Soc., 1919, 115, 933.

78. Graebe & Caro, Ber., 1880, 13, 99.

79. Braude, Ann.Reports, 1945, 42, 128.

80. Ward, Biochem.J., 1923, 17, 903.

81. Morton & Rodgers, J.Chem.Soc., 1925, 127, 2698.

82. Fox, J.Chem.Soc., 1910, 117, 1119.

83. Jacobs, Craig & Rothen, Science, 1936, 88, 166.

84. Kirby, J.Chem.Soc., 1945, 528.

85. Carr & Pyman, J.Chem.Soc., 1914, 105, 1591: Hesse, Ann., 1914, 405, 1.

86. Ibid., 1894, 282, 208.

87. Späth & Burger, Monats., 1927, 47, 733.

88. Späth and Bernhauer, Ber., 1925, 58, 200.

89. Henry, op.cit., 732.

90. Ibid., 85.

91. King & Ware, J.Chem.Soc., 1941, 331.

92. Barger & Girardet, Helv.Chim.Acta, 1941, 24, 504.

93. Witkop, Ann., 1943, 554, 83.

94. Goutarel & Berton, Compt.rend., 1943, 217, 71.

95. Steiner, Compt.rend., 1923, 176, 244.

96. Rosenfeld & Kolesnikov, Ber., 1936, 69, 2022.

97. Prelog, Helv.Chim.Acta., 1948, 31, 588.

98. Witkop, J.Am.Chem.Soc., 1948, 70, 3712.

99. Woodward, Brehm & Nelson, ibid., 1947, 69, 2250.

100. Goutarel & Berton, Compt.rend., 1948, 226, 1379.

101. Djerassi & Ryan, J.Am.Chem.Soc., 1949, 71, 1000.

102. Clarke, Handbook of Organic Analysis, London, 1946, 280.

103. Kiliani, Arch.d.Pharm., 1896, 234, 273.

104. Karrer & Schmid, Helv.Chim.Acta, 1947, 30, 2081.

105. Raymond-Hamet, Compt.rend., 1941, 212, 135.

106. Cole, J.Physiol., 1903, 30, 315.

107. Rossi, Del Boca & Lobo, Anales de Farm. y Bioquim, 1932, 30, 51; Am.Chem.Abstr., 1932, 26, 5703.

108. Voisenet, Bull.Soc.chim., 1905, 33, 1198.

109. Henry, op.cit., 520.

110. Clarke, op.cit., 283.

111. Rossi, Del Boca & Lobo, Anales de Farm. Y Bioquim, 1931, 2, 192; Am.Chem.Abstr., 1932, 26, 2553.

112. Werner, Ber., 1909, 42, 4324.

113. Gaebel, Arch. d.Pharm., 1910, 248, 207, 112.

114. Labat, Bull.soc.chim., 1909, 5, 743.

115. Culvenor, Goldsworthy, Kirby & Robinson, J.Chem.Soc., 1950, 1130.

116. Henry, op.cit., 182.

117. Harvey, Miller & Robson, J.Chem.Soc., 1941, 153.

118. Clemo & Swan, ibid., 1946, 617.

119. Dewar & King, Nature, 1941, 148, 25.

120. Henry, op.cit., 427.

121. Ibid., 428.

122. Clarke, Macbeth & Stewart, J.Chem.Soc.Proc., 1913, 29, 161.

123. Macbeth, J.Chem.Soc., 1915, 107, 1824.

124. Hammick & Young, 1936, 1463.

125. Barger, Madinaveitia & Streuli, J.Chem.Soc., 1939, 510.

126. Scholz, Compt.rend., 1935, 200, 1624.

127. Fourneau & Benoit, Bull.soc.chim., 1945, 12, 934.

128. Christie, Kropman, Leisegang & Warren, J.Chem.Soc., 1949, 1700.

129. Spiegel, Ber., 1915, 48, 2077.

130. Kirby, J.Chem.Soc., 1949, 735.

131. Kropman & Warren, ibid, 1950, 700.

132. Kupal, Ber., 1908, 41, 819.

133. Henry, op.cit., 558.

134. Busch, Ber., 1902, 35, 1656.

135. Schoental, J.Chem.Soc., 1950, 867.

136. Pregl, Quantitative Microchemistry, London, 1937, 171.

137. Numm & Rapson, J.Chem.Soc., 1949, 3151.

138. Fessler & Shriner, J.Am.Chem.Soc., 1936, 58, 1384.

139. Henry, op.cit., 24.

140. Ibid., 147.

141. Pictet & Kram, Ber., 1910, 43, 1329.

142. Fieser & Fieser, Chemistry of the Natural Products Related to Phenanthrene, Baltimore, 1949, 408.

143. Jacobs & Sato, J.Biol.Chem., 1948, 57, 175.

144. Henry, op.cit., 278.

145. Ibid., 248.

146. Ibid., 388.

147. Ibid., 540.

148. Ibid., 524.

149. Ibid., 486.

150. Ibid., 498.

151. Ibid., 554.

152. Henry, Kirby & Shaw, J.Chem.Soc., 1945, 524.

153. Prelog & Balenovic, Ber., 1941, 74, 1508.

154. Oudemans, Ann., 1879, 197, 48.

155. Cushny, Manual of Pharmacoloby & Therapeutics (Edited by Edmunds & Gunn), London, 1934, 542.

156. Johnson, Ann.Rep., 1949, 46, 206.

157. Henry, op.cit., 489.

158. Ibid., 501.

159. Chatterjee & Karrer, Helv.chim.Acta., 1950, 33, 802.

160. Briggs & Locker, J.Chem.Soc., in press.

161. Henry, op.cit., 224.

162. Strain, Organic Chemistry. An Advanced Treatise, by Gilman, New York, 1942, Vol.2, 1390.

163. Jackman, Macbeth & Mills, J.Chem.Soc., 1949, 1719. c.f. Human & Mills, Nat., 1946, 158, 877.

164. Glasstone, Introduction to Electrochemistry, New York, 1942, 322.

165. Karrer & Enslin, Helv.Chim.Acta, 1949, 32, 1390.

166. Ruzicka & Stoll, ibid., 1924, 7, 271.

The Zerewitinoff Estimation of Active Hydrogen Atoms.

Introduction.

In an attempt to determine the number of active hydrogen atoms in various Mitragyna alkaloids by means of methyl magnesium iodide in phenetole, it was found that results varied considerably with temperature and that, in general, whole number values were obtainable only on refluxing the reaction mixture, cf. Table 1.

Table 1.

Alkaloid	Number of Active Hydrogen Atoms at Room Temp.	50-60°	100°	160°C.
Mitragynine	1.89	2.26	2.84	2.97
Rhynchophylline	0.87	1.53	1.81	3.08.

A thorough search was therefore made of the literature to ascertain what was known of the various types of active hydrogen atoms at various temperatures. Since, however, in some cases the results of different workers on the same compound seemed contradictory, and since many results were very much different from what one would expect, a series of estimations was done on 38 substances, each at four standard temperatures. These estimations were done with both the methyl magnesium iodide and the substance dissolved in phenetole. In a smaller series of compounds in which abnormal results had occurred either in the literature or in the main investigation, further estimations were done not

only with both reagent and substance in butyl ether, but also with reagent in one solvent and substance in another.

HISTORICAL.

In the 45 years that have passed since Zerewitinoff adapted the reaction of Grignard reagents with substances containing active hydrogen atoms to the estimation of these atoms, many substances have been examined for these. It is proposed to discuss the previous work under the two main heads of "Methods", and "Results Obtained".

Methods.

In this section it is proposed to deal with first the Grignard reagents used, then the solvents, and finally the temperature. In assessing the value of a particular reagent, solvent, etc., one is faced with the difficulty of too many variables. It is useless saying that, merely because substance A shows one active hydrogen atom in solvent B and none in C, then C is a better solvent than B. Closer examination of the methods used would probably show that the Grignard reagents and reaction temperatures were also different. In this paper discussion will, as far as possible, be limited to unequivocal cases.

Reagents.

Although probably 90% of workers have used methyl magnesium iodide, ethyl magnesium bromide has been used a

little[1,2,3], and also ethyl magnesium iodide[4]. In the latter case the ethane was estimated gravimetrically.

More revolutionary is the use of such alkyls as zinc diethyl, boron tri-n-propyl and mercury dimethyl[5]. Although Nelson[5] says that, given sufficient time, zinc diethyl is quite satisfactory, Haurowitz[6] states that it gives only 60-95% of the expected amount of methane. Since, however, Nelson and Haurowitz were working not only with different solvents, but also with different types of substances, this apparent contradiction is understandable.

Recently lithium aluminium hydride has been used quite successfully[7,8]. With substances capable of keto-enol tautomerism, this reagent seems to give somewhat low results.

Solvents.

Although ethyl ether was initially considered to be the only possible solvent for Grignard reagents[9], Lehman and Basch[10] showed their contempt for this idea by using ether in the preparation of the reagent, and then distilling off the ether prior to doing the reaction in pyridine. At present, choice of solvent seems purely empirical. Frequently the solvent for the substance is different from that for the reagent. Sometimes a mixture of several solvents is used for the substance[11,12], and sometimes none at all[2,7]. The same substance, e.g., resorcinol (see Table 2), often

gives different results with different solvents.

Table 2.

Solvent for Reagent	Solvent for Resorcinol	Temp.	No of Active Hydrogens	Ref.
Butyl ether	iso-amyl ether	90°	1	13
nil	pyridine	90°	1	10
ether	nil	0°	1.5	7
amyl ether	amyl ether	140°	2	14
pyridine	amyl ether	room	2	15

Although most authors ignore the possible influences of solvent, some work on this aspect has been done. Lieff, Wright & Hibbert, for instance, have estimated twenty-three substances in amyl ether, xylene, dioxane and pyridine.[16]

Some attempts have been made to explain abnormalities. Fuchs, Ishler & Sandhoff[13] attribute the failure with polycarboxylic and polyhydric acids to insolubility of the substance. Lehman & Basch[10] suggest a further contributing factor may be that the reaction product is insoluble, and so the reaction takes place only at the surface. According to Kohler, Stone & Fuson[17] three difficulties may arise:-

(1). Relative insolubility.

(2). Insolubility of intermediates - said "rarely to interfere with the determination and sometimes to disappear on prolonged heating, but not infrequently to make it impossible to complete the reaction."

(3). Occurrence of successive reactions. These appear most frequently in cases involving oxidations and reductions, and reveal themselves by a slow and diminishing evolution of gas. As a result one cannot say when the reaction is complete.

Certain solvents call for special comment.

(a) Ether. As pointed out by Hibbert & Sudborough[14], the two disadvantages of ether are due to its low boiling point. Not only can one not use high temperatures, but there is also considerable variation of vapour pressure with slight variation in temperature.

(b) Pyridine has four distinct disadvantages:-

(i) It is extremely tedious and difficult to purify.

(ii) At elevated temperatures pyridine itself allegedly forms methane with methyl magnesium iodide - with methyl magnesium iodide in amyl ether and a substance in pyridine one may get a "blank" result of up to 50% of the amount expected from the substance[10].

(iii) In contrast to this evolution of excess methane giving high results, the solubility of methane in pyridine leads to low results at low temperatures.

(iv) The volume of methane is hard to read. After first falling to a minimum, it then tends to rise.

In reply to a scathing condemnation by Tanberg[18] on the use of pyridine as a solvent, Zerewitinoff[19] makes the

following points in answer to the second disadvantage:-

(a). If all the methyl iodide is not removed one would get methyl pyridine iodide, which would in turn react to give pyridine, magnesium iodide and ethane.

(b). If the pyridine was dried by boiling with barium oxide, one might get dihydropyridine, which contains active hydrogen. Altogether one is inclined to agree with Fuchs, Ishler & Sandhoff[13] that while pyridine has no special advantages, it is less precise.

(c). n-Amyl Ether. Although this is said to be difficult to purify[20], it has not been found so in this investigation.

(d). Anisole. At higher temperatures anisole may react even quantitatively with methyl magnesium iodide to form ethane[21].

(e). Phenetole. Although phenetole does not seem to decompose as does anisole, methane is slightly soluble in phenetole, e.g., 12.5 ml. phenetole allegedly dissolve 4.4 ml. methane at room temperatures[20]. However, provided one always uses the same amount of solvent and reagent, the constant error due to solubility will be compensated in the "blank"

(f). N-Alkyl Morpholines. N-methyl- and -ethyl morpholines have been used with lithium aluminium hydride[8]. It is held that N-ethyl morpholine is the best solvent for the estimation of active hydrogen atoms with this reagent.

Temperatures.

Although most compounds containing active hydrogen atoms react with Grignard reagents at room temperature, others are more resistant, and so temperatures up to 140° have been used. In many cases there is a definite parallel between the temperature used and the number of active hydrogen atoms shown, e.g., amides[22] and primary amines (various workers) show one active hydrogen atom at room, and two at higher temperatures. As is obvious from the case of resorcinol (summarised in Table 2 p. [illegible]), the influence of solvent must not be ignored. Some attempt has been made, e.g., by Zerewitinoff[22] and by Schmitz-Dumont & Hamann[11,12] to investigate the effect of temperature, but much remains still to be done. As would be expected, a hydrogen atom inert or only partially active at lower temperatures is often fully activated at higher temperatures. However, in the absence of detailed information on the solubility of a particular substance and of its reaction product with the Grignard reagent, one cannot say how much of the apparent inertness at lower temperatures is genuine inertness, and how much is due merely to insolubility.

Results Obtained.

Possibly the best way of summarising the numerous results given in the literature is to discuss the substances

under such heads as Alcohols and Phenols, Amines, etc.

(a) Alcohols and Phenols.

Although alcohols usually show the expected number of active hydrogen atoms, the lower alcohols give low values[23]. The only other abnormal results described are with certain complex sugar derivatives, e.g., methyl 2:3:4:6-tetramethyl-α- and -β-glucose pyranosides[16].

Among the phenols apparently contradictory results are obtained in many cases, e.g., hydroquinone[7,13,14,15], toluhydroquinone[13,25], resorcinol[7,10,13,14,15], phloroglucinol[10,13,15], and picric acid[10,13].

As might be expected, mercaptans show one active hydrogen atom for each thiol group[22].

(b) Amines.

In general, primary amines, aromatic and aliphatic, show one active hydrogen atom at room and the second at higher temperatures, i.e., 85-140°[2,20,23,24,26]. Although a secondary amine shows its active hydrogen atom at room temperatures[20,22,23,26], the lower amines resemble the corresponding alcohols in giving low values[23]. These values, however, may be raised either by prolonged standing or by heating. Tertiary amines show no active hydrogen[22].

(c) Acids.

In general, aliphatic and monocarboxylic aromatic acids show one active hydrogen per carboxyl group[4,13,16,25,27]. There are, however, references to the non-activity of a number of fatty acids[22] and of o-, m- and p-toluic acids[13]. Of the alleged inactivity of polycarboxylic aromatic acids[13], more will be said later.

(d) Esters and Acyl Derivatives.

The effect of esterification is to remove the active hydrogen atoms of the carboxyl groups[14,15]. Besides the expected effect of removal of the active hydrogen atom from the group acylated, introduction may of course create active hydrogen atoms by bringing about the possibility of keto-enol tautomerism, e.g., in aceto-acetic ester[13,14,22].

(e) Amides and Ureas.

As already mentioned, amides behave exactly like primary amines. Ureas are slightly different. At room temperature urea and thiourea show two active hydrogen atoms, and at 85°, not four but three atoms[22]. Phenyl-urea and -thiourea show two active hydrogen atoms independent of temperature[3].

(f) Compounds with Active Methylene Groups.

Comment on such compounds as acetoacetic or malonic ester is unnecessary. Anhydrides with vicinal methyl or methylene groups show active hydrogen atoms[28], doubtless

because of activation by the carbonyl groups[12].

(g) Heterocyclic Compounds and Alkaloids.

With these compounds one obtains simply the results expected from a consideration of the various groups present. Thus while pyrrole[4,3], indole[4], α-[3] and β-[4] methylindoles, and carbazole[4], for instance, all behave as imino compounds to show one active hydrogen atom, N-methylpyrrole and -indoles being tertiary amines, show none[4]. Alkaloids in general again react as would be expected from the groups present. Some however show considerable variation with temperature, e.g., calycanthine shows two at room temperature and four at 90°[28].

Discussion of Present Investigation.

Methods.

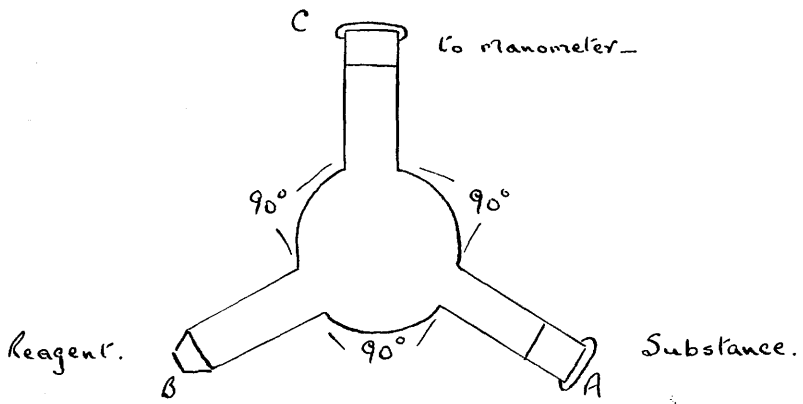
The purpose of the present investigation was to study under standard experimental conditions the estimation of active hydrogen in a fairly divergent series of compounds. Since the Mitragyna alkaloids gave widely differing results according to the temperature used, particular attention was paid to the influence of temperature. Thirty-eight substances were therefore investigated, each at the four standard temperatures: (a) room temperature, (b) 50-60°, (c) 100°, and (d) 160°. These reactions were done with phenetole as solvent for both substance and reagent. In some cases where

unexpected results or ones in conflict with those in the literature had been obtained, the substances were investigated also both with butyl ether as the common solvent and with reagent in butyl ether or phenetole and substance in another. The results with these compounds are given in Table 4 and will be discussed in detail later.

Apparatus.

The standard apparatus for Zerewitinoff estimations was modified only in the arrangement for adding the reagent to the solution of the substance. A three-way Q-Q adaptor (Fig.I) was used

Fig.I



The flask containing the substance in solution was placed on the arm A, and that with the reagent on arm B. To add reagent to substance, the whole adaptor was rotated about the axis of C sufficiently to allow the addition to take place.

Results.

As already mentioned, most of the results in phenetole

were as expected. In several cases, however (examples are given in Table 3), the value for active hydrogen atoms increased steadily towards a whole number, usually attained only at 160°. Although the higher values may be due to increased

Table 3.

Substance	Number of Reactive Hydrogen Atoms at				Solubility of Substance	Solubility of Reactn. Prod.
	room temp.	50-60	100	160°		
Succinimide	0.16	0.16	0.16	0.78	Insol.	Insol.
Piperidine	0.62	0.79	0.90	1.11	Sol.	"
Brucine	0.05	0.06	0.18	0.51	Insol.	Sol.
Strychnine	0.36	0.36	0.45	1.02	"	Insol.
Phthalic Acid	0.32	0.33	0.49	2.10	"	"

activity on heating, they may well be due to incomplete reaction of the undissolved material or to interference by the insoluble reaction product. The fact that various experiments on the same substance at the same temperature gave different results suggests a physical rather than a chemical explanation. Further evidence was given by an experiment with isophthalic acid. The acid was let stand with methylmagnesium iodide in phenetole for three hours, during which time methane equivalent to 0.82 gram atoms of active hydrogen was collected. Within 20 minutes of starting mechanical stirring a further 0.30 equivalents had beencollected, and ultimately a value of 1.86 was obtained. It would seem, therefore, that low results

may often be due to lack of solubility. Although some workers[e.g. 8] do not dissolve the substance but merely add it to a solution of the reagent, it would seem advisable, before adding the reagent, always to endeavour to dissolve the substance.

Certain results merit more detailed consideration.

(a) 2-Nitro-4-Methoxy-Aniline.

Although one would expect this, as a primary amine, to show one active hydrogen atom at room temperatures, and two at 100°, actually the values were 1.40 and 1.53 respectively. The high initial result may be explained as due to the influence of the adjacent nitro group. Since the substance was not completely soluble, and since the reaction product was a very sticky glue, the low final result is almost certainly due to insolubility.

(b) Malonamide.

The low results for malonamide seem inexplicable. Despite the insolubility of the malonamide itself, one would expect at 160° a value of 5 or 6 (4 from NH_2 groups, and 1 or 2 from the CH_2), rather than the total of 0.61 obtained.

(c) Strychnine.

Although the values found for strychnine were somewhat lower than those of Wieland & Hölscher[29], it is noteworthy that these are very erratic, viz:-

in anisole 1.64, 1.30, 1.21 and 1.08 at 20°

in pyridine 0.73, 0.66 at 20°; at 80°, 1.99, 1.74, 1.60 and 1.19.

(d) Pyridine.

As already mentioned, when pyridine is used as solvent in Zerewitinoff estimations, there may be a high "blank". Zerewitinoff[19] attributes this not to any activity of pyridine but to the formation and subsequent decomposition of methyl pyridinium iodide, and this view is supported by the failure, in this investigation of either pyridine, α- or β-picoline, or δ-collidine to show active hydrogen. Contrary to the theory of Zerewitinoff, it was found that even when methyl iodide was added to the pyridine, no active hydrogen was formed.

(e) Fluorene and Fluorene Derivatives.

Although one would expect fluorene to show active hydrogen, the values with the phenetole Grignard reagent were very low. At room temperature with the fluorene dissolved in phenetole or anisole no hydrogen was evident, and at 160° the value in phenetole was still 0.00. With both Grignard reagent and substance in butyl ether, higher values (0.15 and 1.10) were obtained. Although addition of reagent to the phenetole solution caused formation of a precipitate, there was no precipitate formed with the anisole solution, and even if insolubility was important, it would not explain

Table 4.

Substance	Phenetole Grignard and Phenetole	Anisole	Amyl Ether	Ether	Pyridine	Bu$_2$O Butyl Ether	Grignard and Ether	Fuchs, Ishler & Sandhoff (13)
Catechol	1.73	-	-	-	-	-	-	1.99
	1.82**	-	-	-	-	-	-	-
Resorcinol	1.79	1.98	1.45	1.98	0.10	2.01	2.02	0.98
	1.92*	-	1.84*	-	1.13**	-	-	-
Hydroquinone	1.02	1.40	0.47	2.05	1.99	2.09	0.81	0.00
	1.95*	1.79*	1.55*	-	-	-	-	-
Phloroglucinol	-	-	-	2.99	-	2.23	2.93	0.00
	-	-	-	-	-	2.99*	-	-
Phthalic Acid	0.32	0.32	-	1.93	-	2.02	1.95	0.00
	2.10	2.14	-	-	-	-	-	-
Isophthalic Acid	0.55	-	-	-	-	0.31	0.00	0.00
	1.20*	-	-	-	-	1.54*	-	-
Terephthalic Acid	1.27	-	-	-	-	0.65	-	0.00
	2.04*	-	-	-	-	1.86*	-	-
Ethyl Malonate	1.71	-	-	-	-	1.28	-	0.66***
	2.05*	-	-	-	-	1.77*	-	-
Ethyl Acetoacetate	-	-	-	-	-	1.03	-	0.99
	-	-	-	-	-	1.13*	-	-
Fluorene	0.00	0.00	-	-	-	0.15	-	-
	0.00*	0.00**	-	-	-	1.10*	-	-
Indole	1.01	-	-	-	-	1.09	-	-
	1.97*	-	-	-	-	1.44*	-	-

* at 160°
** at 100°
*** miscalculated by Fuchs, Ishler & Sandhoff at 1.00.

complete failure to collect methane on refluxing. The different result in butyl ether may be explained as due to the influence of solvent on the rate of formation of active hydrogen by the fluorene. That fluorene 9-carboxylic ester gave only 0.27 equivalents of active hydrogen, and that only at 160°C is even more remarkable. Again while formation of precipitate on reagent addition may partially explain the low values, this is not the complete explanation. Introduction of bromine atoms in the 2- and 7-positions to give the dibromo fluorene and ester increased considerably the amount of active hydrogen - at 160° to about one equivalent. Although one would expect the order of reactivity in producing active hydrogen atoms to be fluorene < fluorene-9-carboxylic ester < 2:7-dibromfluorene < 2:7-dibromfluorene-9-carboxylic ester, one would not expect the differences to be quite so marked.

(f) Indole.

Indole represents the one possibly high result in the whole series of compounds examined. As shown in Table 4, the second active hydrogen atom appears only at higher temperatures, but is given both in phenetole and butyl ether. This hydrogen may well be in the β-position, for sketole (β-methyl indole) shows only one active hydrogen atom.

(g) Malonic Ester.

Although malonic ester is described as having 0.6, 0.7 and 2 active hydrogen atoms[7,13,22,27], in this investigation it was found to show two active hydrogens at 160° with either phenetole or butyl ether as solvent. Since even at room temperature the values were greater than one, there is no question of the second atom being activated merely by heating. Although the 0.6 obtained with lithium aluminium hydride in ether[7] may be due to a very slow rate of enolization of the ester in that solvent, in view of the result obtained in this investigation with butyl ether as solvent, such an explanation cannot be advanced for the result of Fuchs, Ishler & Sandhoff[13]. While they found 0.66 hydrogen atoms at 90°, I found 0.96 and 1.36 at 50-60° and 1.26 and 1.77 at 100°. The variable results in butyl ether are probably due to the insolubility of the reaction product.

(h) Phthalic Acids.

Although Fuchs, Ishler & Sandhoff[13] say the phthalic acids contain no active hydrogen, in this investigation it was found, as shown in Table 4, that the acids in various solvents and with both the butyl ether and the phenetole Grignard reagents showed values ranging from 0.31 to 2.02 active hydrogen atoms at room temperatures, and from 1.20 to 2.04 at 160°. The reason for the variable results is again

almost certainly the insolubility of the acids and their reaction products.

(i) Polyhydric Phenols.

The various literature results for polyhydroxy benzenes are summarised in Table 5.

Table 5.

Phenol	Tempera-	Solvents	No. of Active Hs.	Ref.
Catechol	70	Butyl ether	2	13
Resorcinol	70	Butyl ether-iso-amyl ether	1	13
	90	Pyridine-	1	10
	0	Ether*	1.5	7
	140	Amyl ether	2	14
	room	Pyridine-amyl ether	2	15
Hydroquinone	0	Ether*	0	7
	70	Butyl ether	0	13
	room	Ether	2	5
	"	Pyridine-amyl ether	2	15
	140	Amyl ether	2	14
Phloroglucinol	room	Pyridine-amyl ether	3	15
	70	Butyl ether	0	13
	90	Pyridine-	1	10

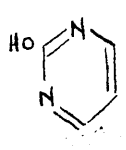
* With lithium aluminium hydride.

In this investigation these substances were examined fairly thoroughly in various solvents. As is shown in Table 4, p.186, the expected value of 3 for phloroglucinol and 2 for the others was found at room temperature in 11 out of the 17 combinations examined. In 3 out of the remaining 6, the expected value was found at a higher temperature.

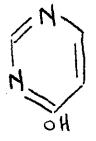
Fuchs, Ishler & Sandhoff[13] attribute their results to insolubility of the substances. Lehman & Basch[10] point out that the values are probably due also to the insolubility of the reaction product and the consequent fact that the reaction takes place only at the surface. It is further suggested that the low results with resorcinol and phloroglucinol are due to ketonization of the unreactive hydroxyl groups.

At first sight there seems to be much evidence in support of the ketonization theory. As Karrer[30] points out, resorcinol is not so very different from phloroglucinol, which can show ketonic properties.

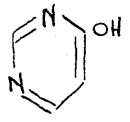
Again, as Lythgoe points out[31], the 2-, 4- and 6-hydroxypyrimidines (I, II, III)



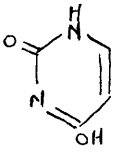
I



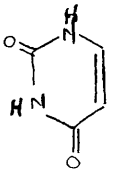
II



III



IV



V

show no phenolic properties. On ultra-violet spectroscopic evidence, Austin[32] concludes that in alcohol at least, urecil is in the monoketonic form IV. Arndt[33] formulates urecil as the diketo compound (V), and suggests that, unless forbidden by resonance considerations, the lactam structure is generally preferable for hydroxypyrimidines. On the other hand, it is doubtful how far one may compare

this lactim-lactam isomerism with the keto-enol tautomerism being discussed for the polyhydric phenols.

There is, however, much evidence against the theory that low values are due to the phloroglucinol reacting mainly in the ketonic form. If phloroglucinol existed partly in the keto form, and if the rate of enolization was slow with respect to the rate of reaction of the enol form to liberate its active hydrogen, then one might say that the low values were due to the extreme relative slowness of enolization. But the fact that other workers have found higher values destroys the validity of this argument. One may of course postulate that the results are due to the influence of the solvents on the rates of reaction. If this is so, it seems strange that, although butyl ether must slow the rate considerably (no active hydrogen atoms found) and pyridine nearly as much (one active hydrogen), the addition of amyl ether to the pyridine should so increase the rate of enolization as to cause the expected active hydrogen value of three to appear.

Further evidence against the ketonic theory is provided by the ultra-violet spectrum. The spectrum, not only of phloroglucinol, but also of catechol, resorcinol and hydroquinone, is typically phenolic and offers no evidence for ketonic groups[34]. It would therefore seem that although

phloroglucinol at least can react in the ketonic form, the normal state of these polyhydric phenols is enolic rather than ketonic.

The final argument against the ketone theory is that in the present investigation the values found for phloroglucinol were 2.23 at room temperature and 2.99 at 160°. Since both the phloroglucinol and the reaction product are insoluble in butyl ether, the low result in butyl ether at least, if not in pyridine also, is due to insolubility.

In the case of hydroquinone the arguments on enolisation, solvent and solubility apply as well as they do for phloroglucinol. There is the further consideration that if hydroquinone gives low values, catechol should give ones at least as low. While the electronic considerations applying to the para-compound should apply equally to the ortho-, with the latter there is also the possibility of chelation or of steric hindrance causing a further lowering of the active hydrogen value.

In the case of resorcinol also there is the argument from the ultra-violet spectrum. Moreover the X-ray crystal structure[35] gives no evidence for a keto group, but, on the contrary, is what one would expect for m-dihydroxybenzene.

Conclusion.

The purpose of this investigation was to examine the influence of temperature in Zerewitinoff estimations. With one possible exception, any values found higher than the corresponding ones in the literature were due to the insolubility of substance and/or reaction product. In the latter case, although the result may well be due mainly to the formation of a protective coating around particles of undissolved substance, there may well be a purely physical effect of adsorption of evolved methane in the network of the precipitate. Certain results lower than those in the literature may also be explained on the basis of insolubility.

Insufficient evidence was accumulated to allow comment on the increase in reactivity of various types of active hydrogen atoms at higher temperatures. From a study of the literature values, however, one is inclined to agree that with amides and primary amines, while the first hydrogen atom is active at room temperatures, the second becomes so only at higher temperatures. The one possibly abnormal result - that of indole - may well be due to the activation of the β -hydrogen atom at 160°.

EXPERIMENTAL.

Method.

Reagents.

Most of the estimations were done with both the methyl magnesium iodide and the substance dissolved in phenetole. In a few cases, the substance was dissolved in anisole, amyl ether or pyridine. In other cases, butyl ether was used as solvent for reagent and substance, and finally a few estimations were done with the reagent in butyl ether and the substance in ethyl ether. Anisole, phenetole and amyl and butyl ethers were purified by refluxing for five hours with sodium and then standing overnight, followed by distillation first from the sodium and then from phosphorus pentoxide. Since the butyl ether gave a peroxide test with vanadic acid, the ether was first freed from peroxides by treatment with sodium hydroxide and silver nitrate. The pyridine, which had been standing for two years over potassium hydroxide, was distilled before use. The ethyl ether was dried by standing over sodium wire.

Methyl magnesium iodide was prepared in the usual way by warming on the steam bath a mixture of 35.5 gm. freshly distilled methyl iodide, 6 gm. magnesium, 100 gm. of the appropriate ether, and a crystal of iodine. The solution was let stand several hours, then decanted, let stand overnight, and again decanted. To avoid the danger of

deterioration of Grignard reagent due to frequent exposure to atmospheric moisture during transfer of a portion of the reagent to the apparatus, the solution was kept in 25 ml. flask It was found unnecessary to work in an atmosphere of nitrogen. Specimens for analysis were, where possible, vacuum-dried for one hour at 100°C.

Apparatus.

Although the methane was collected over mercury in the usual way, the reaction part of the apparatus was modified. 2 ml. of the reagent was placed in a 5 ml. flask attached to arm A (Fig.1) of a three-way Q Q joint and 0.05 to 0.2 gm. of the substance to be examined was dissolved in 2 ml. solvent in a 10 ml. flask attached to arm B. In cases of difficult solubility or insolubility, 4 ml. solvent was used. By means of arm C, the reaction part of the apparatus was attached to a glass tube leading to the manometer where the methane was collected. To add the reagent to the solution of substance, the adaptor was rotated about the axis of arm C until the reagent ran into the reaction flask. This could be immersed in a bath at any given temperature.

In reactions at room temperature (which varied over the series from 16 to 28°C) methane was collected until two readings five minutes apart showed no change in volume. Unless otherwise mentioned, in the estimations at 50-60° and

100°, the bath containing the reaction vessel was kept at the given temperature for five minutes. In the final estimation for each substance, unless otherwise stated, the reaction mixture was gently refluxed for five minutes.

Results.

Table 6. (Grignard reagent and substance in phenetole).

Substance	Active Hydrogen Found		Literature Values			Expected Value	Ref
	Temp.	No.	Temp.	No.	Solvent		
A. Hydroxyl Compounds							
Ethyl Salicylate	23°	1.03	room	1	amyl ether	1	15
	50-60	1.03					
	100	1.03					
	160	1.04					
Catechol (b)	22	1.73	70	2	butyl ether	2	13
	50-60	1.73					
	100	1.82					
	160	1.82					
Resorcinol (b)	21	1.79	70	1	butyl ether	2	13
					iso amyl "		
			room	2	pyridine/ amyl ether		15
	50-60	1.83	90	1	pyridine/ ether		10
	100	1.87	140	2	amyl ether		14
	160	1.92	0	1.5	ether (d)		7
Hydroquinone (a), (b), (c)	20,22	1.02 0.95	room	2	pyridine amyl ether	2	15
	50-60	1.02 0.97	room	2	ether		5
	100	1.02 0.97 1.14(d)	70	0	Butyl ether		13
	160	1.03 1.48 1.95	140	2	amyl ether		14
			0	0	ether (α)		7
8-Hydroxy Quinoline (b)	18	0.77				2	
	50-60	0.77					
	100	0.77					
	160	0.79					

Substance	Active Hydrogen Found			Literature Values			Expected Values	Ref
	Temp.	No.		Temp.	No.	Solvent		
B. Acids								
Benzoic Acid	20	1.08		room	1	dioxane	2	10
	50-60	1.08		room	1	ether		10
	100	1.10						
	160	1.10						
Phthalic Acid (a),(b),(c)	25	0.32		70	0	butyl ether	2	13
	50-60	0.33						
	100	0.49						
	160	2.10						
Isophthalic Acid (a),(b),(c)	18	0.55		70	0	butyl ether	2	13
	50-60	0.58						
	100	0.78						
	160	1.20						
Terephthalic Acid (a),(b),(c)	18	1.27		70	0	Butyl ether	2	13
	50-60	1.33						
	100	1.67						
	160	2.04						
C. Esters.								
Ethyl Malonate	22	1.71		room	2	pyridine	2	27
	50-60	1.71		room	2	pyridine		22
			&	100		amyl ether		22
	100	1.71		70	0.7	butyl ether	13	13
	160	2.05		0	0.6	ether (d)		7
Ethyl Salicylate	23	1.03		room	1		1	14
	50-60	1.03						
	100	1.03						
	160	1.04						
Fluorene	25	0.00						
9-Carboxylic Ester (a),(b),(c)	50-60	0.00						
	100	0.00						
	160	0.27						
2:7-Dibrom-fluorene 9-Carboxylic ester (b)	18,21	0.57	0.96					
	50-60	0.67	0.96					
	100	0.73	0.98					
	160	0.90	1.03					

Substance	Active Hydrogen Found			Literature Values			Expected Values	Ref
	Temp.	No.		Temp.	No.	Solvent		
D. Amides and Imides								
Formamide (b),(c)	24,28	0.28	0.43					
	50-60	0.29	0.43					
	100	0.44	0.43					
	160	0.97	1.45					
Malonamide (a),(d)	21 22	0.16	0.00	room	2.25	pyridine	2 or 4	22
	50-60	0.16	-	85	4.25	amyl ether	4 or 6	
	100	0.16	-					
	160	0.11	0.17					
Benzamide (a),(f)	25	1.23	1.29	room	1	pyridine	1	22
	50-60	1.28	-	85	2	amyl ether		
	100	1.35	-				2	
	160	-	1.99					
Succinimide (b),(c)	24	0.16					1	
	50-60	0.16						
	100	0.16		50-60	1	?pyridine isoamyl ether		22
	160	0.78						
Phenylurea (f)	23	2.66		room	2	pyridine	2	22
	50-60	2.72		85	3	amyl ether		
	100	2.87					3	
	160	2.89						
E. Amine								
2-Nitro-4-Methoxy aniline (b)	22	1.40						
	50-60	1.40						
	100	1.53						
F. Heterocyclic Compounds.								
Indole (b)	23,24,	1.01	1.04	140	1	?pyridine isoamyl ether	1	4
	24		0.98					
	50-60	-	1.30					
			1.12					
	160	-	1.97					
			1.80(c)					

Substance	Active Hydrogen Found				Literature Values			Expected Values	Ref
	Temp.	No.			Temp.	No.	Solvent		
Skatole	21,21,24	1.00	1.06	1.05	140	1	?pyridine isoamyl ether	1	4
	50-60	1.00	-	-					
	100	1.00	-	1.11					
	160	-	-	1.14					
Carbazole	18	0.91			room	1	?pyridine isoamyl ether	1	4
	50-60	0.95							
	100	0.95							
	160	0.96							
Piperidine (b)	24	0.62	0.71					1	
	50-60	0.79							
	100	1.11	0.78(c)						
Pyridine	26	0.00						0	
	50-60	0.00							
	100	0.00							
Pyridine + excess Methyl Iodide	25	0.00							
	100	0.02							
α-Picoline	22	0.00						0	
	50-60	0.00							
	100	0.00							
	160	0.08							
γ-Picoline	25,19	0.02	0.00					0	
	50-60	0.06	0.00						
	100	0.06	0.00						
	160	0.07	0.00						
s-Collidine	26	0.00						0	
	50-60	0.00							
	100	0.00							
	160	0.00							
8-Hydroxy-quinoline (b)	18	0.77						1	
	50-60	0.77							
	100	0.77							
	160	0.79							
Isoquinoline	26	0.07						0	
	50-60	0.08							
	100	0.08							
	160	0.08							

Substance	Active Hydrogen Found			Literature Values			Expected Values	Ref.
	Temp.	No.		Temp.	No.	Solvent		
G. Substances Containing Active Methylene Groups.								
Ethyl Malonate	22	1.71					2	
	50-60	1.71						
	100	1.71						
	160	1.71						
Malonamide	21,22	0.16	0.00				4	
	50-60	0.16	-					
	100	0.16	+				6	
	160	0.61	0.17					
Fluorene (c)	19	0.00					1	
	50-60	0.00						
	100	0.00						
	160	0.00						
Fluorene 9-Carboxylic Ester (b),(c)	25	0.00					1	
	50-60	0.00						
	100	0.00						
	160	0.27						
2:7-Dibrom-fluorene (b)	17	0.58					1	
	50-60	0.72						
	100	0.79						
	160	0.83						
2:7-Dibrom-fluorene 9-carboxylic ester (b)	18,21	0.57	0.96				1	
	50-60	0.67	0.96					
	100	0.73	0.98					
	160	0.90	1.03					
H. Alkaloids								
Atropine (a),(c)	24	0.00		room	1	pyridine	1	24
	50-60	0.00		85	1	pyridine		
	100	0.02						
	160	0.18						
Brucine (a),(c)	26	0.05		room	0.2	pyridine	1	29
	50-60	0.06						
	100	0.18						
	160	0.51						

Substance	Active Hydrogen Found		Literature Values			Expected Values	Ref.
	Temp.	No.	Temp.	No.	Solvent		
Calycanthine (f)	27	2.19	room	2	pyridine	?	24
	50-60	2.34	95	4	pyridine		
	100	3.08					
	160	3.79					
Cinchonine	23	0.86	room	1.25	pyridine	2	24
	50-60	0.96	85	1.25	pyridine		
	100	1.17					
	160	1.66					
Morphine	24	0.10	room	2	pyridine	2	24
	50-60	0.37	85	2	pyridine		
	100	0.72					
	160	2.00					
Narcotine	27	0.00	room	0	Pyridine	0	24
	50-60	0.00					
	100	0.00					
	160	0.00					
Strychnine (a),(b),(c)	25	0.36	room	0.7-1.6	pyridine	?	29
	50-60	0.36	85	1.2-2.0	pyridine		
	100	0.45					
	160	1.02					
Yohimbine (b),(g)	16	1.38	room	2	pyridine	2	36
	50-60	1.60					
	100	1.86					
	160	2.00					

(a) Substance insoluble
(b) Insoluble product formed on addition of reagent
(c) Heated 15 minutes at 160°
(d) With lithium aluminium hydride
(e) Substance dissolved in hot phenetole
(f) Substance insoluble but gradually dissolves after addition of Grignard reagent
(g) Estimated as hydrochloride, which contained three active hydrogen atoms.

Table 7.

(Showing determinations of Active Hydrogen with Grignard Reagent in Phenetole, but Substance in another Solvent).

Substance	Solvent	Active Hydrogen Found Temp.	No.
Resorcinol	(i) Anisole	26	1.98
	(ii) Amyl ether	25	1.45
		50-60	1.78
		100	1.81
		160	1.84
	(iii) Ether (b)	21	1.98
	(iv) Pyridine (b)	22	0.05 0.02 0.10
		50-60	0.05 0.03 -
		100	0.13 0.03 -
Hydroquinone	(i) Anisole	26	1.40
		50-60	1.55
		100	1.79
	(ii) Amyl Ether (b)	25	0.47
		50-60	0.49
		100	0.60
		160	1.55
	(iii) Ether (b)	21	2.05
	(iv) Pyridine	22	1.99
Phloroglucinol	(i) Ether (b)	20	2.99
Phthalic Acid	(i) Amyl Ether (a)	25	0.32
		50-60	0.33
		100	0.49
	(c)	160	2.14
	(ii) Ether (a),(b)	22	1.93
Fluorene	Anisole	20	0.00
		50-60	0.00
		100	0.00

Table 8.

(Reagent and Substance in Butyl Ether).

Resorcinol (b)	20,25,18	1.32 1.98 2.01
	50-60	1.34
	100	1.61
	140	1.75

Substance	Temp.	No.
Hydroquinone (b)	21,27,25	0.50[a] 2.09 1.92
	50-60	0.61
	100	1.41
	140	2.00
Phloroglucinol (a),(b)	24	2.23
	50-60	2.37
	100	2.99
Phthalic Acid (a) (c)	19,19,22,18,20,21	0.87 0.88 0.66 2.02 1.12 0.11
	50-60	1.78 - 0.21 - 0.25
	100	2.04 0.59 1.18 0.39
	140	1.23 1.68 1.97
Isophthalic Acid (a), (c)	21	0.31
	50-60	
	100	0.71
	140	1.14
		1.54 (after 30 mins. further heating)
Terephthalic Acid (a)	21	6.65
	50-60	-
	100	0.67
	140	1.86
Malonic Ester (b)	19,25	0.90 1.28
	50-60	0.96,1.36
	100	1.26 1.77
	140	1.34 -
Acetoacetic Ester (b)	25	1.03
	50-60	1.06
	100	1.13
Fluorene (b)	24,26	0.00 0.15
	50-60	0.74 0.22
	100	0.97 0.27
	140	1.10 0.58
Indole (b)	27,26	0.78 1.09
	50-60	0.78 1.14
	100	0.85 1.25
	140	1.07 1.44.

Table 9.

(Substance in ether: reagent in butyl ether).

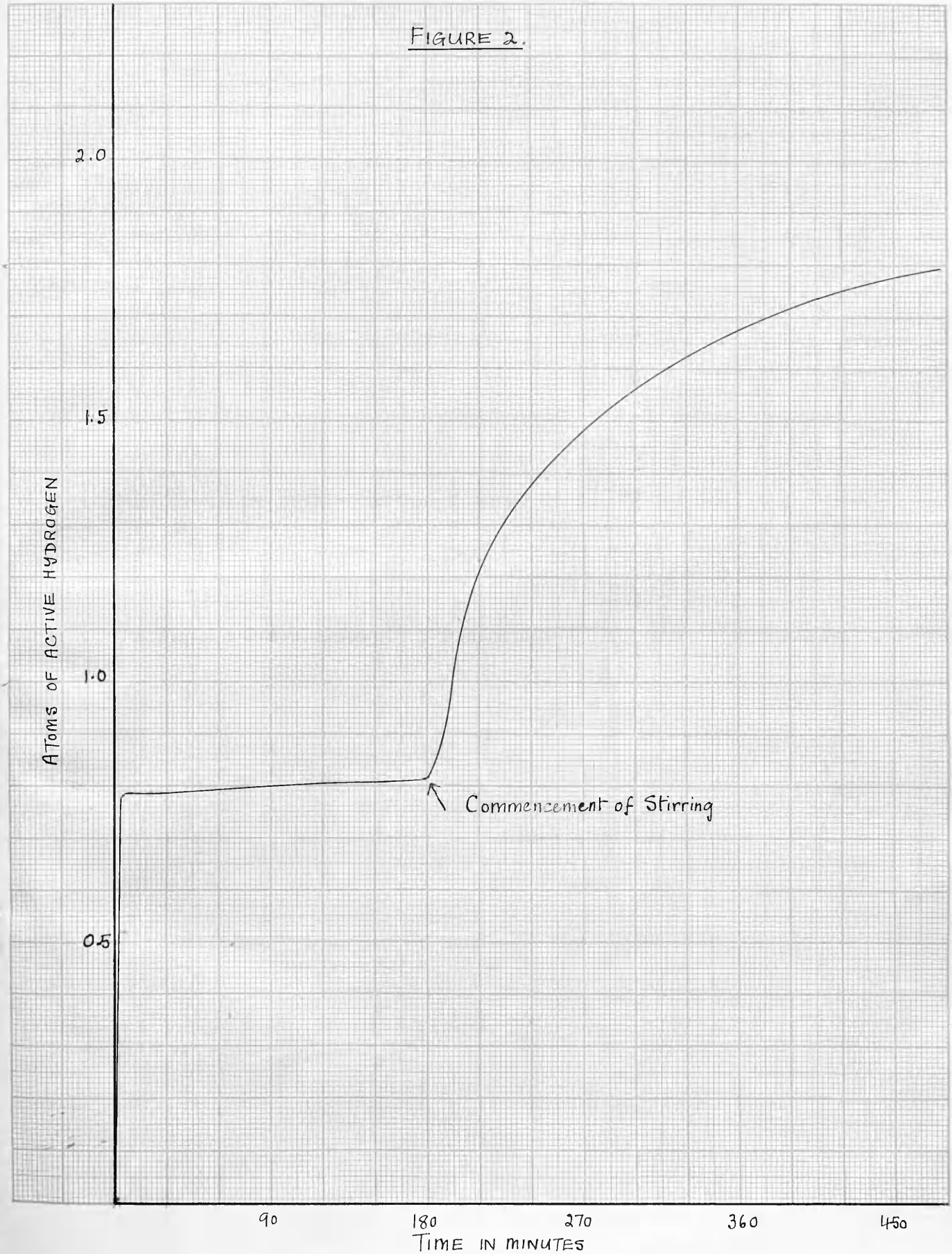
Substance	Temp.	No. of Active Hs.
Resorcinol (b)	20	1.96, 2.10, 2.02
Hydroquinone (b)	20	0.81
Phloroglucinol (b)	20	2.93
Phthalic Acid (a)	20	1.95
Isophthalic Acid (a)	21	0.00

To examine the effect of stirring on the evolution of methane by a substance giving an insoluble reaction product, isophthalic acid in phenetole was let stand with methyl magnesium iodide also in phenetole, and the volume of methane evolved read at intervals. The results are shown in Table 10, and graphically in Fig.2.

Table 10.

Time in mins.	Vol. Methane evolved	Vol for One Equiv.	No. Active Hs.
0	0	10.8 ml.	
5	8.3		0.78
20	8.5		0.79
60	8.5		0.79
120	8.7		0.81
180	8.9		0.82
At this stage mechanical stirring was begun.			
185	9.3		0.86
190	9.5		0.88
200	12.1		1.12
210	13.3		1.23
220	13.8		1.28
250	15.0		1.39
275	16.3		1.51
300	16.9		1.57
350	17.8		1.65
400	18.7		1.73
470	19.3		1.79
540	19.7		1.83
705	20.1		1.86

FIGURE 2.


2.0
1.5
1.0
0.5
ATOMS OF ACTIVE HYDROGEN
Commencement of Stirring
90
180
270
360
450
TIME IN MINUTES

References.

1. Oddo & Vassallo, Gazz.chim.ital., 1912, 42, 204, Am.Chem.Abstr., 1913, 7, 341.
2. Moureu & Magnonac, Compt.rend., 1914, 158, 1624.
3. Ciusa, Gass.chim.ital., 1920, 50, 53, Am.Chem.Abstr., 1921, 15, 837.
4. Oddo, Ber., 1911, 44, 2048.
5. Nelson, Iowa State Coll. J.Sci., 1937, 12, 145, Am.Chem.Abstr., 1938, 32, 3756.
6. Haurowitz, Mikrochemie, 1929, 7, 88.
7. Krynitsky, Johnson & Carhart, J.Am.Chem.Soc., 1948, 70, 486
8. Hochstein, ibid., 1949, 71, 305.
9. Blaise, Compt.rend., 1901, 132, 839.
10. Lehman & Basch, J.Indust. & Eng.Chem., Anal.Ed., 1945, 17, 428.
11. Schmitz-Dumont & Hamann, J.prakt.Chem., 1933, 139, 162.
12. Ibid, ibid., 167.
13. Fuchs, Ishler & Sandhoff, J.Indust. & Eng.Chem., Anal.Ed., 1940, 12, 507.
14. Hibbert & Sudborough, J.Chem.Soc., 1904, 85, 933.
15. Zerewitinoff, Ber., 1907, 40, 2033.
16. Lieff, Wright & Hibbert, J.Am.Chem.Soc., 1939, 61, 865.
17. Kohler, Stone & Fuson, ibid., 1927, 49, 3181.
18. Tanberg, ibid., 1914, 36, 335.
19. Zerewitinoff, Ber., 1914, 47, 2417.
20. Sudborough & Hibbert, J.Chem.Soc., 1909, 95, 477.
21. Houben-Weil, Die Methoden der Organischen Chemie, Leipzig, 1924, 4, 780.

22. Zerewitinoff, Ber., 1908, 41, 2233.

23. Hibbert, Proc.Chem.Soc., 1912, 28, 15;
J.Chem.Soc., 1912, 101, 328.

24. Zerewitinoff, Ber., 1910, 43, 3590.

25. Braude & Stern, J.Chem.Soc., 1946, 404.

26. Shtuber & Dobromyslova, J.Applied Chem. U.S.S.R., 1938, 11, 704: Am.Chem.Abstr., 1938, 32, 7031.

27. Kawai & Sugiyama, Ber., 1938, 71, 2443.

28. Barger, Madinaveitia & Streuli, J.Chem.Soc., 1939, 510.

29. Wieland & Hölscher, Ann., 1932, 500, 70.

30. Karrer, Organic Chemistry, Amsterdam, 1947, 428.

31. Lythgoe, Quarterly Reviews, 1949, 3, 200.

32. Austin, J.Am.Chem.Soc., 1934, 56, 2141.

33. Arndt, Rev.Fac.Sci. Istanbull, 1944, A9, 19, quoted by Lythgoe, op.cit., 203.

34. Hartley, Dobbie & Lauder, J.Chem.Soc., 1902, 81, 929.

35. Robertson, Proc.Roy.Soc., 1936, 157, 79.

36. Leonard & Elderfield, J.Org.Chem., 1942, 7, 556.