THE QUATERNARY ALKALOIDS

OF

ASPIDOSPERMA PEROBA F. ALLEM. EX SALD.

A THESIS

submitted to

THE UNIVERSITY OF GLASGOW

by

MUHAMMAD QAISUDDIN

in fulfilment of the

requirements for the degree of

DOCTOR OF PHILOSOPHY

July, 1964

The Department of Pharmacy,
The Royal College of Science
and Technology.
Glasgow.

S U M M A R Y

Recently, much interest has been shown in the tertiary alkaloids obtained from many species of Aspidosperma (Apocynaceae) but comparatively few reports have appeared on the quaternary alkaloids of this genus, a neglect possibly due to the greater difficulty of isolating water-soluble bases from natural mixtures. The present work gives a review of known Aspidosperma alkaloids and reports on the partial separation of quaternary alkaloids from an extract of the stem bark of Aspidosperma peroba F. Allem. ex Sald. (= A. polyneuron M. Arg.).

The mixture was shown by paper chromatography and thin-layer chromatography to contain at least five alkaloids. The alkaloid macusine B, previously reported in Strychnos toxifera (Loganiaceae), was isolated as the thiocyanate and converted to the known corresponding tertiary alkaloid normacusine B. Both compounds were characterised by physical data, infrared and ultraviolet spectra, and preparation of derivatives.

From the mixed thiocyanate which had yielded macusine B, a methanol-soluble fraction gave a second quaternary compound which was isolated as the iodide ($C_{21}H_{27}N_2O_3I$) for which m.p., ultraviolet and infrared spectra, and optical rotation are quoted.

Attempts were made to separate the remaining alkaloids, precipitated as reinecktates and converted to chlorides, by adsorption

chromatography on alumina followed by counter-current distribution or by partition chromatography using a range of conditions and many solvent systems. In particular, certain fractions from the alumina columns were subjected to distribution on a steady-state machine (by courtesy of Messrs. Quickfit and Quartz Ltd.) and preparative separations were also attempted using paper chromatography on large paper rolls in a Chromax pressure mantle.

No satisfactory separation of the quaternary chlorides was achieved, therefore a mixture containing three of the compounds was treated with ethanolamine to convert them to tertiary bases. From the reaction mixture one crystalline compound was isolated but this proved to be an ethanolamide. This compound was treated with methanolic hydrochloric acid to give the corresponding methyl ester and then this tertiary base converted to its methochloride using methyl chloride. Information is given on the ethanolamide and the tertiary base which are shown to be typical 2:3 disubstituted indoles.

Macusine B nitrate has been shown to exhibit very feeble neuromuscular blocking activity as measured on the isolated frog rectus abdominis preparation.

The author is deeply indebted to Dr. F. Fish, under whose guidance this work was carried out, for his direction and advice which have proved invaluable. He wishes to thank Professor J.B. Stenlake for his keen interest, helpful suggestions and encouragement, and other members of the Pharmacy Department Staff, especially Dr. W.D. Williams, for helpful advice; also the Association of Commonwealth Universities for financial support.

E R R A T A

Page 3 line 20 - for "similating" read "simulating"

Page 92 add H to N in the formula for 2,3-disubstituted indo

Page 136 line 4 - for "residue" read "residues"

CONTENTS

C O N T E N T S

INTRODUCTION

DISCUSSION

DISCUSSION (Continued)

EXPERIMENTAL

PRELIMINARY EXTRACTIONS AND SEPARATIONS

MACUSINE B

NORMACUSINE B

EXPERIMENTAL (Continued)

INTRODUCTION

THE GENUS *ASPIDOSPERMA*

The family Apocynaceae, comprising 155 genera and about 1,000 species[1], is known for its richness in alkaloid- and glycoside-bearing plants. Of the three sub-families, the Plumerioideae consists almost exclusively of alkaloid-bearing plants including species of the genera *Aspidosperma* and the closely related *Rauwolfia*. The *Aspidosperma* are classified in the tribe Alstonieae because of fruit structure, and into the sub-tribe Aspidospermatinae on seed structure.

Hybridization takes place readily and this has considerably increased the difficulties of botanical systematisation of the genus. The earlier taxonomic works are due to C.F.P. Martius (1824), A. de Candolle (1844) and J. Muller Argoviensis. In 1948, the French botanist Pichon[2], reported approximately 100 species and classified them into eleven series. A critical revision of the genus by Woodson[3] in 1951, reduced the number of species to 52 and the series to 9, many of the older species being shown to be synonymous. More recently (1955), Ducke[4] has made critical notes on some of the species.

Aspidosperma species are trees from about 2 to 60 metres high and most contain a milky or reddish latex. They are indigenous to tropical and sub-tropical regions of Central and South America and dispersed from the drier

regions of Brazil, Paraguay and Argentina to the inundated river margins of the Amazon valley[3,8,9].

The woods of Aspidosperma vary in toughness, texture and colour and many are economically important timbers, being used for many kinds of construction work. The wood and bark of several Aspidosperma species are good sources of tannin. The barks of many species are locally credited with medicinal properties and have found uses in folk-medicine[7]. The bark of A.quebracho-blanco Schlecht. is used in diseases of the liver, in affections of the respiratory organs, as a tonic, an analgesic, and has been recommended in fever, especially malaria[8,9,10]. The bark of A.peroba F. Allem.ex Sald.(=A. polyneuron M. -Arg.) is used in popular medicine under the name 'Peroba rosa' or 'Palo rosa' in the treatment of diarrhoea and against malaria[11]. It has also been credited with a curative action against leprosy[8]. 'Ubira-re-puíta' used by the Argentinain natives as a remedy for snakebite and as a febrifuge is the bark of A. chakensis Spegazzini[12].

NOTES ON THE PHARMACOLOGY OF ASPIDOSPERMA BARKS

The first reported pharmacological work on Aspidosperma barks revealed one of the most characteristic effects of their tertiary alkaloids when Penzoldt,[15] in 1879, observed that 2.5 grams of quebracho bark killed a dog by inhibiting respiration.

Both Wood (1910)[14] and Cow (1914)[13] compared aspidospermine, aspidosamine, quebrachine (yohimbine), and quebrachamine and found them to have similar qualitative effects which can be seen to be due to actions on the central and peripheral nervous systems. These effects have been summarised[16] as a fall in blood pressure, marked and persistent increase in both the rate and depth of respiration, together with clonic convulsions and muscular weakness. However, the respiratory effect has been attributed to intense local irritation, mainly by quebrachine, on parts of the lung. Sub-lethal doses paralyse the nerve cells of the brain, spinal cord, and autonomic nervous system. Lethal doses paralyse the sympathetic, vagal, and motor nerve endings, thus similating the effects of curarising agents and nicotine; death results from paralysis of the respiratory centre, the motor nerves still responding to electrical stimuli.

Later, similar results were obtained by Floriani using the barks of A. polyneuron[17] and A. quirandy Hassl.,[18] and

the alkaloid aspidospermine[16] which, in addition, was shown to produce diuresis, slight variations in the erythrocyte count, emetic and anti-thermic effects. It has also been reported[30] to reduce the muscle tone and inhibit contraction in the intestine and to exert adrenolytic effects, though not as strongly as yohimbine.

More recently, Banerjee and Lewis examined the total alkaloids from a number of Aspidosperma species and they demonstrated hypotensive, adrenolytic, acetyl-cholinolytic, histaminolytic and mild antipyretic effects in A. oblongum A.DC.[31,32] and A. excelsum[33] Benth. Alkaloids of A. album (Vahl) R. Ben. and A. megalocarpon M.-Arg.[34] had an acetyl-cholinolytic action but potentiated the effects of adrenaline while alkaloids of A. ulei Mgf.[35] had both acetyl-cholinolytic and adrenolytic effects, together with some central stimulating action.

In 1956-57, Raymond-Hamet[36,37] showed that the alkaloid quebrachamine, like sparteine, potentiates some of the adrenaline effects in dog; it increases the hypertensive and renal-vasoconstrictive actions of average doses of adrenaline and converts the hypotensive effects of small doses into hypertension.

In a recent report on yohimbine, described as a classic adrenolytic agent, Malone and Roth[38] referred to confirmation of its central nervous system activity and also to its effect as an antimetabolite of serotonin. These workers

demonstrated that the persistent and characteristic blepharoptosis (eyelid closure) induced by reserpine can be potentiated, in mice, by simultaneous administration of yohimbine. Yohimbine itself, however, showed no reserpine-like sedative-blepharoptotic activity in non-lethal dosage.

While various Aspidosperma barks have been reported to be effective against malaria and other protozoal infections, Becker[33] failed to show such activity. However, Banerjee and Lewis[34,35] demonstrated *in vitro* amoebicidal activity for alkaloids of some of the species which they examined.

There are no reports in the literature on the pharmacological effects of the few quaternary alkaloids so far isolated from *Aspidosperma* species.

TERTIARY ALKALOIDS OF *ASPIDOSPERMA* SPECIES

General

In recent years, interest in the chemistry of the genus *Aspidosperma* has increased considerably and the last four years have witnessed the successful structural elucidation of an array of indole alkaloids of this genus, ranging from the relatively simple harman-3-carboxylic acid to complex hexacyclic N-acyldihydroindoles. Rapid progress in this field can be attributed to the following factors:-

(a) The importance of the *Aspidosperma* as a possible source of therapeutically-potent alkaloids, since it is a genus botanically related to *Rauwolfia*.

(b) The introduction of X-ray crystallographic analysis, nuclear magnetic resonance spectroscopy, and mass spectrometry in structural chemistry.

(c) The availability of gas chromatography as a tool for the detailed analysis of complex natural mixtures.

(d) Interest in biogenetic pathways of these complex compounds and also in a chemical basis for the taxonomic classification of plants.

Research works on this genus are scattered over a period of more than 80 years but a more or less systematic search for its alkaloids started only a decade ago[18,30,31,88]. Prior to this, isolation and chemical studies were conducted

sporadically and among the earlier workers, mention may be made of Fraude[33], Hesse[34], Rothlin[35], Ewins[36], Field[37], Floriani[9] and Orazi[38].

The earlier work has been reviewed by Henry(1949)[39], Marion(1952)[40], Palmer(1954)[30], Bisset(1958)[6] and, more exhaustively, by Schmutz(1961)[10]. The latest, and comparatively recent, review dealt with the chemotaxonomy of the then known alkaloids, but a tremendous amount of work has been done since that time with the result that a further 54[x] Aspidosperma alkaloids have been isolated and characterised. Of these, the five alkaloids aricine, reserpiline, isoreserpiline, β-yohimbine[43] and isoreserpiline-Ψ-indoxyl[45] had been previously found in Rauwolfia species. β-Yohimbine is present also in Amsonia elliptica Roem.et Schult. (Apocynaceae)[43] and in Corynanthe johimbe K. Schum. (Rubiaceae)[44], while isoreserpiline is present also in Ochrosia elliptica Labill. (Apocynaceae)[44]. Eburnamenine had been previously reported in Hunteria eburnea Pichon[46] and is also present in Rhazya stricta Decaisne[46], both Apocynaceous plants.

---

[x] Includes only one quaternary compound, macusine B, the isolation and characterisation of which is described in this thesis and has been published recently (1964)[41].

The alkaloids, 1-methyldeacetylaspidospermine, deacetylaspidospermine, demethylaspidospermine[47], (-)-pyrifolidine[48,49], deacetylpyrifolidine[48], 1,2-dehydro-aspidospermidine[50], and dihyrocorynanthead[51] were identical with chemically-prepared derivatives of known alkaloids; the optical antipodes of (-)-pyrifolidine[48] and (-)-guatambuine[10] were also known. Structures of the majority of the remaining compounds have been clearly established.

In addition to the above 54 characterised alkaloids, 17 bases from A. oblongum[88] and eight from A. quebracho-blanco[88] have been reported. The molecular weights for all these alkaloids have been worked out, and further work on their chemistry is in progress. The skeletal features of the 17 A. oblongum alkaloids have been indicated from mass spectrometrical analysis and two of the compounds, both of molecular weight 412, are considered to be reserpiline and isoreserpiline. Some of the remainder may also prove to be known alkaloids but others will probably be new compounds.

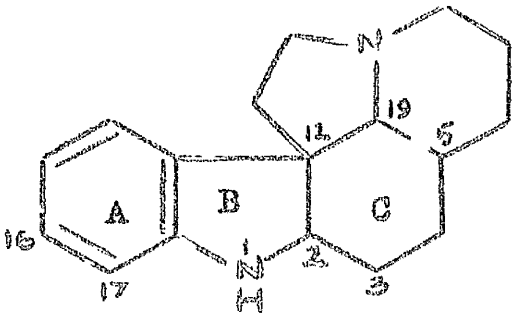
Classification of Aspidosperma Alkaloids.

The Aspidosperma alkaloids are derivatives of indole forming a complex class of naturally-occurring bases. However, they can be broadly classified into the five main

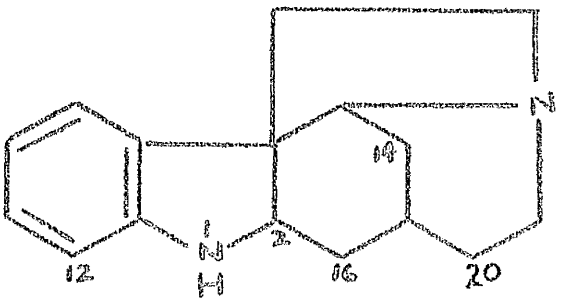
groups: Dihydroindole alkaloids, Indole alkaloids, Pyrido-carbazole alkaloids, Oxindole alkaloids, and alkaloids of unknown structure.

A. Dihydroindole alkaloids:- This group, comprising 43 compounds, can be further divided into two subgroups according to the number of rings in the molecule.

(i) Pentacyclic dihydroindoles:- Two types of alkaloids are recognised in this subgroup, namely the Aspidospermine type[83] having skeleton I, and the Aspidospermatine-akuammicine type[83,84] having skeleton II.



I



II

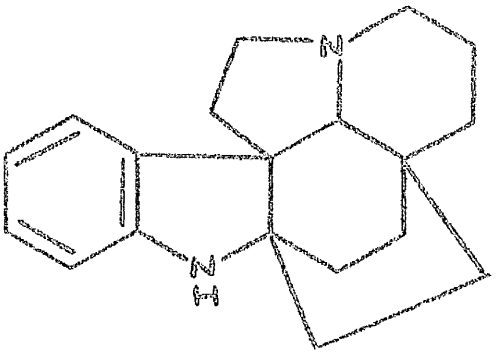
(a) The Aspidospermine type:- Of these 24 alkaloids, the simplest is aspidospermidine[83] having a $-C_2H_5$ substituent at C5. All the alkaloids of this type can be regarded as derivatives of I differing only in their substituents at 1,3,5,16 and 17. Dehydroaspidospermidine differs from all other compounds within this group by having a double

bond in the 1-2-positions (an indolenine structure).

(b) The Aspidospermatine-akuammicine type alkaloids are eight in number. Four members are 1- and/or 12-substituted aspidospermatidine[63] (=CH.CH_3 at C14) and one is a dihydroaspidospermatine (-CH_2.CH_3 at C14). The remaining two members, compactinervine[64] and tubotaiwine[65], have a double bond in the 2-16-position (indoline structure) and differ in substituents at C14 and C20.

(ii) Hexacyclic dihydroindoles:- These alkaloids can be placed in three categories, namely the pyrifoline[66], aspidoalbine[67] and ajmaline[68] types.

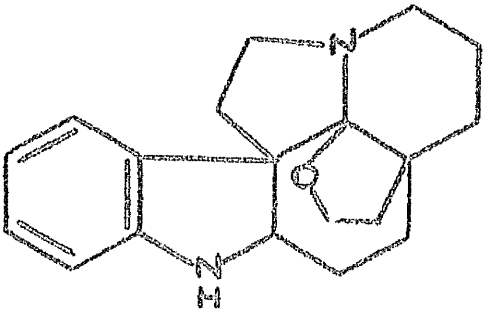
(a) The pyrifoline type alkaloids number six and they have skeleton III, which represents the simplest member, aspidofractinine[69]. The remaining members are all substituted aspidofractinine.



III Aspidofractinine

(b) The aspidoalbine type: Four such compounds are known and these are based on an aspidospermine-like skeleton with an additional tetrahydrofuran ring. The

simplest compound is fendleridine[60] (IV) and the remainder are all fendleridine derivatives.



IV

(c) The ajmaline type is represented in the Aspidosperma by only one alkaloid, namely, quebrachidine[61].

B. Indole alkaloids:- This group contains 32 alkaloids. The structures of 15 have been established and for the remaining 17 the skeletal features have been worked out[62]. The indole alkaloids can be placed into three main subgroups according to the number of rings present in their molecules.

(i) Tricyclic indoles:- There is only one member of proved structure, harman-3-carboxylic acid[62], which in conjunction with a carbohydrate moiety forms a glyco-alkaloid. A compound which appears from its ultraviolet spectrum to be very similar, and also occurs as a glyco-alkaloid, is queborachacidine[63].

(ii) Tetracyclic indoles:- This subgroup includes the corynantheine type[64] of alkaloids, namely dihydrocorynantheol[65]

10-methoxydihydrocorynantheol and its 19,20-dehydro derivative[66,67] together with 12 of the above 17 alkaloids. In addition (-)quebrachamine[68] is a tetracyclic indole with an aspidospermine-like skeleton in which the ring C is open in the 12-19-position (in I).

(iii) Pentacyclic indoles:- This subgroup embraces 15 members among which four structural types are encountered, the yohimbine[48] type, reserpiline[48] type, sarpagine[69] type and the eburnamenine[48] type.

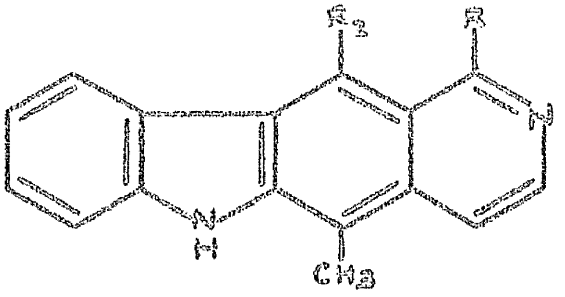
(a) The yohimbine type includes, in addition to yohimbine[100] β-yohimbine and its 11-methoxy derivative [68].

(b) The reserpiline type comprises reserpiline, isoreserpiline[68] and aricine[65] together with 5 alkaloids[63] of molecular weights 352, 382, 412, 382 and 412. Many of the above two types of alkaloid occur in the related genus Rauwolfia[48].

(c) Sarpagine type. Only three members with the sarpagine skeleton are known in the Aspidosperma and they all occur in the same species, A. polyneuron[70,61]. However this type has been found in related genera of the Apocynaceae[71] and also in the Loganiaceae[72,73].

(d) Eburnamenine[48] is the only alkaloid of its type yet found in the Aspidosperma (A. quebracho-blanco).

C. Pyridocarbazole alkaloids:- These number eleven and all are tetracyclic. With the exception of uleine[16], the only compound of its type yet found in the Aspidosperma, all are derivatives of either ellipticine or olivacine[19], two alkaloids which differ only in the position of their second $-CH_3$ substituent(V).



V

Olivacine $R_1 = CH_3$, $R_2 = H$

Ellipticine $R_1 = H$, $R_2 = CH_3$

D. Oxindole alkaloids:- Carapanaubine[78] is the only oxindole in this genus. In Table 1, it has been placed with isoreserpiline-ψ-indoxyl[68], a compound present in A. discolor A.DC.

E. Alkaloids of unknown structure:- In addition to the 15 (7 un-named and 8 named) alkaloids reported in Schmutz's review, there are at least thirteen alkaloids whose structures are not yet known. These are obscurinervine, obscurinervidine, dihydroobscurinervine, dihydroobscurinervidine from A. obscurinerveum[71]; quebrachacidine[68], 294A, 294B.

308A, 320B, 322B, 324B, 368A and 398A from A. quebracho-blanco[88].

All the tertiary aspidosperma alkaloids of known structures are collected in Table 1; physical data of the five alkaloids, obscurinervine, obscurinervidine and their dihydroderivatives, and quebrachacidine are also included.

Alkaloids of Aspidosperma peroba F. Allem. ex Sald.

A. polyneuron M.-Arg. (=A. perobs F. Allem. ex Sald.) was first investigated in 1909 by Peckolt[76] who obtained about 0.4% aspidospermine. In 1918 Rothlin[86] isolated from the bark the six bases aspidospermine, quebrachamine, quebrachine (yohimbine), hypoquebrachine, aspidospermatine and aspidosamine, all previously reported in A. quebracho-blanco Schlecht by Hesse[84]. In addition to these, he also isolated aspidospermicine and aspidospermanine. Later, in 1938, Floriani[9] confirmed the findings of Rothlin.

It was 20 years after Floriani's work that Antonaccio[7] reported the isolation of two new alkaloids, alkaloid A, later named perobine, and alkaloid B.

A reinvestigation of the species in 1959 by Schmutz and co-workers[11] led to the detection of six bases, three of which were identified as aspidospermine, quebrachamine and yohimbine. Of the remaining three bases, one was

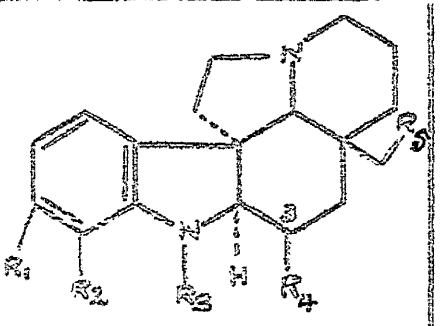
obtained crystalline and was shown to be a new alkaloid, palosine. The other two could not be crystallised.

Recently, Antonaccio et.al.[69] isolated a glyco-alkaloid based on the aglycone harman-3-carboxylic acid, while Djerassi and co-workers[70] isolated the two alkaloids normacusine B and polyneuridine.

Table I

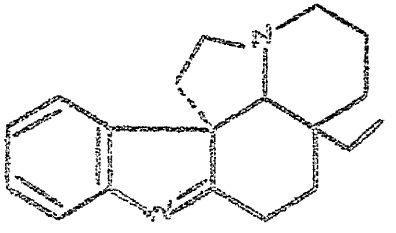
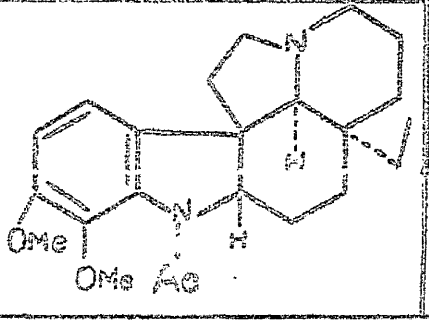
Tertiary alkaloids of the Aspidosperma

Pentacyclic Dihydroindoles.
Aspidosperamine Type.

Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
Aspidospermine $C_{22}H_{30}O_2N_2$	207-209	-92° ($CHCl_3$) -99° (EtOH)	 R_1 R_2 R_3 R_4 R_5 H OMe Ac H Me	Aspidosperma peroba F. Allem. ex. Sald. (=A. polyneuron M.-Arg) A. sessiliflorum F. Allem. A. australe M.-Arg. A. quirandy Hassl. A. pyricollum M.-Arg. A. quebracho-blanco Schlecht.	10,53, 78,79
Deacetylaspidospermine $C_{20}H_{28}ON_2$	109-111	-	R_1 R_2 R_3 R_4 R_5 H OMe H H Me	A. quebracho-blanco Schlecht.	47,53
Demethoxyaspidospermine $C_{21}H_{28}ON_2$	amorph.	-15° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 H H Ac H Me	A. discolor A.DC. A. eburneum F. Allem.	67
Demethylaspidospermine $C_{21}H_{28}O_2N_2$	170	+94° (MeOH)	R_1 R_2 R_3 R_4 R_5 H OH Ac H Me	A. discolor A.DC.	47,67
1-Methyldeacetylaspidospermine $C_{21}H_{30}ON_2$	169-171 (methiodide).	-	R_1 R_2 R_3 R_4 R_5 H OMe CH_3 H Me	A. quebracho blanco Schlecht.	47,53

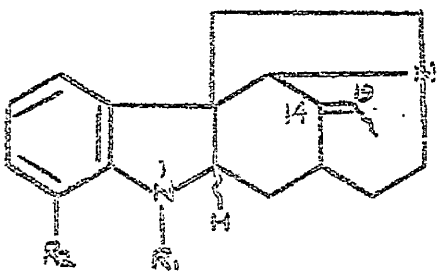
Alkaloid	m.p. °C.	[α]D	Structure					Source	Refs.
Aspidocarpine $C_{32}H_{36}O_5N_2$	168.5-169.5	+140° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.megalocarpon* M.-Arg. *A.album*(Vahl) R. Bent. *A.limae* Woodson	10,80 81,82
			OMe	OH	Ac	H	Me		
Aspidolimine $C_{33}H_{38}O_5N_2$	150-151	+133±3° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.limae* Woodson *A.ternatum*.	81,82
			OMe	OH	EtCO	H	Me		
Limaspermine $C_{32}H_{36}O_3N_2$	175-175.5	+108° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.limae* Woodson	83
			H	OH	EtCO	H	CH_2OH		
Palosine $C_{33}H_{38}O_2N_2$	149-152	-85.9° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.polyneuron* M.-Arg.	10
			H	OMe	EtCO	H	Me		
Demethoxypalosine $C_{32}H_{36}ON_2$	117-120	-20° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.limae* Woodson *A.discolor* A.DC.	55,67, 81
			H	H	EtCO	H	Me		
O-Demethylaspidocarpine $C_{31}H_{36}O_5N_2$	156-158	+125° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.album* (Vahl) R.Bent.	80
			OH	OH	Ac	H	Me		
Cylindrocarpine $C_{32}H_{38}O_4N_2$	168-169	-181° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.cylindrocarpon* M.-Arg.	10,48, 84,85
			H	OMe	C_6H_5CH =CHCO	H	COOMe		
Cylindrocarpidine $C_{33}H_{40}O_4N_2$	118-118.5	-122° ($CHCl_3$)	R_1	R_2	R_3	R_4	R_5	*A.cylindrocarpon* M.-Arg.,	10,48, 84,85
			H	OMe	Ac	H	COOMe		

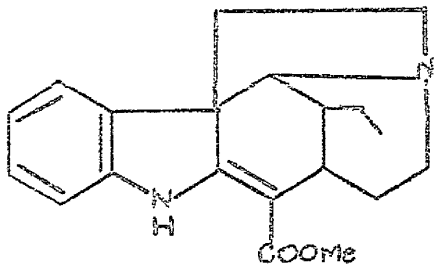
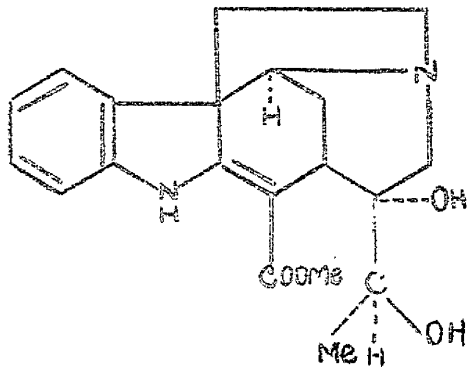
Alkaloid	m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Spegazzinine $C_{31}H_{48}O_6N_2$	105–110	+175.6° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 R_6 H OH Ac 3αOH Me	*A.chakensis* Speg.	10,71 86,87
Spegazzinidine $C_{31}H_{48}O_4N_2$	237–238	+186° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 OH OH Ac 3αOH Me	*A.chakensis* Speg.	86,87
(-)-Pyrifoline $C_{33}H_{52}O_3N_2$	148–150	−93° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 OMe OMe Ac H Me	*A.quebracho-blanco* Schlecht.	53
Deacetylpyrifoline $C_{31}H_{50}O_3N_2$	149.5–151.5	+9° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 OMe OMe H H Me	*A.quebracho-blanco* Schlecht. *A.pyrifolium* Mart.	53,71
Methoxylimaspermine $C_{33}H_{52}O_4N_2$	174–175	−118° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 OMe OH EtCOH CH_2OH	*A.limae* Woodson	55
Limaspedine $C_{31}H_{48}O_4N_2$	177–188	+110° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 H OH Ac H CH_2OH	*A.limae* Woodson	55

Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
Methoxylimapodine $C_{22}H_{30}O_4N_2$	220	+131° ($CHCl_3$)	R_1 R_2 R_3 R_4 R_5 OMe OH Ac H CH_2OH	A.limae Woodson	55
Aspidospermidine $C_{18}H_{26}N_2$	110-112	-	R_1 R_2 R_3 R_4 R_5 H H H H Me	A.quebracho-blanco Schlecht.	53
1-Methylaspidospermidine $C_{20}H_{28}N_2$	-	-	R_1 R_2 R_3 R_4 R_5 H H CH_3 H Me	A.quebracho-blanco Schlecht.	53
1,2-Dehydroaspidospermidine $C_{19}H_{24}N_2$	-	-		A.quebracho-blanco Schlecht.	50,53, 68
(+)Pyrifolidine $C_{23}H_{32}O_3N_2$	147.5-150	+90° ($CHCl_3$)		A.pyrifolium Mart.	10,48, 84

Pentacyclic Dihydroindoles.

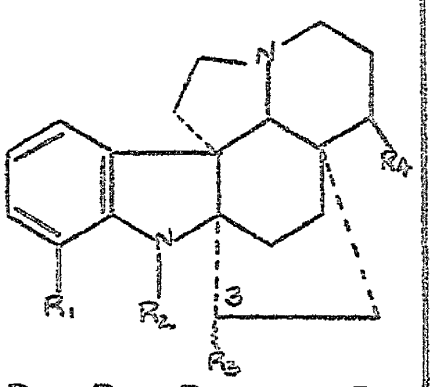
Aspidospermatine-Akuammicine Type.

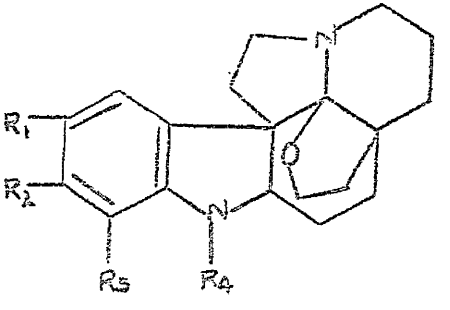
Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
Aspidospermatine $C_{21}H_{30}O_2N_2$	157-159	-73° (EtOH)	 R_1 = Ac; R_2 = OMe	A.quebracho-blanco Schlecht. A.peroba F.Allem. ex Sald. (=A.polyneuron M.-Arg.)	10, 53
Aspidospermatidine $C_{19}H_{28}N_2$	184-186	-	R_1 = H; R_2 = H	A.quebracho-blanco Schlecht.	53
1-Methylaspidospermatidine $C_{20}H_{30}N_2$	-	-	R_1 = Me; R_2 = H	A.quebracho-blanco Schlecht.	53
Deacetylaspidospermatine $C_{19}H_{28}ON_2$	-	-	R_1 = H; R_2 = OMe	A.quebracho-blanco Schlecht.	53

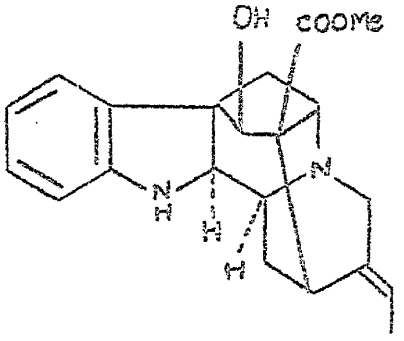
Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
1-Acetylaspidosepermatidine $C_{30}H_{34}ON_2$	-	-	R_1 Ac; R_2 H	A.quebracho-blanco Schlecht.	53
14,19-Dihydroaspidospermatine $C_{31}H_{36}O_2N_2$	-	-	R_1 Ac; R_2 OMe (14,19-dihydro)	A.quebracho-blanco Schlecht.	53
Tubotaiwine $C_{20}H_{24}O_2N_2$	amorph.	+584° ($CHCl_3$)		A.limae Woodson	55
Compactinervine $C_{20}H_{24}N_2O_4$	110-120	-640° (Pyridine)		A.compactinervium Kuhlm	54

Hexacyclic Dihydroindoles.

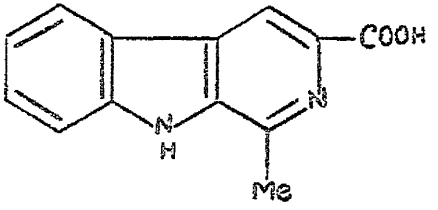
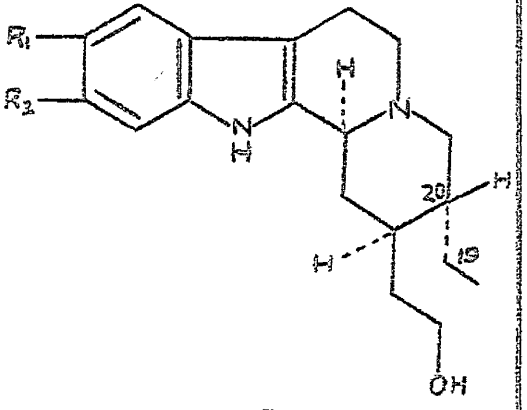
Pyrifoline Type.

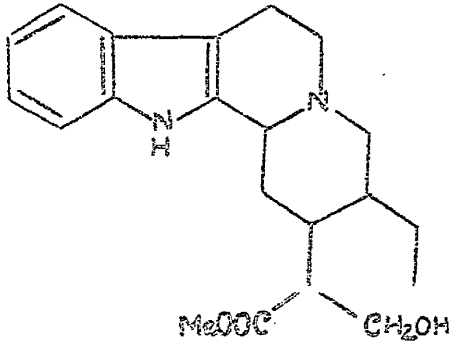
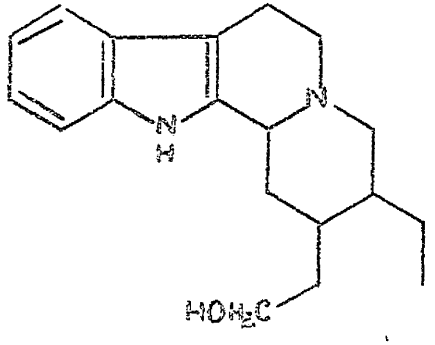
Alkaloid	m.p. °C.	[α]D	Structure	Source	Refs.
Pyrifoline $C_{23}H_{30}O_3N_2$	141.5-143.5	+102° ($CHCl_3$)	 R_1 OMe; R_2 Ac; R_3 H; R_4 OMe	A.pyrifolium Mart.	10,56,59
Refractidine $C_{21}H_{26}O_3N_2$	158-160	-140° ($CHCl_3$)	R_1 H; R_2 CHO; R_3 H; R_4 OMe	A.refractum Mart.	56,59
Refractine $C_{23}H_{28}O_6N_2$	dimorph. 157-159 191-192	-23° ($CHCl_3$)	R_1 OMe; R_2 CHO; R_3 3αCOOMe; R_4 H	A.refractum Mart.	10,59,88
Aspidofiline $C_{21}H_{26}O_3N_2$	190-191	-174° ($CHCl_3$)	R_1 OH; R_2 Ac; R_3 H; R_4 H	A.pyrifolium Mart.	59,89,90

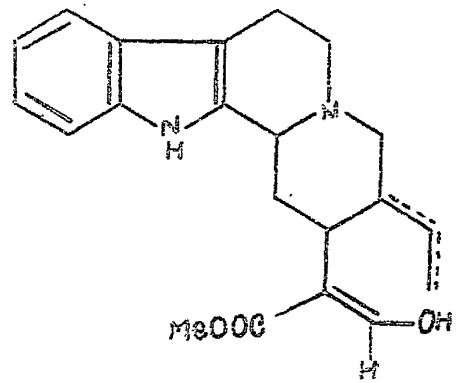
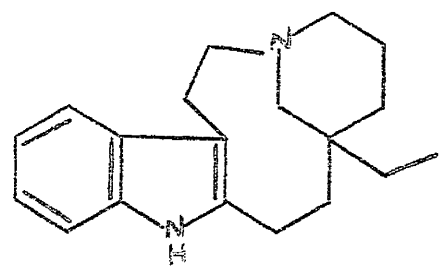
Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
Aspidofractine $C_{22}H_{26}O_3N_2$	193-193.5	-142° ($CHCl_3$)	R_1 H; R_2 CHO; R_3 3αCOOMe; R_4 H	A.refractum Mart.	59,88
Aspidofractinine $C_{19}H_{24}N_2$	-	-	R_1 H; R_2 H; R_3 H; R_4 H	A.refractum Mart.	59.
Hexacyclic Dihydroindoles.					
Aspidoalbine Type.					
Aspidoalbine $C_{24}H_{32}O_3N_2$	170-172	+148° ($CHCl_3$) +159° (MeOH)	 R_1 OMe; R_2 OMe; R_3 OH; R_4 EtCO	A.album (Vahl) R. Bent.	57,80

Alkaloid	m.p. °C.	[α]D	Structure	Source	Refs.
N-Acetyl-N-depropionyl-aspidoalbine $C_{28}H_{30}O_5N_2$	194-195	+174° ($CHCl_3$)	R_1 R_2 R_3 R_4 OMe OMe OH Ac	A.album (Vahl)R. Bent.	80
Aspidolimidine $C_{28}H_{28}O_4N_2$	196-199	+239° ($CHCl_3$)	R_1 R_2 R_3 R_4 H OMe OH Ac	A.limae Woodson	81
Fendleridine $C_{19}H_{24}ON_2$	185-186	-	R_1 R_2 R_3 R_4 H H H H	A.fendleri Woodson	60
Hexacyclic Dihydroindoles. Ajmaline Type.					
Quebrachidine $C_{21}H_{24}O_3N_2$	276-278	+54° ($CHCl_3$)		A.quebracho-blanco Schlecht.	61,71

Tricyclic Indoles.

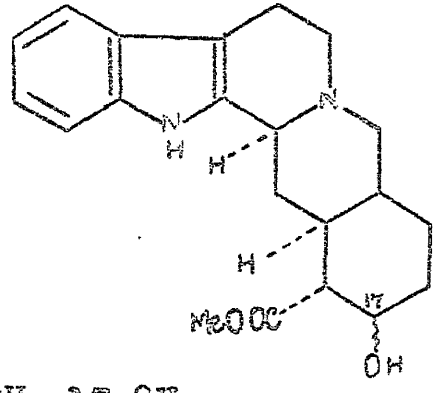
Alkaloid	m.p. °C.	[α]D	Structure	Source	Refs.
Harman-3-carboxylic acid $C_{14}H_{12}O_2N_2$	252-253	—		A. polyneuron M.-Arg.	62
Tetracyclic Indoles.					
Dihydrocory-nantheol $C_{16}H_{22}ON_2$	181-183	-19° ($CHCl_3$) -37° (Pyridine)	 R_1 H R_2 H	A. marcgraviamum Woodson A. auriculatum	51,65

Alkaloid	m.p. °C.	[α]D	Structure	Source	Refs.
10-Methoxydihydro-corynantheol (AD IV) $C_{20}H_{28}O_3N_2$	165-166	-16° (Pyridine)	R_1 OMe; R_2 H	A.discolor.A.DC.	66,67
19,20 - Dehydro-10-methoxydihydro-cornynantheol (AD VI.) $C_{20}H_{26}O_3N_2$	184-185	-65° (Pyridine)	R_1 OMe; R_2 H (19,20-dehydro)	A.discolor.A.DC.	66,67
Alkaloids of Mol. Wts. 354,384,356, 386.	-	-		A.oblongum A.DC.	52
Alkaloids of Mol. Wts. 296,298,326, 328.	-	-		A.oblongum A.DC.	52

Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
Alkaloids of Mol. Wts. 352, 382, 354, 384.	-	-		A.oblongum A.DC.	52
(-)-Quebrachamine $C_{19}H_{24}N_2$	146-147	-109° ($CHCl_3$)		A.quebracho-blanco A.chakensia Speg. A.peroba F.Allem. ex Sald. (A=polyneuron M.-Arg.)	10, 53, 68

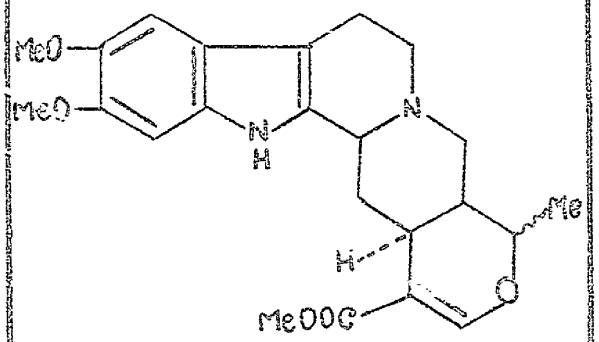
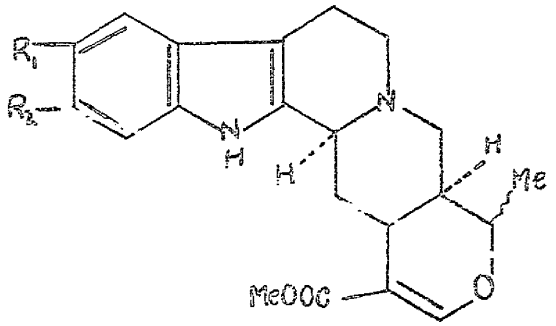
Pentacyclic Indoles.

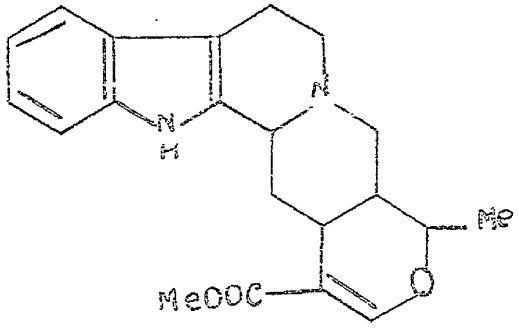
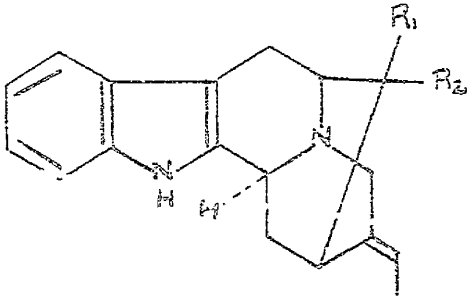
Yohimbine Type.

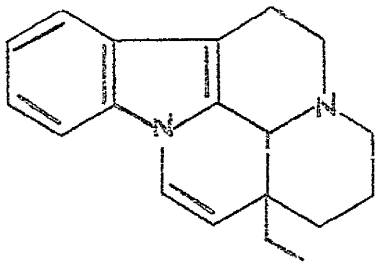
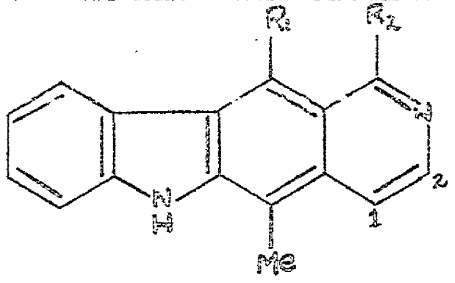
Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
Yohimbine (Quebrachine) $C_{21}H_{26}O_3N_2$	233-234	+106° (pyridine)	 R=H, 17αOH	A.quebracho-blanco Schlecht. A.peroba F.Allem.ex Sald. A.discolor A.DC.	10,44, 53,66, 67
β-Yohimbine $C_{21}H_{26}O_3N_2$	228-229	-14+3° (EtOH)	R=H, 17βOH	A.oblongum A.DC. Aspidosperma species (unidentified)	44,52, 65
11-Methoxy-β-yohimbine $C_{22}H_{28}O_4N_2$	148-149	-	R=OMe, 17βOH	A.oblongum A.DC.	52

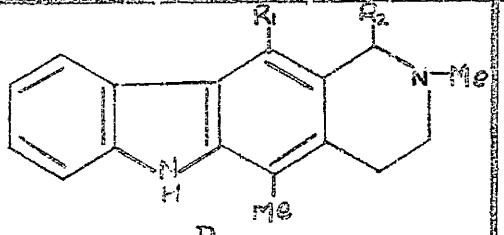
Pentacyclic Indoles.

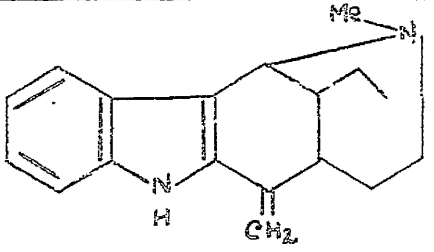
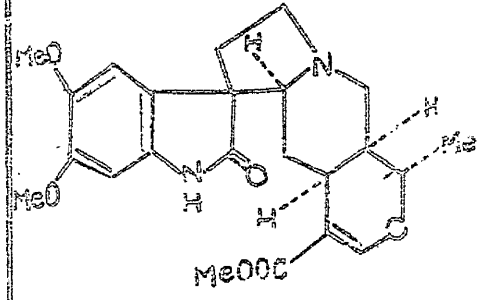
Reserpiline Type.

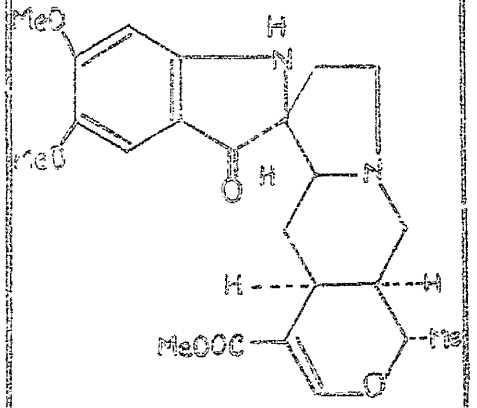
Alkaloid	m.p. °C.	[α]D	Structure	Source	Refs.
Reserpiline $C_{33}H_{38}N_2O_9$	amorph.	-69° (MeOH)		A.discolor A.DC.	44,66
Aricine $C_{33}H_{36}N_2O_4$	184- 185.5	-63° (pyri- dine)	 R1 R2 OMe H	A.marcgravianum Woodson	65,91

Alkaloid	m.p. °C	[a]D	Structure	Source	Refs.
Isoreserpiline $C_{33}H_{38}N_2O_5$	210-212	-84° (pyridine)	R_1 OMe; R_2 OMe	A.discolor A.DC.	66,91
Alkaloids of Mol.Wt. 352,382,412,382,412	-	-		A.oblongum A.DC.	52
Pentacyclic Indoles.					
Harpagine Type.					
Normacusine B $C_{19}H_{22}ON_2$	245-246 270-272 (dimorphic)	+40° (MeOH) +28° (pyridine)	 R_1 CH_2OH; R_2 H	A.polyneuron M.-Arg.	70,73

Alkaloid	m.p. °C.	[α]D	Structure	Source	Refs.
Polyneuridine $C_{21}H_{26}O_5N_2$	245-247	-73° (pyridine) +1° ($CHCl_3$)	R_1 = CH_3OH; R_2 = COOMe	A.polyneuron M.-Arg.	70
Pentacyclic Indoles.					
Eburnamenine Type.					
Eburnamenine $C_{18}H_{22}N_2$	amorph. 196 (picrate)	+183° ($CHCl_3$)		A.quebracho-blanco Schlecht.	45,46.
Pyridocarbazoles.					
Ellipticine $C_{17}H_{14}N_2$	312-314	0°	 R_1 = Me; R_2 = H	A.subincanum Mart.	10

Alkaloid	m.p. °C	[α]D	Structure	Source	Refs.
Dihydroellipticine (=u-Alkaloid D) $C_{17}H_{16}N_2$)	287-93	0°	1,2-dihydroellipticine (see previous compound)	A.ulei Mgf. A.subincanum Mart.	10,92
Olivacine $C_{17}H_{14}N_2$	318-24	0°	R_1 = H; R_2 = Me	A.longipetiolatum Kuhlm. A.olivaceum M.-Arg. A.australe M.-Arg. A.subincanum Mart.	10,92
Dihydroolivacine $C_{17}H_{16}N_2$	307-18	0°	1,2 dihydroolivacine (see previous compound)	A.ulei Mgf.	10
N-Methyltetrahydro-ellipticine (=u-Alkaloid B) $C_{18}H_{20}N_2$	215-18	0°	 R_1 = Me; R_2 = H	A.ulei Mgf. A.subincanum Mart.	10
N-Methyltetrahydro-olivacine (=u-Alkaloid C, (+)-Guatembuine) $C_{18}H_{20}N_2$	249-252	+112± 3° (pyridine)	R_1 = H; R_2 = Me	A.ulei Mgf. A.longipetiolatum Kuhlm. A.australe M.-Arg.	10,74

Alkaloid	m.p. °C.	[α]D	Structure	Source	Refs.
(-)-Guatambuine $C_{18}H_{20}N_2$	247-248	-106±2° (pyridine)	R_1 = H; R_2 = Me	A.australe M.-Arg.	10,74
(±)-Guatambuine $C_{18}H_{20}N_2$	227-228	0°	R_1 = H; R_2 = Me	A.australe M.-Arg.	10,74
Uleine $C_{18}H_{22}N_2$	72-76 (Kofler) 118-121 (Cap.)	+11.5° ($CHCl_3$)		A.ulei Mgf. A.australe A.pyricollum M.+Arg A.olivaceum M.-Arg.	10,74
Oxindoles.					
Carapanaubine $C_{23}H_{26}O_5N_2$	221-223	-101° ($CHCl_3$)		A.carapanauba M.Pichon	75

Alkaloid	m.p. °C.	$[\alpha]_D$	Structure	Source	Refs.
Isoreserpiline -ψ-indoxyl $C_{32}H_{38}O_8N_2$	250-253	-254° ($CHCl_3$)		A.discolor A.DC.	43,66

Alkaloids of Unknown Structure.

Obscurinervine $C_{32}H_{36}O_8N_2$	204-205	-54° ($CHCl_3$)	-	A.obscurinervium	71
Hydroobscurinervine $C_{32}H_{38}O_8N_2$	184-185	-61° ($CHCl_3$)	-	A.obscurinervium	71
Obscurinervidine $C_{32}H_{38}O_8N_2$	206-207	-39° ($CHCl_3$)	-	A.obscurinervium	71

Alkaloid	m.p.°C	[a]D	Structure	Source	Refs
Dihydroobscurinervidine $C_{24}H_{30}O_5N_2$	189-190	-44° ($CHCl_3$)	-	A.obscurinervium	71
Quebrachamidine $C_{26}H_{38}N_2O_{11}$	234-238	-250±15° (EtOH)	-	A.quebracho-blanco Schlecht.	63

QUATERNARY ALKALOIDS IN PLANTS

Although the presence of quaternary alkaloids in plants was realised towards the end of the last century[93], it was the isolation of crystalline d-tubocurarine from tube-curare by King[94] in 1935, and its potential therapeutic promise as a muscle relaxant, that lent impetus to research in this important class of natural products. It also provided a lead for the synthesis of such useful neuro-muscular blocking agents as gallamine, suxamethonium, cyclomethone[95].

Quaternary alkaloids of various types are now known to occur in many plant families. Structural diversities range from relatively simple benzylisoquinolines to the more complex dimeric indole types. Quaternary alkaloids of the aporphine[96,97] and protoberberine[98] types occur in the families, Menispermaceae, Rutaceae, Berberidaceae and Ranunculaceae. The protoberberine type has also been isolated from Papaveraceae, Anonaceae and Convolvulaceae, and aporphines from Magnoliaceae Aristolochiaceae and Lauraceae. Quaternary bis-benzylisoquinoline alkaloids [99,100,101] are common in Menispermaceae, especially in the genus Chondodendron of which several species, but particularly Ch. tomentosum Ruiz. and Pav., are used in the preparation

of curare[102]. This type of compound has also been found in the Anonaceae[103,104]. Benzylisoquinoline quaternaries[105a] have been isolated from Ranunculaceae, Rutaceae, Magnoliaceae and Combretaceae. Quaternary benzophenanthridine[105b] alkaloids have been found in the family Papaveraceae and in the genera Toddalia and Xanthoxylum of the Rutaceae while from the Rutaceous plant Halfordia scleroxyla a quaternary alkaloid with an oxazole structure has been isolated recently [106]. Quaternary dibenzopyrrocolines[107] have been encountered in the genus Cryptocarya (Lauraceae).

By far the largest number of quaternary alkaloids reported are derivatives of indoles and they are distributed mainly in the Loganiaceae. The spectacular advances in the isolation and chemistry of these alkaloids are due to the pioneering work by King and by H.Wieland but, more particularly, to the researches of Karrer and Schmid, and T. Wieland with their various colleagues. The work in this field up to 1960 has been exhaustively surveyed by Burnauer[108], and by Battersby and Hodson[109]. Research on these compounds has continued with further notable contributions by the Karrer and Schmid[110,111] and the T. Wieland[112] groups, and by several other

workers[113,116,118]. As stated previously, tertiary indole alkaloids are particularly abundant among members of the Apocynaceae and, in spite of the tremendous interest which this family has been receiving since the isolation of reserpine in 1952, there have been comparatively few reports on its quaternary alkaloids.

Although the first of these, echitamine, was isolated from Alstonia scholaris R.Br. in 1875[116], and subsequently [117,118,119] from several other species of Alstonia, the structure has only recently been established by X-ray crystallography[120].

In 1961 two pyridicarbazole alkaloids, ellipticine methonitrate and 1,2-dihydroellipticine methonitrate[32], were isolated from Aspidosperma subincanum Mart. in which they occur as minor bases. Recently Bartlett and co-workers [121] reported the isolation, as chlorides, of thirteen quaternary alkaloids from Hunteria eburnea Pichon. Of these thirteen alkaloids, five were the derivatives of known tertiary bases but not of such bases from this plant species. The structure of the sixth alkaloid, huntrabrine methochloride, was proposed on the basis of degradative work, and a partial structure was suggested for hunteracine chloride. The other six alkaloids were insufficiently characterised.

More recently macusine B, previously reported[123a] in Strychnos toxifera (Loganiaceae), has been isolated* from Aspidosperma peroba F.Allem.ex Sald.[91].

Quaternary anhydronium bases have been isolated from a few genera and the structure of most of them established. Examples of such bases[44] are alstoniline from Alstonia; flavopereirine from Geissospermum; alstonine from Alstonia, Rauwolfia and Vinca (Lochnera); serpentine from Rauwolfia and Vinca; and serpentinine from Rauwolfia. Recently a zwitterionic indole compound, flavocarpine, has been isolated from Pleiocarpa mutica[185] and its structure confirmed by synthesis.

A bisquaternary steroidal alkaloid, malouetine[124], was isolated from Malouetia bequaertiana (Apocynaceae) in 1960.

Probably many other Apocynaceous plants contain quaternary alkaloids and their presence has been indicated in Aspidosperma limae Woodson[88], in a Diplorhynchus species[185] and also[123] in Callichilia monopodialis Stapf., Hedranthera barteri Pichon, and Pleiocarpa pycnantha Stapf.

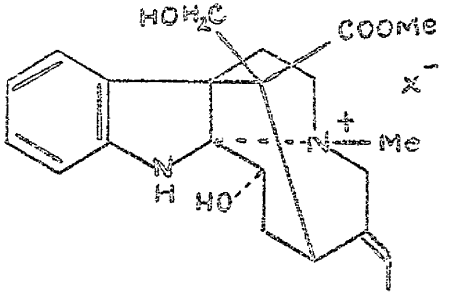
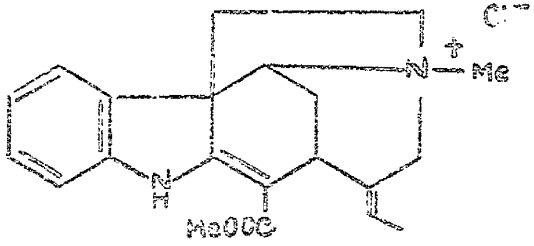
Information on the known quaternary bases of the Apocynaceae is collected in Table 2.

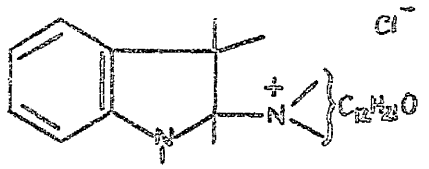
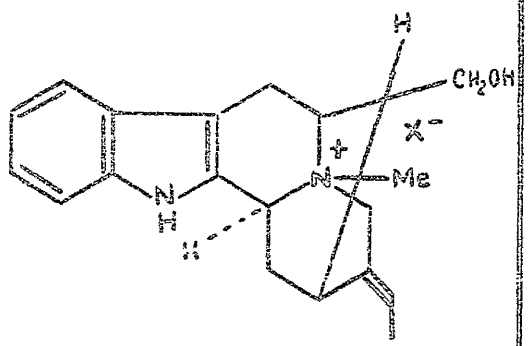
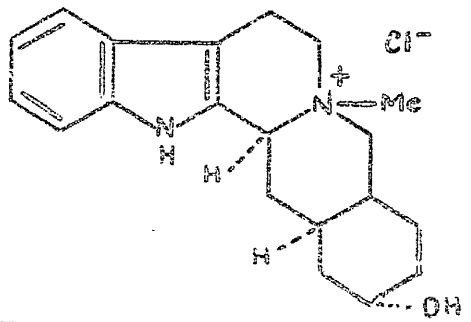
* This isolation forms part of the present work.

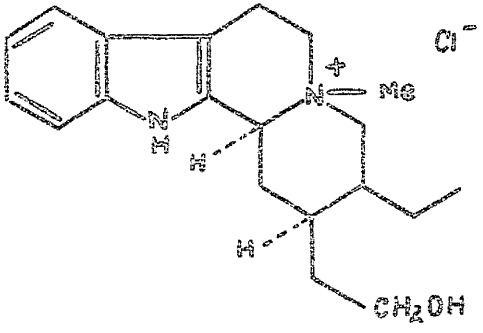
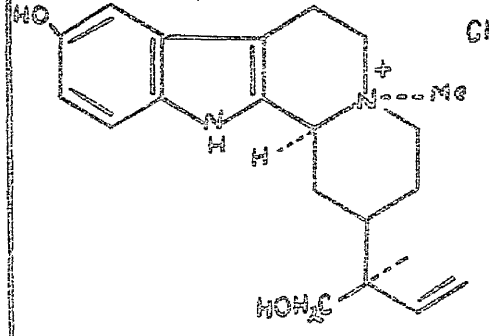
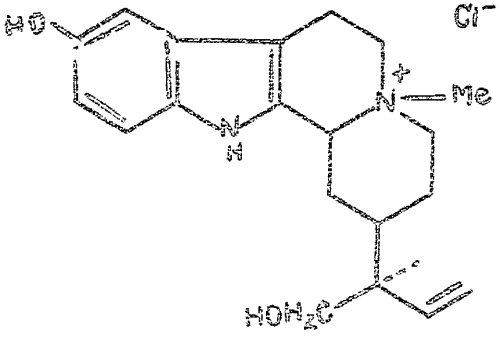
Table 2.

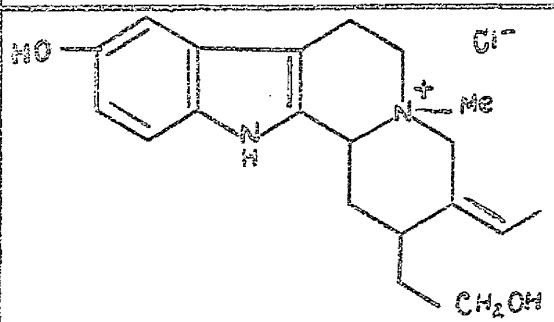
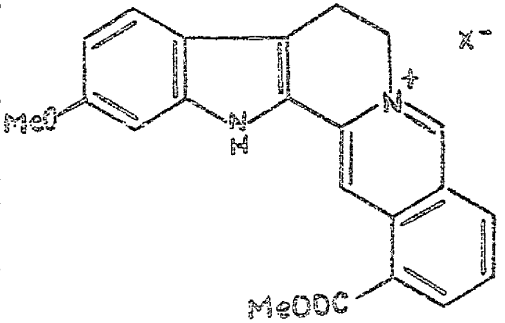
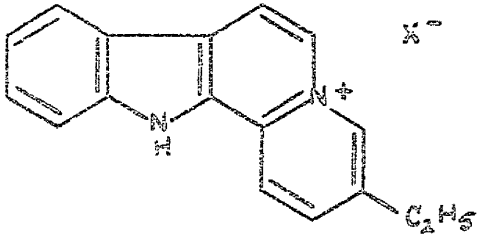
Quaternary Alkaloids of the Apocynaceae.

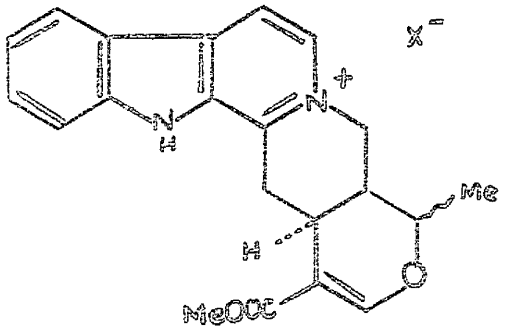
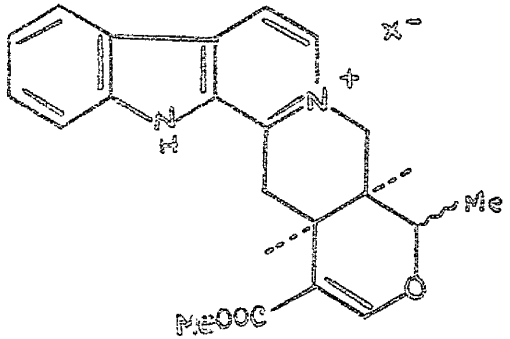
Dihydroindoles.

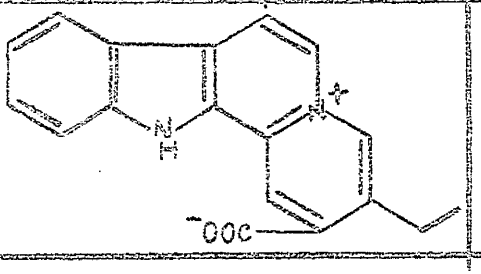
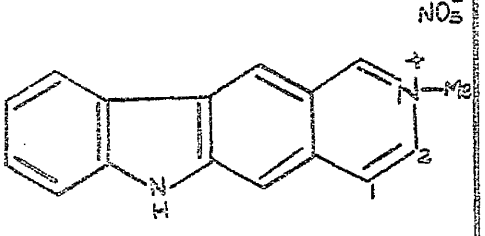
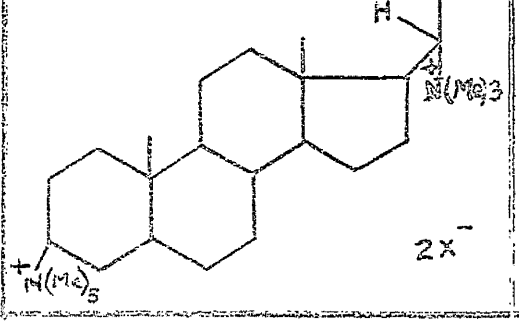
Alkaloid	m.p. °C	[α] D	Structure	Source	Refs.
Echitamine $C_{19}H_{27}O_5N_2^+ X^-$	295 (chloride) 267 (iodide) 176 (nitrate) 275 (sulphate)	-58° (Chloride in water) -51.6° (sulphate in water) -51.4° (nitrate in water)		Alstonia scholaris R.Br. A.gilleti De Wild. A.spathulata Blume A.angustiloba Miq. A.verticillosa F. Muell. A.congensis Engl. A.spectabilis R.Br.	117, 118, 119, 120
Ajmalicine methochloride $C_{21}H_{25}O_2N_2^+ Cl^-$	271-272	-567° (MeOH-water)		Hunteria eburnea Pichon	121

Alkaloid	m.p. °C	[α] D	Structure	Source	Refs.
Hunteracine chloride $C_{20}H_{23}ON_2^+ \ Cl^-$	343-344	-91° (27.5% water-MeOH)		H. eburnea	121
Indoles					
Mucusine B $C_{19}H_{23}ON_2^+ \ X^-$	248-249 (chloride) 283-285 (thiocyanate) 142-147 (nitrate) 280-281 (iodide) 224-226 (picrate)	+15.6 +1.7° (chloride in water) +16° (nitrate in MeOH) +17° (iodide in MeOH)		Aspidosperma peroba F. Allem. ex Sald.	41,73
Yohimbol methochloride $C_{22}H_{27}ON_2^+ \ Cl^-$	264-265	+53° (MeOH)		Hunteria eburnea Pichon	121

Alkaloid	m.p. °C	[α] D	Structure	Source	Refs.
Dihydrocoory-mantheol methochloride $C_{20}H_{29}ON_2^+ Cl^-$	296-297	+101°		H.eburnea	121
Hunterburmine α-metho-chloride $C_{20}H_{27}O_2N_2^+ Cl^-$	335 294-295 (methio-dide)	-		H.eburnea	121
Hunterburmine β-metho-chloride $C_{21}H_{27}O_2N_2^+ Cl^-$	307-308 277-280 (methio-dide)	+105° (chloride in 27.5% water-MeOH)		H.eburnea	121

Alkaloid	m.p. °C	[α] D	Structure	Source	Refs.
Huntrabrine methochloride $C_{20}H_{27}O_2N_2^+ \quad Cl^-$	285–287	+54° (water)		H.eburnea	121
Alstonidine $C_{22}H_{18}O_3N_2$ $C_{22}H_{19}O_3N_2^+ \quad X^-$	372	0°		Alstonia constricta F. Muell.	44
Dlevopereirine $C_{17}H_{16}N_2$ $C_{17}H_{18}N_2^+ \quad X^-$	233–235	0°		Geissospermum laeve Baill.	44

Alkaloid	m.p. °C	[α] D	Structure	Source	Refs.
Alstonine $C_{21}H_{20}O_3N_2$ $C_{21}H_{21}O_3N_2^+ \quad X^-$	205-210	+141° (hydrochloride in water)		Alstonia constricta Rauwolfia hirsuta Jacq. R.obscura K. Schum R.vomitoria Afzel. Vinca rosea L.	44
Serpentine $C_{21}H_{20}O_3N_2$ $C_{21}H_{21}O_3N_2^+ \quad X^-$	175-177	+292° (MeOH)		Vinca rosea Rauwolfia canescens L. R.fruticosa Burck R.heterophylla Willd. ex Roem. et Schult. R.lingustrina Willd. ex Roem. et Schult. R.micrantha Hook f. R.sellowii Muell. Arg. R.serpentina Benth. ex Kuntz R.sumatrana Jack	44

Alkaloid	m.p. °C	$[\alpha]_D$	Structure	Source	Refs.
Flavocarpine $C_{18}H_{16}O_2N_2$	307-308	0°		Pleiocarpa Mutica Benth.	123
Pyridocarbazoles					
Ellipticine methonitrate $C_{18}H_{17}N_2^+ \ NO_3^-$	293-304	–		Aspidosperma subincanum Mart.	92
1,2-Dihydroellipticine methonitrate $C_{18}H_{18}N_2^+ \ NO_3^-$	301-303 273-275 (methopicrate)	–	see above (1,2-dihydro-compound)	A. subincanum	92
Steroidal alkaloid					
Malouetine $C_{37}H_{58}N_2^{++} \ X_2^{--}$	264 (picrate)	+3° (chloride in water)		Malouetia bequaertiana	124

Alkaloid	m.p. °C	[α] D	Structure	Source	Refs.
Alkaloids of unknown structure.					
Serpentinine $C_{21}H_{22}O_3N_2$ $C_{21}H_{23}O_3N_2^+ X^-$	265-70	+52° (MeOH)	-	R.degeneri Sheriff R.lingustrina R.maulensis Sheriff R.sandwicensis A.DC. R.serpentina R. tetraphylla	44
Alkaloid - F	242-243 (chloride)	-	-	Hunteria eburnea Pichon	121
Alkaloid - H	300 (chloride)	-	-	H.eburnea	121
Alkaloid - I	278-280 (chloride)	-	-	H.eburnea	121
Alkaloid - J	291-293 (chloride)	-	-	H.eburnea	121

Alkaloid	m.p. °C	[α] D	Structure	Source	Refs.
Alkaloid - K	207-208 (chloride)	—	—	H.eburnea	121
Alkaloid - N	263-266	—	—	H.eburnea	121

METHODS FOR EXTRACTION AND SEPARATION OF QUATERNARY BASES

As practically all alkaloid-producing plants elaborate a mixture of closely related alkaloids, together with large amounts of readily extractable non-alkaloidal material, a constant problem in this type of work is the separation of the mixed bases from suitable extracts, followed by fractionation to yield individual alkaloids. The conventional method for the isolation of tertiary alkaloids is to extract the suitably basified plant material with a water-immiscible solvent then remove the alkaloids, as salts, by shaking the extract with dilute aqueous acid: the acid solution is basified and the alkaloids, as free bases, shaken out with an organic solvent, commonly ether or chloroform, or a mixture of both. Alternatively, the dried plant material can be extracted with ethanol or methanol, either under acid conditions or under neutral conditions; in the latter case, after removal of most of the solvent, the residual extract is treated with dilute aqueous acid. Both tertiary and quaternary alkaloids are removed by this method and the former can be obtained by basifying the acid solution and shaking with an organic solvent. Because of their water-solubility, the quaternary bases remain in the aqueous phase and can only be recovered

by separation as complexes with mercuric chloride or potassium mercuric iodide or, more commonly, as insoluble salts such as picrates or reineckates. Such mixed salts can sometimes by fractionated directly, but, alternatively, they may be converted to chlorides and these are separated. Occasionally, isolation of individual quaternary compounds may be achieved by fractional crystallation from suitable solvents or by microsublimation but these conventional methods often fail to achieve adequate separations and resort has to be made to various chromatographic methods, to countercurrent distribution, or to a combination of such techniques to achieve separations hitherto impossible.

Adsorption chromatography has been used successfully in the separation of many tertiary bases but Battersby and Hodson[109] have criticised the use of alumina columns for the attempted separation of quaternary alkaloid reineckates because well-separated bands on such columns contained the same alkaloids. Similarly, alumina columns were ineffective for separation of mixed quaternary chlorides [132]. However, the method is useful, as shown in the present work, for the removal of impurities from mixed reineckates or chlorides prior to their separation by other methods.

Partition chromatography is also suitable for

separation of tertiary bases as shown, for example, by Partridge and Evans[127] and, later, by Chilton and Partridge[128]. It is much more useful than adsorption methods when dealing with quaternary compounds and it is now established that the most efficient and satisfactory method of resolution for quaternary alkaloids is that developed by Schmid and Karrer[129], and by T.H. Wieland[130] utilising cellulose columns and a variety of solvent systems for the separation of calabash curare alkaloids. Partition and other methods for the separation of South American Indian and Strychnos curare alkaloids have been well reviewed by Marini-Bettolo and Casinovi[131]. Bartlett and co-workers[131] have satisfactorily separated the quaternary alkaloids of Hunteria eburnea Pichon on cellulose, using the monophasic solvent systems acetone/water or acetone/ethyl acetate/water.

Although partition chromatography on cellulose columns has been a major step forward in the fractionation technique, it is only by working under strictly controlled conditions with very large amounts of starting materials and, in the process, discarding several and often appreciable mixed fractions, that reasonable quantities of pure alkaloids can be obtained. It is extremely difficult to realise worthwhile preparative separations on columns when employing small quantities of materials. In such cases,

preparative paper chromatography has been successfully employed to yield small quantities of pure alkaloids, for example, in the separation of quaternary bases from Strychnos solimoesana, S.guianensis, S.subcordata[131] and Aspidosperma subincanum[92].

Recently the introduction of the ChroMax Pressure Mantle (LKB-Produkter AB Stockholm) carrying a long paper roll on which partition chromatography is achieved, has given improvements over the usual columns prepared with powdered cellulose for the separation of some compounds but no reports have appeared on alkaloid separation by this means. Some use has been made of both the Chromax system and ordinary cellulose columns in this present work.

Another useful method for the separation of complex mixtures of natural products is based on counter-current distribution. As with partition chromatography, this technique was developed by Martin and Synge who used it in an attempt to separate the components of amino acid and peptide mixtures. The instrumentation and wider application of this analytical technique is largely due to the pioneer work of Craig and in recent years a number of efficient and easily operable counter-current machines have been developed. By the use of suitable solvent pairs,

particularly with buffer solutions of different pH as the aqueous phase, it is sometimes possible to effect separation of bases from complex mixtures and the application of this technique to the alkaloid field has been reviewed by Casinovi[152]. This worker succeeded in resolving a total extract from Strychnos amazonica by repeated distribution between ethyl methyl ketone and water. The advantage of this method lies in the possibility of checking, by suitable means, the course of fractionation at any stage in the process. It is continous and there is no danger of diffusion which may occur in, and reduce the efficiency of, chromatographic methods either as a result of interruptions in flow or the long duration of the process. Also absent are irreversible adsorption phenomena and wall effects which in column chromatography may bring about a loss of substance and thus reduce efficiency. Further improvements in this type of separation have been made possible by steady-state distribution. In the Craig-type apparatus the mixed solutes are introduced at one end of a bank of tubes and the upper phase only of a two-solvent system moves forward from the same end to separate and distribute the solutes over several tubes. However, in the steady-

state machine (Quickfit and Quartz Ltd.) the solute mixture is fed to the centre of a bank of tubes and both liquid phases are admitted continously, one at each end of the train and discharging each at the opposite end to give two separate series of fractions. This permits continuous operation, numerous transfers, and more satisfactory separations. The disadvantages of these methods lie in the complexity of the equipment and the fact that, with many solvent systems, emulsions are easily formed under the experimental conditions. Control of emulsions is especially difficult when dealing with crude natural extracts and more particularly with quaternary compounds, as shown by experience in this work.

The peculiar physico-chemical properties of quaternary alkaloids and the complexity of natural mixtures of these make difficult, even nowadays, their isolation as pure products.

DISCUSSION

INTRODUCTION TO THE PRESENT WORK

The object of this investigation was the isolation and identification of the quaternary alkaloids from the stem bark of a South American Aspidosperma species already known for its comparative richness in tertiary alkaloids, chiefly aspidospermine. There are indications that many plant species known as sources of tertiary bases also contain quaternary alkaloids and many of these may be of pharmacological interest and possible therapeutic value.

PRELIMINARY EXTRACTIONS AND SEPARATIONS

The botanical origin of the bark used as the initial starting material was authenticated[183] for Smith, Kline and French Ltd. at source. It was quoted as Aspidosperma peroba F. Allem. ex Sald. which is synonymous[3] with A. polyneuron M. Arg., the name under which much of the previous work on the chemistry of this species has been reported.

The actual starting material used in this present work was supplied as the butanol-soluble fraction (B, Figure 9) remaining after preliminary separation of tertiary bases from an ethanolic extract of the dried bark. It had obviously formed a very significant proportion of the total alkaloids, the crude tertiary and crude quaternary mixtures each representing about 0.4% weight of the dried bark. The quaternary residue was shown to be extractable with water and dilute mineral acids to give solutions strongly positive to various alkaloidal reagents. Different methods were used in attempts to precipitate the alkaloids and so obtain a preliminary purification of the mixture.

Attempted Separation of quaternary bases as trichloro-acetates.

A method has been described[184] for the isolation and

purification of quaternary ammonium compounds from aqueous solutions by treatment with trihalo-acetic acid to give compounds of low water-solubility, which are reported to precipitate in almost quantitative yields. This method, tested qualitatively, did not yield appreciable quantities of workable precipitate from aqueous extracts of the crude quaternary mixture and it was, therefore, abandoned in favour of the commonly used separation as reineckates[122,136,156].

Preparation and purification of crude mixed reineckate.

The ready precipitation as reineckates provided a simple method of separating the quaternary compounds from aqueous or aqueous-acid extracts of the crude material but some brown colouring material was also precipitated and had to be removed subsequently by chromatography of acetone solutions on alumina. Use was made of acid-washed alumina in an attempt to prevent the formation of diacetone alcohol and triacetone alcohol which usually[136,137] contaminate fractions of acetone eluants from alumina columns but, despite this precaution, the later fractions were still contaminated with those compounds which then prevented the formation of completely dry residues when some fractions of eluant were concentrated.

Elution with acetone quickly removed the bulk of the

alkaloids and gave a number of fractions relatively free from contaminating colour. Subsequent elution with acetone/ethanol, acetone/ethanol/acetic acid and then aqueous acetic acid removed further small quantities of alkaloidal material but these were increasingly contaminated with non-alkaloidal colouring matter.

Fractions shown to be identical by paper chromatography were bulked as indicated in Table 3.

Paper chromatography of alkaloid reineckates.

Despite the criticisms[109] that adsorption chromatography does not give satisfactory separations of quaternary alkaloid reineckates, alumina columns have, in some cases, given partial fractionation[136,138] and it was necessary to determine whether or not any separation had been achieved in this case. In order to check the elution pattern of alkaloids from the column, preliminary experiments were conducted with total mixed reineckates to determine the best conditions for separation on paper chromatograms. None of the commonly-used solvent systems gave satisfactory results, all giving continuous streaking along the entire length of run. The streaking may have been caused by impurities, therefore similar experiments were conducted using a purified fraction of reineckate obtained from the alumina column. Both ascending and circular chromatography with different

Table 3.

Chromatography of mixed reineckate on alumina.

Combined Fractions	Eluant			Residue	
	Solvent	Volume (ml.)	Colour	Weight (g.)	Remarks
A	acetone	1400	pink	4.564 (+0.236g. crystals)	rose-pink solid. The fraction on concentration deposited pale pink crystals (0.236g.) which were separated before taking the fraction to dryness
B	acetone, acetone containing 1-5% ethanol.	1200 1600	almost colourless	0.436	brownish-pink paste
C	acetone containing 5-10% ethanol	1500	yellowish pink	0.373	brownish-red paste
D	acetone containing 10-25% ethanol	3100	yellowish pink	0.705	brownish-red paste
E	25% ethanol in acetone: acetic acid, 95:5	1200	yellowish brown	1.750	dark brown solid
F	5% aqueous acetic acid	1000	brown	unobtainable	dark brown paste

solvents did not give entirely satisfactory results, therefore the solvent system acetone/benzene/chloroform/water (60:15:10:20) was formulated and used in descending chromatography which gave quicker separation than upward development. The organic phase, to which 3% methanol was added, gave good resolution.

The bulked fractions A-F were chromatographed using the latter solvent and the results are given in Table 4.

Table 4.

Combined fractions	R_f values	Observations
A	0.51	elongated area
	0.92	strong and compact
B	0.51	elongated area
	0.92	strong and compact
C	0.34 }	faint and not distinctly separated
	0.50 }	
	0.60	slightly elongated
	0.84	strong and compact
D	0.92	compact
E	-	remained at starting line, trace only
F	-	remained at starting line, trace only
Crytals from fraction A	0.51	elongated area

Although the individual fractions which were combined in C had all shown a spot with R_f 0.92, no area with this R_f value was revealed in chromatograms of the combined fraction. However, it was suspected that the same material was responsible for the spot of R_f 0.84 in C. Thus this material appeared to be present in all the fractions (except E and F), a result which provides supporting evidence for Battersby's[109] criticism that well-separated bands contain the same compounds when alkaloid reineckates are chromatographed on alumina.

There were indications in the major part of the eluant (fractions A-C) of at least four different compounds and although, at first, it appeared that fraction D might contain a single alkaloid this material was later converted to chloride and then shown, by paper chromatography, to be a mixture.

Because there was very little movement of the solute in combined fractions E and F, using the above solvent system, those deeply coloured fractions, containing most of the impurities from the mixed reineckate, were chromatographed using a butanol/acetic acid/ water mixture and there were indications of three alkaloidal compounds but in trace amounts only (see page 110).

The final conclusion was that while some partial fractionation may have been achieved on alumina, there was no separation of any single compound. However, some purification

had undoubtedly occurred and combined fraction A was sufficiently pure to deposit crystals. Paper chromatography, using the acetone benzene/chloroform/water system, showed this crystalline material to have R_f value 0.51 thus corresponding to the compound of the same R_f value in the main acetone eluates from the alumina column.

After removal of the crystals (subsequently shown to be macusine B thiocyanate) the remainder of reineckate fraction A was converted to chloride (mixed chloride C, pages 75, 123.) and this eventually yielded compound T3A.

MACUSINE B

The pale pink crystals from fraction A gave, after two successive recrystallisations from methanol, colourless needles, m.p. 292-295° (decomp., Gallenkamp hot plate), 283-285° (decomp., Reichert hot plate). The infrared spectrum of this compound (Figure 1) showed bands at 750 (ortho-disubstituted benzene ring), 2090 (CN) and 3200-3450 cm.$^{-1}$ (OH,NH). From the presence of the significant sharp band at 2090 cm.$^{-1}$ the compound was thought to be a thiocyanate salt and this was confirmed by positive tests with ferric chloride and silver nitrate. The ultraviolet spectrum (Figure 2) with $\lambda_{max.}^{EtOH}$ 222 mμ (log ε 4.55), 273(3.87), 283(3.85), 290(3.72) was similar to that of macusine B chloride[122] being characteristic of a 2,3-disubstitued indole[122,130]. The empirical formula $C_{21}H_{25}N_3OS$, based on elementary analysis of the compound, fitted exactly with that of the thiocyanate salt of macusine B isolated from Strychnos toxifera by Battersby et al.[122]. The equivalent weight (367.1, by Volhard titration) also fitted well with the calculated value (367.4) for macusine B thiocyanate.

Earlier, Wieland and co-workers[130], and then King[136], isolated a similar compound after passing reineckates of the alkaloids from calabash curare and Strychnos toxifera bark through alumina columns. Neither King nor the German workers suspected their compound to be a thiocyanate and both gave analyses in support of the formula $C_{23}H_{28}O_3N_3$. They noted

FIGURE 1

I.R. SPECTRUM OF MACUSINE B THIOCYANATE

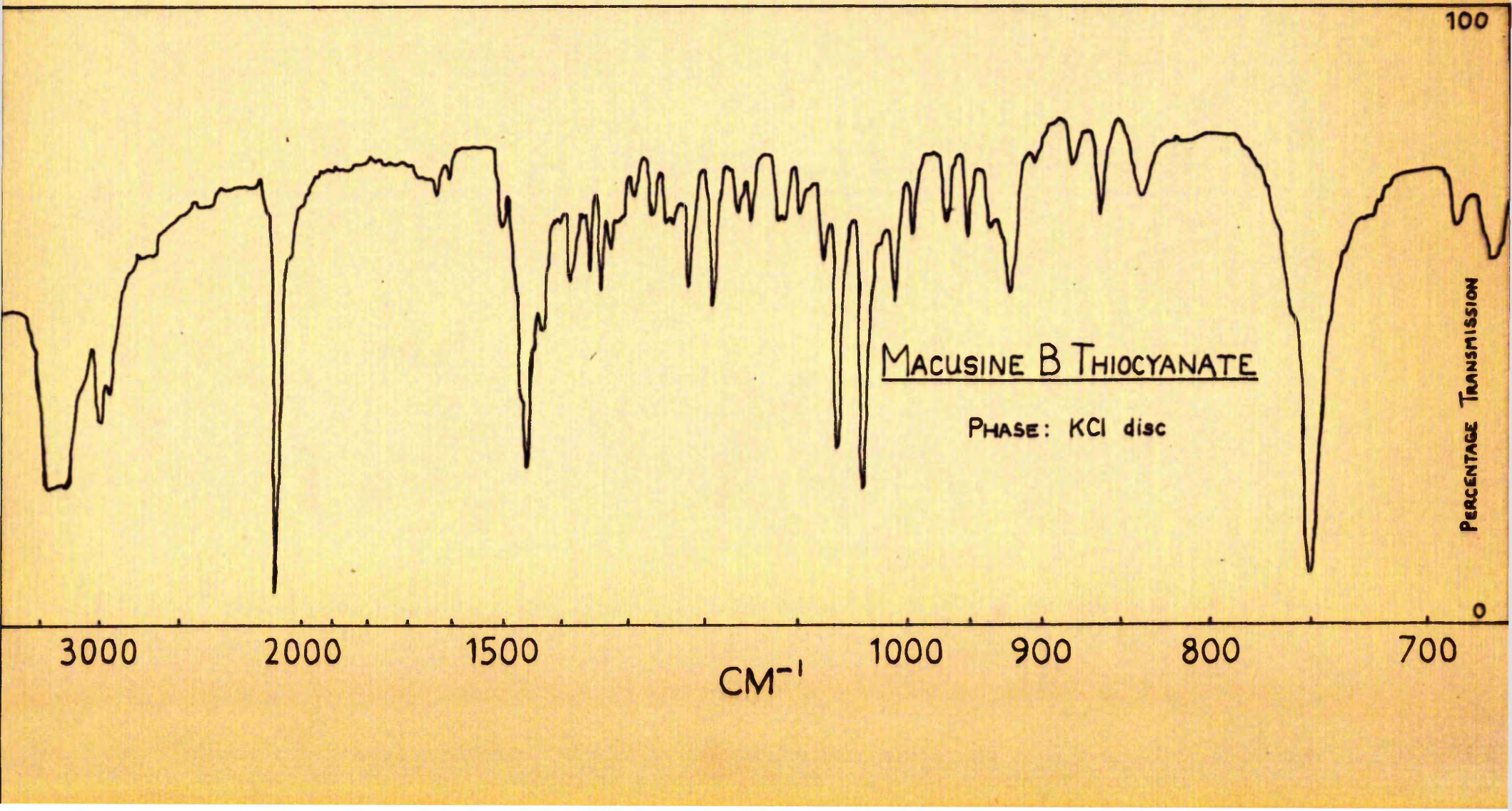
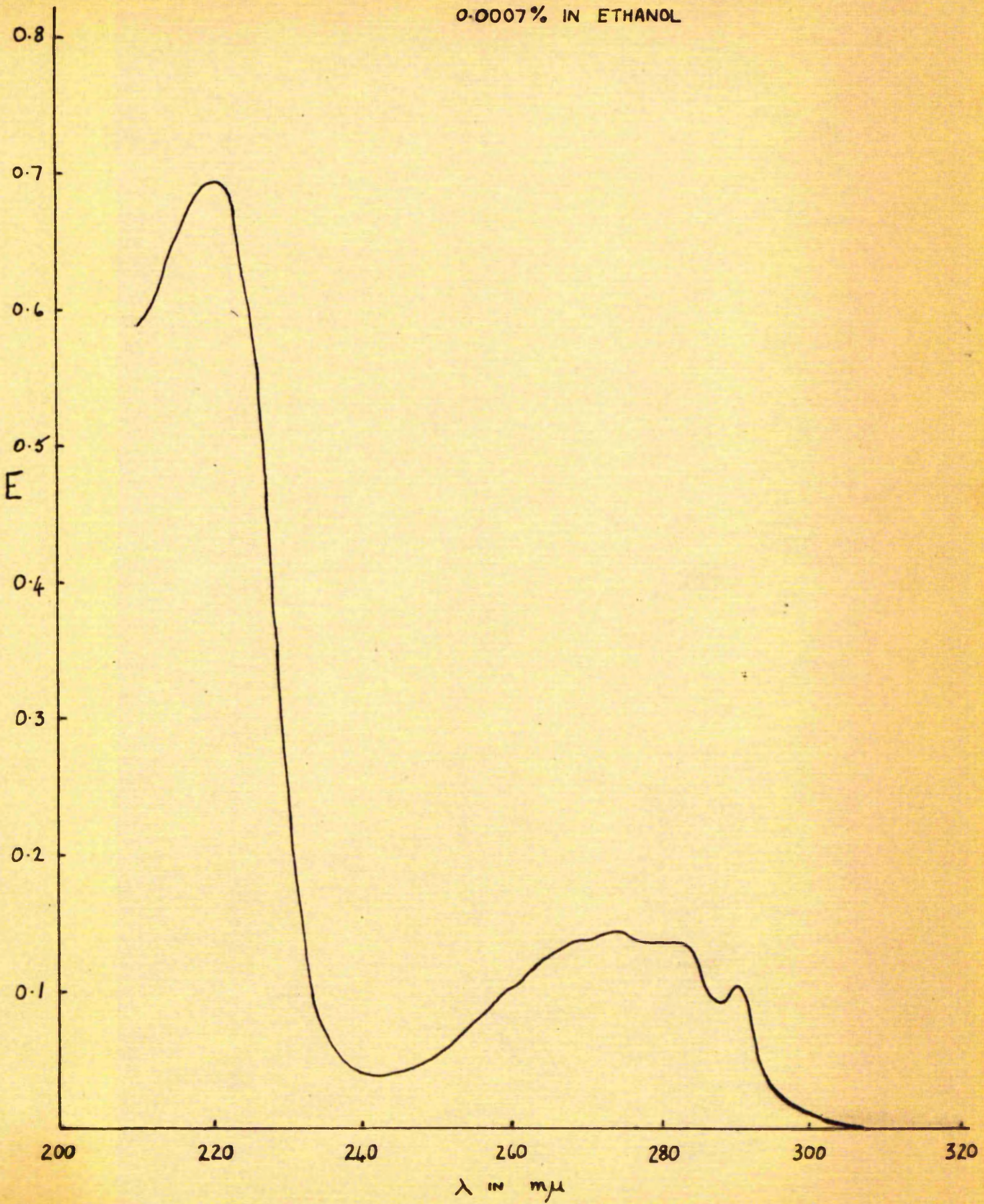


FIGURE 2

U.V. SPECTRUM OF MACUSINE B THIOCYANATE

0·0007% IN ETHANOL



melting points, 275-278° and 292° (decomp.) respectively, while Battersby et al.[123] quoted m.p. 302° (after sintering and darkening at 286°). In this work, as reported above, slightly different melting points were obtained on two separate instruments and the m.p. 283-285° is regarded as the most accurate.

On the basis of the infrared spectrum determined for King's sample, Battersby and co-workers concluded that this compound was probably identical with macusine B thiocyanate. The analytical figures given by King also provide a good fit for $C_{21}H_{28}N_2OS$. Further, the thiocyanate salt encountered in the present investigation gave a bright yellow colour with nitric acid similar to that reported by King for his compound.

The above facts, and the recent isolation[70] from the same plant (A. polyneuron) of the corresponding tertiary base, normacusine B, made it highly probable that the compound separated from fraction A was the thiocyanate salt of the quaternary alkaloid macusine B. The anion must obviously have come from the decomposition of reineckate.

Battersby et al. initially isolated macusine B thiocyanate from a partition column of a chloride mixture which had been prepared from the mixed reineckate salts of Strychnos toxifera alkaloids and they later prepared it by precipitation with ammonium thiocyanate from an aqueous solution of its chloride. This later technique was also used in the present work for the precipitation of thiocyanates from an aqueous solution of the

crude starting material. After removal of other thiocyanates by washing with aqueous methanol and cold methanol, the remaining macusine B thiocyanate was recrystallised from boiling methanol. The crystals gave the same colour tests, melting point and infrared spectrum as those of the compound obtained from fraction A of the mixed reineckate. The other mixed thiocyanates were reserved for further investigation and subsequently yielded compound Q2.

The tentative identification of macusine B thiocyanate was confirmed by the preparation of nitrate, iodide and picrate (later chloride also) and by conversion to the corresponding tertiary base, normacusine B. The melting points and optical rotations of the chloride and iodide were in good agreement with the values reported in the literature[122,139]. Stauffacher[151] isolated tombozine and showed it to be identical with normacusine B; consequently his tombozine methiodide ($C_{20}H_{24}IN_2O$) is the same as macusine B iodide. Analytical figures for the prepared iodide agreed well with those calculated for the above formula. The chloride gave positive results for a number of qualitative colour tests described[122] for macusine B chloride although with sulphuric acid and ferric chloride the colour did not become green on standing, as reported by Battersby, but remained blue.

Using chloride, nitrate, or thiocyanate, absence of violet-blue colour with p-dimethylaminobenzaldehyde reagent gave

support to the spectral evidence for a 2,3-disubstituted indole nucleus[140].

The nitrate and picrate salts of macusine B had not been reported in the literature; however, analyses of the prepared nitrate and picrate, and the equivalent weight of the picrate (539.4), fitted the formulae required for macusine B nitrate ($C_{20}H_{28}N_8O_4$) and macusine B picrate ($C_{26}H_{27}N_5O_9$, M. Wt. 537.5), respectively.

NORMACUSINE B

The conversion of macusine B thiocyanate into normacusine B was accomplished by refluxing the former with ethanolamine at 170-175° as described by Hunig and Baron[141]. These workers have successfully applied this method of N-dealkylation to certain quaternary ammonium salts such as tetramethylammonium iodide, trimethylanilinium iodide, methylethylpiperidinium iodide, dimethyltetrahydroisoquinolinium iodide.

$$(R_4N)^+ X^- + NH_2CH_2CH_2OH = R_3N + RNHCH_2CH_2OH + HX$$

In other cases, however, such as quaternary morphinan and tropine salts some decomposition due to ring opening was observed.

Tomita and Takano[142] have used the method with success for N-demethylation of the methiodides of non-phenolic benzyltetrahydroisoquinoline-type and aporphine-type bases. For example, dl-laudanosine methiodide, O,O-dimethylcorytuberine methiodide, and isotetrandrine dimethiodide were successfully converted to their corresponding tertiary bases.

The reaction is independent of the nature of the anion[141] and in the present instance the method was very successful, the yield of normacusine B prepared from macusine B thiocyanate being 70% of the theoretical value. The tertiary base was extracted with ether and recrystallised from acetone. After removal of two crops of crystals the mother liquor, which became dark brown in colour on standing, gave an indication of traces of three alkaloidal compounds other than

normacusine B when tested on thin-layer chromatograms. This may indicate some slight decomposition but the trace compounds were not examined.

Normacusine B was characterised by comparison of its physical data with those reported in the literature and by the preparation of its known crystalline acetate. The hydrochloride and picrate, not previously reported in the literature, were also prepared but the hydrochloride could not be crystallised.

Normacusine B has been shown to be identical with several other reported compounds and collected information on some of these is given in Table 5. It can be seen that this substance is dimorphic occurring as either prisms (m.p. ca. 245°) or needles (m.p. ca. 272°). Desmethoxylochnerine and deoxysarpagine are also identical with tombozine[130] and, therefore, with normacusine B.

The melting point and rotation of the prepared acetate also agreed well with the figures quoted for normacusine B acetate and other acetates with which it has been shown to be identical (Table 6).

The analyses of normacusine B, its picrate and acetate, and the equivalents weights of the base and picrate fitted well with those required by normacusine B ($C_{19}H_{22}ON_2$) and its corresponding salts.

Table 5.

Physical data on different samples of Normacusine B and identical compounds.

Alkaloid	m.p. °C	$[\alpha]_D$	max. (mμ) (log ε)	Refs.
Normacusine B (Djerassi)	245-247(prism) 270-272	+40°(MeOH)	225 (4.45) 280 (3.80) EtOH 289 (3.70)	70
Normacusine B (Battersby)	245(rhombic) 275(needles)	-	-	73
Normacusine B (present sample)	243-245(prism) 271-273(needles)	+33.1°(MeOH)	227 (4.49) 281 (3.98) EtOH 290 (3.88)	41
Desformoakuammidinol	232-238(prism) 275(needles)	+35±2°(MeOH)	-	143
Tombozine	270-272	+37±2°(EtOH)	222 (4.60) 280 (3.87) MeOH 289 (3.77) Sh 272 (3.85)	139
Diplorrhyne	235(prism) 270-271(needles)	+35°(MeOH)	-	71

Table 6.

Some physical data on different samples of Normacusine B acetate.

Sample	m.p. °C	$[\alpha]_D$	Refs.
Normacusine B acetate (Djerassi)	212-215	+12° (MeOH)	70
Normacusine B acetate (present sample)	221-223	+11.8° (MeOH)	41
Desformoakuammidinol-O-acetate	223	+9° (MeOH)	143
O-Acetyl tombozine	220-222	+11±3° ($CHCl_3$)	139

ATTEMPTED SEPARATIONS OF OTHER QUATERNARY BASES

Preparation and paper chromatography of chlorides.

Since no separation of bases as reineckates could be achieved on alumina, various batches of reineckates were converted to chlorides by the Kapfhammer method as described by Dutcher[135].

Where successful separations of quaternary bases have been achieved in the past the various researchers[121,122,129,130,] have worked with mixed chlorides which have been fractionated on columns of powdered cellulose, using a variety of solvent systems. Several of these systems were tried in paper chromatography experiments to determine which might be useful, firstly, to show the number of compounds present in the mixtures of chloride and, secondly, as potential solvents for partition chromatography of these chlorides on cellulose columns.

An attempt was made to resolve the crude mixed chlorides A and B on paper chromatograms using the solvent systems listed on page 124. Using both downward and upward development no satisfactory resolution was obtained. In all cases considerable streaking was observed, one spot diffusing so badly into the other that very poor zone definition resulted. The solvent systems, ethyl acetate/pyridine/water (7.5: 2.3: 1.65) and water-saturated ethyl methyl ketone with 2% methanol, both with downward development, gave indications of two major and three or four minor bases. The results, as shown in Table 7, appear to show good resolution but too much reliance can not

be placed on the R_f values because exact determination was difficult due to the considerable streaking observed. However it was quite apparent that both the chloride mixtures behaved in the same way even though A had been prepared after removal of the precipitated macusine B and other thiocyanate salts.

Table 7

Solvent system	Crude chloride mixture	R_f values	Relative strength on chromatograms
Ethyl methyl ketone/pyridine/water (7.5:2.3:1.65)	A	0.04 0.16 0.24 0.33 0.42	++ + +++++ ++++ +
	B	0.04 0.15 0.21 0.32 0.44	++ + +++++ ++++ +
Water saturated ethyl methyl ketone with 2% methanol	A	0.03 0.13 0.24 0.39 0.60	+ + +++++ ++++ +
	B	0.03 0.13 0.24 0.38 0.62	+ + +++++ ++++ +

The chloride mixtures A and B were brown in colour and since coloured impurities may have been responsible, to some extent, for the 'tailing' of alkaloidal compounds, these mixtures were subjected to purification on alumina columns. With chloride mixture A elution from alumina was begun with ethanol and the first runnings were colourless but also

alkaloid-negative. The first alkaloidal fractions were yellow in colour and elution was continued until the eluant became only faintly positive to Dragendorff's reagent. Elution was continued with methanol which removed more alkaloidal material but this was increasingly contaminated with yellowish-brown colouring matter. Elution was completed with aqueous methanol which, whilst faintly alkaloidal, was very strongly coloured (Table 8).

Table 8

Fractions of alkaloid chlorides (A) from alumina column.

Fraction	Eluant			Residue	
	Solvent	Volume (ml.)	Colour	Weight (g.)	Remarks
A-1	ethanol	1350	golden yellow	2.01	yellow solid
A-2	methanol	1300	yellowish brown	1.45	brown solid
A-3	50% methanol	950	brown	1.01	dirty brown solid

The different fractions collected were tested by paper chromatography using the solvent system, ethyl acetate/acetone/water (50:45:17)[221] which, with upward development, gave better resolution than the other solvent systems also tried. The results (Table 9) indicated that the main fraction A-1 contained three major alkaloids with traces of three other bases.

Table 9.

Paper chromatography of alkaloid chlorides A using ethyl acetate/acetone/water (50:45:17).

Fraction	Compounds separated	
	R_f values	Relative Strength on chromatograms
A-1	0.16 0.23 0.35 0.52 0.65 0.76	± +-+-+ +-+-+-+-+ +-+-+-+ + ±
A-2	0.04 0.22 0.30	± ± ±
A-3	0.05	±
A (total chloride)	0.06 others undeterminate due to streaking	±

± Signifies a very faint positive seen on some, but not all, papers examined.

The alkaloid of R_f 0.76 was only present in minute traces and could not always be detected. Fraction A-2 contained three trace alkaloids, two of which (R_f 0.22 and 0.30) are probably the same as those of R_f 0.23 and 0.35 in A-1. The other trace alkaloid (R_f 0.04), not usually detectable in A-1, was probably the same as the compound of R_f 0.05 in fraction A-3. Since these compounds were present in small amounts in very dirty residues; they were not examined further.

Fraction A-1 was also tested by thin layer chromatography (page 125)which, while not giving such good resolution as paper chromatograms, seemed to confirm the presence of six compounds. This material (A-1) was reserved and later subjected to partition chromatography on a chromax column.

It was considered that some fractionation between the quicker and slower-moving (on paper) compounds might be possible by reducing the volume of ethanol eluant used on an alumina column. Therefore, mixed chloride B was chromatographed on alumina (Table 10), but only 200 ml. of

Table 10.

Fractions of alkaloid chlorides (B) from an alumina column.

Fraction	Eluant			Residue	
	Solvent	Volume (ml.)	Colour	Weight (g.)	Remarks
B-1	ethanol	200	yellow	1.20	pale yellow solid
B-2	methanol	500	yellowish brown	0.254	brown solid
B-3	50% methanol	400	brown	0.180	dirty brown solid

ethanol was collected before continuing the elution with methanol and, subsequently, aqueous methanol.

The three fractions, after concentration, were chromatographed on paper using the same conditions as for chlorides A and the results are shown in Table 11.

Table 11.

Paper chromatography on alkaloid chlorides B using ethyl acetate/acetone/water (50:45:17).

Fraction	Compounds separated	
	R_f values	Relative strengths on chromatograms
B-1	0.36 0.52 0.65 0.77	+++++ ++++ ± ++
B-2	0.06 0.18 0.25 0.36	+ ± + ++ ±
B-3	0.06 0.18	+ ±

It can be seen that, as expected the major components were obtained in fraction B-1 free from contamination with alkaloids of lower R_f value as well as being reasonably free from colouring matter. Comparing the two ethanol fractions from chlorides A and B it was noted that the compound of R_f 0.77, was in greater concentration in B than in A; this is consistent with B representing the total mixed chloride from the initial starting material whereas A represents only part of the original mixture.

The object of passing the mixed chlorides through alumina columns had been to remove coloured compounds but the concurrent separation of compounds of lower R_f values from the major alkaloids in B-1 prompted the re-chromatography of a portion

of this fraction on alumina. Small volumes (5 ml.) of ethanol eluant were collected separately and each tested on paper chromatograms. The results showed that all the compounds of B-1 were eluted together, no further fractionation having been achieved.

The remainder of fraction B-1 was reserved and later subjected to steady-state distribution. Fractions B-2 and B-3 were not further examined.

The mixed chloride C was much cleaner than samples A and B and it was tested on paper chromatograms without the preliminary cleaning on a column of alumina. Two major alkaloidal components with R_f values 0.35 and 0.54 and a smaller amount of a compound with R_f 0.65 were detected thus showing that sample C was the simplest of the three chloride mixtures and corresponded to the ethanol eluant (B-1) of mixed chloride B except for the absence of the compound with R_f 0.77.

Mixed chloride C was subsequently used for the conversion of its quaternary bases to the corresponding tertiary compounds.

Partition chromatography of mixed chlorides on cellulose columns.

Although there had been some streaking on paper, some of the solvents were considered potentially - useful for separation of the chlorides on columns of cellulose powder; as Bartlett et.al.[121] point out, solvents which do not afford resolution on paper, sometimes give good separations when used on columns.

In preliminary experiments, the simplest chloride mixture (C) was chromatographed on a small column of cellulose powder (previously washed with 8-hydroxyquinoline solution to remove possible traces of metal ions[148]) using ethyl methyl ketone solvent as employed by the Karrer-Schmid[138] and Battersby groups[138]. It was also chromatographed on a larger cellulose column using the acetone/water system developed by Bartlett and co-workers[121]. Apart from the separation of negligible amounts of a fast-moving component, no satisfactory resolution of the mixture was obtained.

Paper roll chromatography.

Paper roll columns for partition chromatography were introduced by Hagdahl and Danielsson[146] in 1954. When used inside a pressure mantle, as in the ChroMax system (LKB Produkter AB, Stockholm), these columns are reported to give

conditions of separation resembling those on a single sheet of chromatography paper. Moreover, using these specially-prepared rolls the major difficulty and main variable of column partition chromatography, namely packing, is avoided. Since the solvent system ethyl acetate/acetone/water (50:45:17) had been the most satisfactory on paper sheets it was used in the paper roll chromatography of mixed-chloride fraction A-1. This was a reasonably clean mixture, previously shown to contain at least six components. To avoid pressure fluctuations within the mantle over the long period of experiment, this was conducted in a constant-temperature room (22-23°C) having first ascertained that separations on paper sheets were as satisfactory at this temperature as at laboratory temperature (18-20°C).

The elution pattern of alkaloids from the ChroMax column was followed by examination of selected fractions on paper chromatograms; similar fractions were bulked, taken to dryness and weighed (Table 12). During concentration of these fractions, as soon as the organic solvents were removed a white deposit appeared in the aqueous concentrate; this material, which interfered with further concentration, was found to migrate to the interface when the liquid was shaken with light petroleum. A clear aqueous layer could then be separated and taken to dryness . An infrared

spectrum of the interfering material was similar to that of the polythene used to enclose the paper column within the pressure mantle.

The various residues were checked against the original mixture (A-1) on paper chromatograms and although Rf values for the same compound varied somewhat from one paper to another, careful examination of the chromatograms made interpretation possible; the collected results from several papers are given in Table 13.

Fraction (k) contained, in a large volume of eluant, small amounts of a compound (Rf 0.06) which gave a faint brown stain with Dragendorff's reagent but which could not be detected in the original mixture; the small residue was deeply coloured. Apart from this, there was no isolation of any single compound although partial fractionation had been achieved. The two major compounds were free from other substances and concentrated mainly in the heaviest and cleanest fraction (c). They were present also in reasonable amount in (d) but were here contaminated with the compound next in significant amount in the original mixture (Rf 0.23, Table 9). This particular compound was present in greatest amount in fraction (g) and persisted into fraction (i). Here, and in fraction (j) there was some difficulty in interpreting trace amounts of what appeared to be two other alkaloids, one of which (Rf 0.12-0.14) was sometimes faintly

discernible in chromatograms of the original mixture. However these were in comparatively trivial amounts and were neglected. The attempts to crystallise chlorides from the two simplest mixtures (c and g.) of major compounds were unsuccessful. These mixtures were later converted to iodides and picrates and attempts were made to obtain individual compounds by fractional crystallisation. A very small amount of impure crystalline material was obtained only in the case of the iodides from (c). The infrared spectrum of this crystalline material was similar to that of an iodide (Q2) reported later in this thesis (page 98).

Table 12 .

Bulked fractions of quaternary chlorides eluted from a CroMax column (partition chromatography of mixed chloride A-1).

Fraction	Volume of eluant (ml.)	Weight of residue (g.)	Colour of residue
a	460	0.044	brown
b	210	0.26	brown
c	360	0.710	yellow
d	290	0.119	light brown
e	180	0.032	brown
f	170	0.034	brown
g	720	0.128	brown
h	440	0.021	brown
i	540	0.022	deep brown
j	1350	0.024	deep brown
k	2000	0.050	deep brown

TABLE 13.

Distribution of compounds in various fractions from a ChroMax column (partition chromatography of mixed chloride A-1).

Rf value (on paper) of compound.	Total chloride A-1	Fractions a	b	c	d	e	f	g	h	i	j	k
0.76-0.78	±	+										
0.62-0.65	+	+	+									
0.48-0.55	+++ ++		+	+++ ++	++ ++							
0.32-0.41	+++ ++			+++ ++	++ ++	+	+	+	±			
0.23-0.29	++				+	++	++	+++	+	+		
0.18-0.22	+									+	±	
0.12-0.14	±									+	+	
0.06												+

Counter-current separation of quaternary chlorides.

The unsuccessful partition column chromatography had been attempted using a virtually monophasic solvent system. For counter-current separations a two-phase system with marked immiscibility is essential. The three undernoted solvent systems were investigated and partition coefficients, in these, for the mixed chloride B were as follows:-

1. Ethyl methyl ketone/water, K = 0.2;
2. Ethyl methyl ketone/ethyl acetate/water, K = 0.2;
3. n-Butanol/water, K = 1.0

In all cases emulsions quickly formed on shaking, doubtless due mainly to the presence of quaternary compounds. However, with solvents 1 and 3 the emulsion dispersed on standing for 10-15 minutes and the former was selected for use on a Craig machine. After very few transfers, emulsion formation became a serious problem and could not be overcome even by extending the settling time to several hours or by addition of a silicone antifoam agent. This experiment had to be abandoned.

For the attempted separation of compounds in mixed chloride B-1, use was made of the simple butanol/water solvent in a steady-state machine in which a settling time of 15 minutes was adequate. The distribution of compounds in the effluents was followed at intervals during a total of 448 transfers; after 330 transfers one component appeared to have finished coming off with the upper phase, while two components were

still coming off together in the lower phase. The total upper phase effluent and total lower phase effluent up to this point, the several succeeding fractions of effluent, and mixed solvents remaining in each tube after the final transfer were separately concentrated and checked for alkaloid distribution by both thin-layer and paper chromatography.

In the results shown on pages 87 and 88 , respectively, the effluent samples refer to fractions of each phase collected from the machine between the specified number of transfers, the upper phase having moved in a positive direction: each tube sample represents the evaporated total contents of several adjacent tubes, those in the plus bank being on the positive side of tube 0 situated at the centre of the cell train.

Results obtained on intermediate fractions not shown in Table 15, were identical with those obtained on neighbouring fractions.

The weights of dried residues obtained from the various fractions (Table 14) can not be taken to indicate relative amounts of alkaloids present in each, since there was a variable distribution of impurities, all the samples being coloured to some extent. It must also be remembered that the relatively large residue in upper phase 1-330 was present in a large volume of effluent compared with the succeeding batches of effluent and the tube contents (50 ml.).

The two methods of screening gave comparable results although paper chromatography (in which more concentrated solutions were applied) showed plus fractions 35-40 up to U 331-368 alkaloid positive, whereas TLC gave no results.

It can be seen that the two fast-running (on paper) components moved in a positive direction in the machine and that the compound of R_f 0.77, while contaminated in the first main batch effluent, occurred as the single alkaloid in several positive fractions. The residues from plus 21-25 to U 379-388, inclusive, when combined (37 mg.) and dissolved in methanol yielded, on concentration, a colourless precipitate of non-alkaloidal material; the trace amounts of alkaloid remained in the yellow methanolic solution and could not be crystallised.

The two major alkaloids moved together less quickly in the negative direction and were present in all fractions from the minus bank. In theplus bank tubes 0-10 the compound with R_f 0.54 (on paper) appeared separate from compound of R_f 0.35 but was here contaminated with compound of R_f 0.77.

An increased number of transfers may have separated

the two compounds present in these fractions but the weights obtained would have been negligible. Compound of R_f 0.26, not detectable in the original mixture, possibly because of very low concentration, was revealed in trace amounts in the main aqueous effluent (L1-330).

Even though the material used in this experiment was relatively clean and represented only a part of the original natural mixture, seeming to contain only two major components, no satisfactory separation was achieved.

Table 14.

Residues from steady-State machine solvent fractions.

Lower phase (L) bulked effluent after transfers	Residue weight (mg.)	Upper phase (U) bulked effluent after transfers	Residue weight (mg.)
1 to 330	46.1	1 to 330	110.9
331 to 368	80.4	331 to 368	12.6
369 to 388	25.6	369 to 388	7.2
389 to 408	50.2	389 to 408	4.0
409 to 428	44.2	409 to 428	3.6
429 to 448	13.8	429 to 448	3.2
Minus bank		Plus bank	
-46 to -50	66.9	46 to 50	3.5
-41 to -45	47.4	41 to 45	3.6
-36 to -40	25.5	36 to 40	2.0
-31 to -35	17.6	31 to 35	2.6
-26 to -30	6.8	26 to 30	4.3
-21 to -25	7.9	21 to 25	3.1
-16 to -20	7.4	16 to 20	3.2
-11 to -15	7.2	11 to 15	3.2
-6 to -10	6.6	6 to 10	4.4
-1 to -5	6.6	0 to 5	7.1

Diagrams of thin-layer chromatograms showing alkaloid distribution in solvent fractions from a steady-state machine.

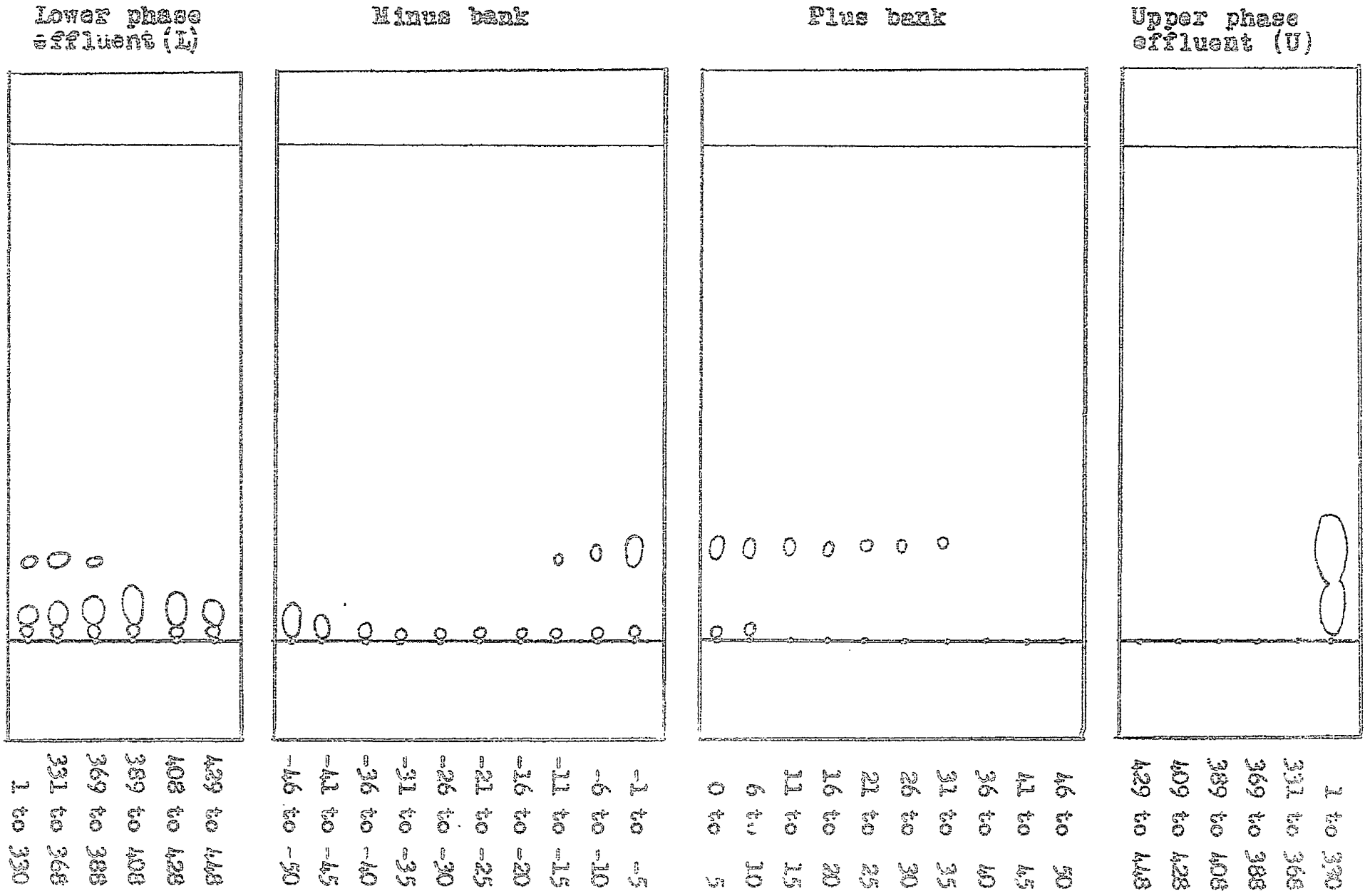


Table 15.

Distribution of quaternary alkaloid chlorides in solvent fractions from a steady-state machi...

Paper chromatography results.

R_f values	Distribution in original mixture (B-1)	Lower phase bulked effluent after transfers				Mixed solvent still in machine after 448 transfers							Upper phase bulked effluent after transfers		
						Minus bank			Plus bank						
		1 to 330	331 to 368	389 to 408	429 to 448	-46 to -50	-31 to -35	-6 to -10	0 to 5	6 to 10	11 to 15	46 to 50	369 to 388	331 to 368	1 to 330
0.77	+++							+	+	+	+	+	+	+	+
0.65	+														+
0.54	++++	++	++	+++	+++	+++	+++	++	+	+					
0.35	+++++	++	++	+++	+++	+++	+++	++							
0.26		+													

EXAMINATION OF TERTIARY BASES PREPARED FROM QUATERNARY SALTS

N-Demethylation of quaternary chlorides.

Attempts to separate the major quaternary alkaloids, other than macusine B, had failed using purely physical methods therefore chemical methods of separation were considered. On the assumption that these alkaloids might be N-methyl quaternaries, analogous in structure at the quaternary centre to macusine B, it was decided to attempt conversion to the corresponding N-demethyl tertiary bases which could then be separated by physical methods, and possibly re-quaternised.

For this purpose the mixture of quaternary chlorides C, which on paper chromatograms gave an indication of three compounds, was demethylated by refluxing with ethanolamine[144]. The reaction mixture, on basifying, yielded a chloroform-soluble solid which, when chromatographed on alumina, yielded fractions which were combined according to their behaviour on thin-layer chromatograms (Tables 16 and 17). Altogether nine components of different R_S values (reference normacusine B = 1.0) could be detected. Of these, only two components, normacusine B and a compound with R_S 0.83, were in major amounts and the others were in traces. Of the seven minor components, three (R_S 0.16, 0.76, 1.2) were probably the same as those with similar R_S values found in normacusine B mother liquor (page 119).

Table 16.

Fractions containing tertiary bases from an alumina column.

Combined Fraction	Eluant		Residue	
	Solvent	Volume (ml.)	Weight (g.)	Remarks
1	chloroform	150	0.29	fawn coloured solid
2	"	175	0.017	pale brown solid
3	"	125	0.053	fawn coloured solid
4	"	775	0.097 (+0.107g crystals)	fawn coloured solid. The fraction on concentration deposited colourless crystals which were separated before taking the fractions to dryness
5	10% ethanol in chloroform	125	0.035	pale brown solid
6	10% ethanol in chloroform	125	0.034	orange-red solid
7	ethanol	450	0.018	deep red, glassy solid

Table 17.

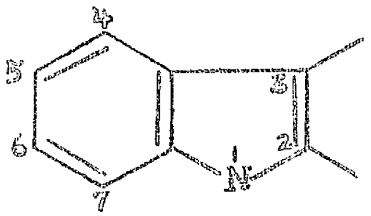
Thin-layer chromatograms of tertiary bases from an alumina column (Reference Standard = Normacusine B).

Combined fractions	R_S values	Relative strengths
1	0.54	±
	1.0	+++++
	1.1	+
	1.19	±
2	0.62	±
	0.85	+
	1.0	+
	1.09	+
	1.20	±
3	0.61	±
	0.83	+++++
	1.0	+
	1.14	+
4	0.62	+
	0.83	+++++
	1.14	+
5	0.62	±
	0.76	±
	0.83	±
6	0.16	+
7	0.16	+

Of the remaining four, one (R_S 1.14), was probably the methyl ester corresponding to the amide also separated from the reaction mixture, vide infra).

Fraction 1 contained largely normacusine B, of which 0.12g. was recovered. Fractions 3 and 4 yielded a crystalline compound which analysed very well for $C_{23}H_{25}N_3O_2$. The product, a tertiary base, showed in the infrared spectrum (Figure 3) typical amide carbonyl absorption at 1640 $cm.^{-1}$ and amide NH absorption at 1540 $cm.^{-1}$; it was concluded that this must have been derived from a quaternary salt carrying an ester substituent, by concurrent N-demethylation and ethanolamide formation. This conclusion was confirmed by examination of the infrared tracing of the unresolved parent mixture of quaternary salts (C) which showed evidence of ester carbonyl absorption at 1724 $cm.^{-1}$.

The ultraviolet absorption of the amide (Figure 4) showed maxima at 226 $m\mu$(log ε 4.53), 284(3.88), 293(3.79) characteristic of a 2,3-disubstituted indole.[71 139]



The implied lack of substitution in the 4,5,6 and 7

The implied lack of substitution in the 4,5,6 and 7

positions of the indole nucleus is supported by a strong absorption band in the infrared spectrum at 740 cm.$^{-1}$ characteristic of aromatic systems with four adjacent hydrogen atoms. A medium intensity band at 830 cm.$^{-1}$ is almost certainly due to the presence of a trisubstituted double bond.

The amide was converted to the corresponding methyl ester (T3) by acid-catalysed methanolysis and the product gave an R_S value of 1.15 on chromatoplates (normacusine B = 1.0) Although satisfactory analytical data could not be obtained on the ester, the spectral evidence (Figures 5 and 6) taken in conjunction with that for the amide (Figures 3 and 4) and normacusine B (page 68) together with the analytical data on the amide, allow speculation on a tentative structure for the methyl ester (T3) in relation to the known structures of normacusine B and polyneuridine.[70]

Macusine B (page 62), normacusine B and polyneuridine have all been identified in extracts from Aspidosperma peroba.

Possible structures which might be considered for the methyl ester and its ethanolamide are therefore (I) and (II), respectively, as shown on the next page.

FIGURE 3

I.R. SPECTRUM OF COMPOUND T3A

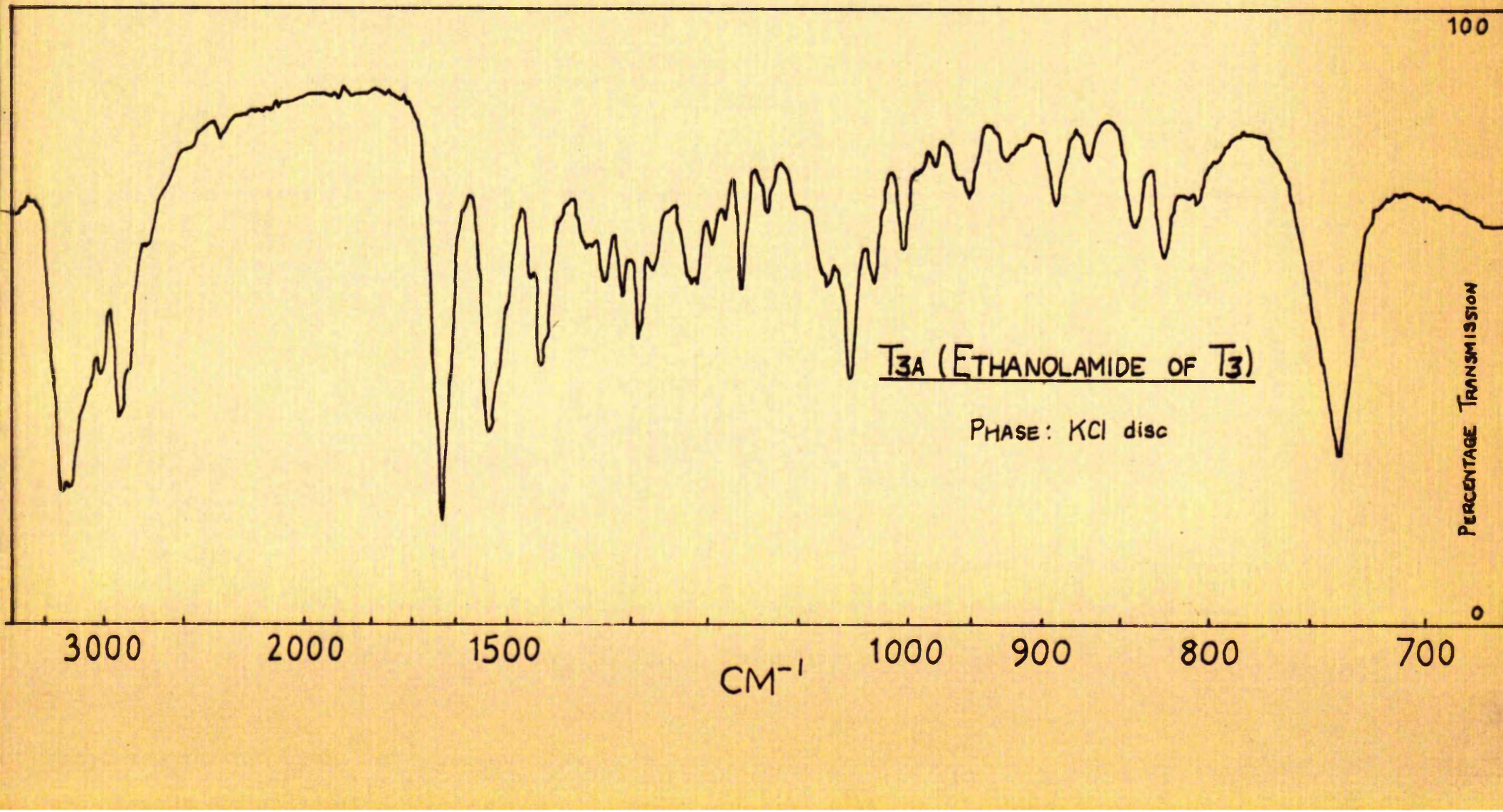


FIGURE 4

U.V. SPECTRUM OF COMPOUND T3A

(ETHANOLAMIDE OF T3)

0·001134 % IN ETHANOL

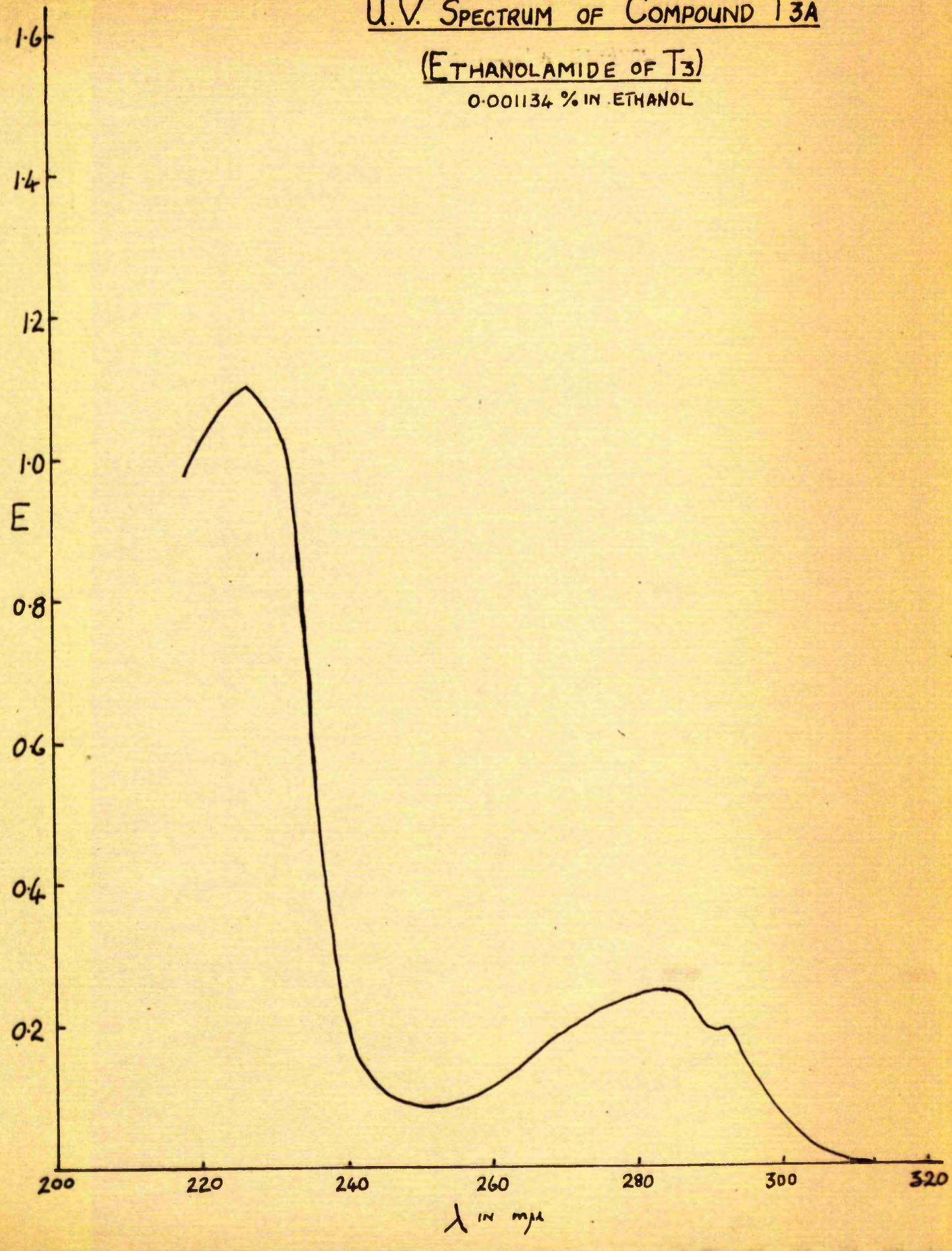


FIGURE 5

I.R. SPECTRUM OF COMPOUND T3

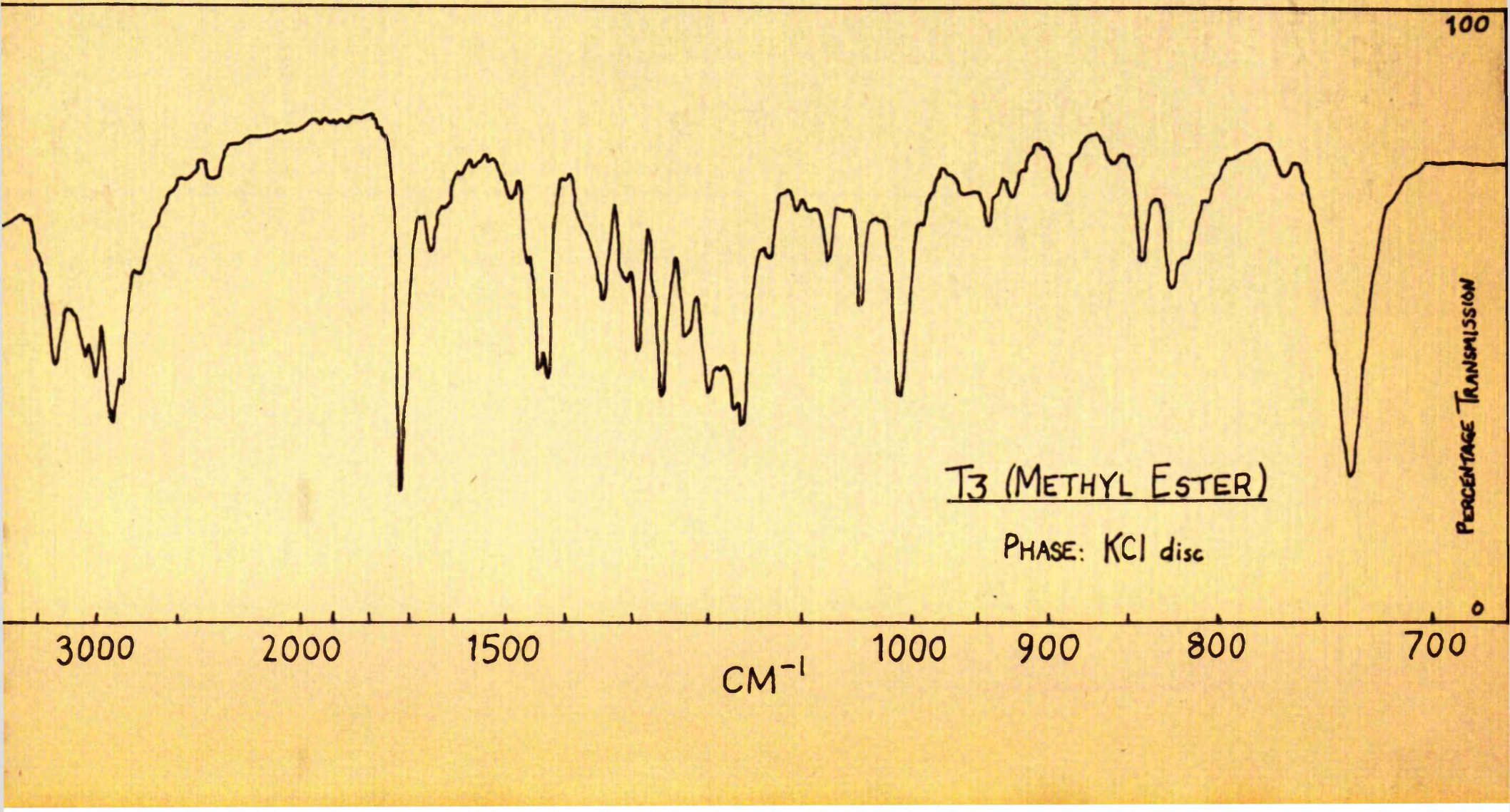
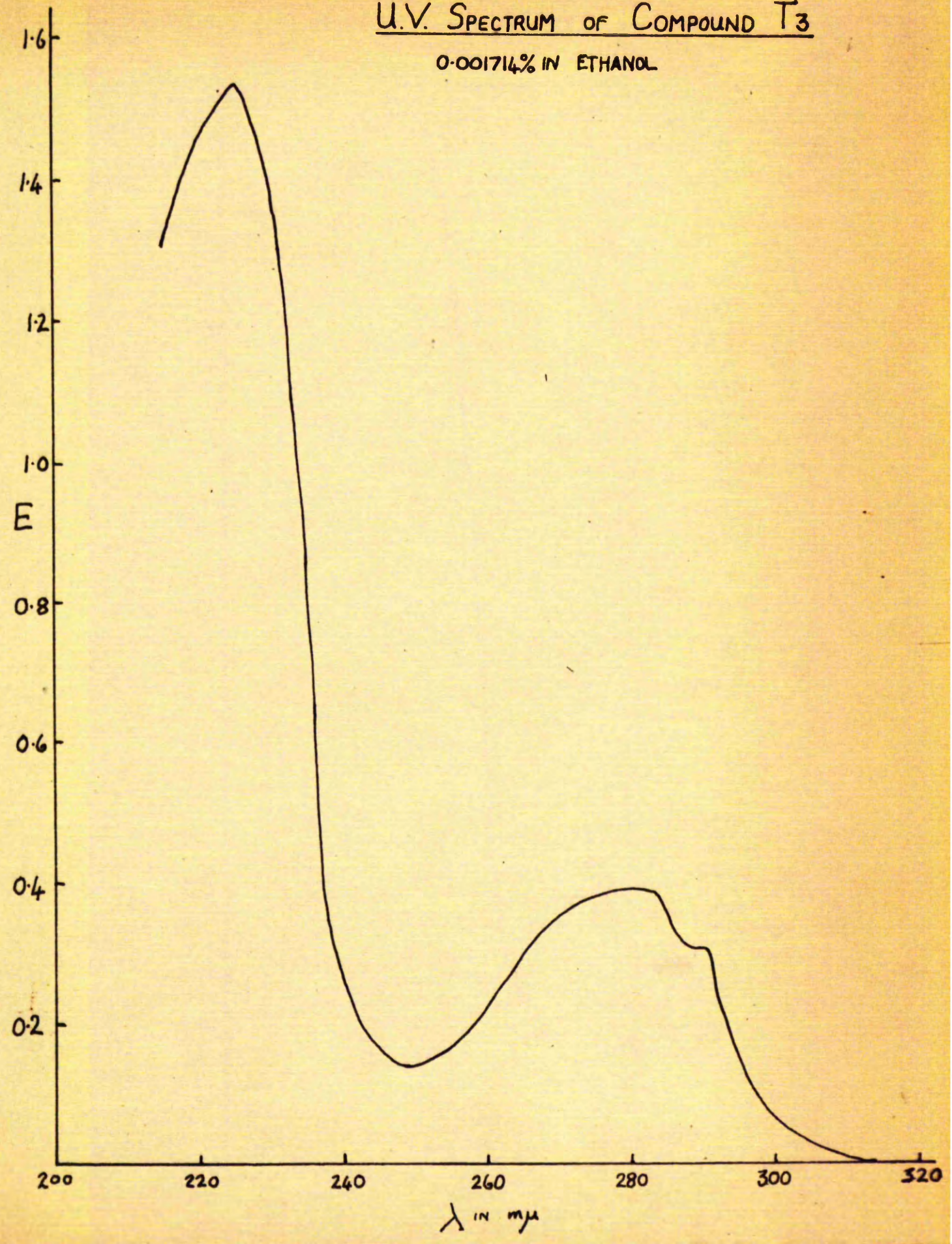
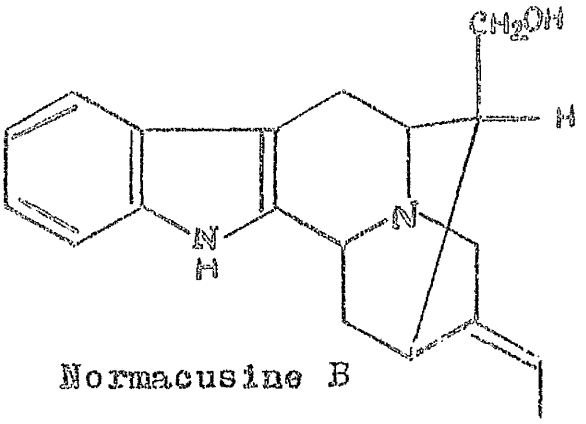


FIGURE 6

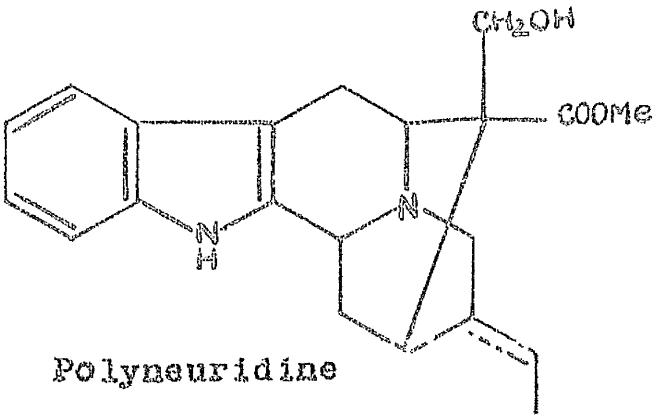
U.V. SPECTRUM OF COMPOUND T3

0·001714% IN ETHANOL

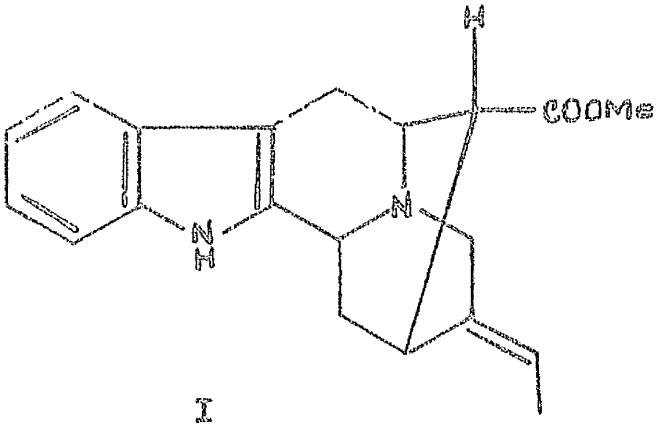




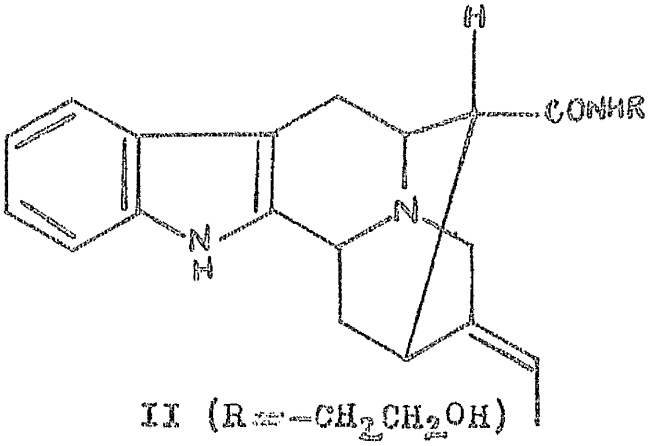
Normacusine B



Polyneuridine



I



II (R = $-CH_2CH_2OH$)

The analytical data obtained for the ethanolamide fit the latter and this is supported by the physico-chemical data discussed above. The methyl ester was obtained only in milligram amounts and microanalysis gave unsatisfactory results but ultraviolet and infrared spectra provided useful support for the main functional groups. The ultraviolet absorption spectrum was closely similar to that of the corresponding amide, showing maxima at 225 mμ (4.46), 283(3.86), 291(3.79) characteristic of the indole

nucleus without substitution in the 4,5,6 and 7 positions. This conclusion is also supported by the strong absorption band in the infrared at 740 cm.$^{-1}$. A medium band at 825 cm.$^{-1}$ and a low intensity absorption inthe 1650 cm.$^{-1}$ region are characteristic of the trisubstituted double bond. Ester carbonyl absorption at 1724 cm.$^{-1}$was also characteristic.

An attempt to obtain supporting evidence from the nuclear magnetic resonance spectrum of the ester T3, in deuterochloroform, was only partially successful. The small quantity of sample available gave only a poorly resolved spectrum compared with that of normacusine B used as a model compound for comparison.

Methochloride obtained by re-quaternisation of tertiary base T3

Methochloride obtained by re-quaternisation of tertiary base T3

The methyl ester T3 was quaternized by heating with methyl chloride under pressure[18] to give the corresponding methochloride, but only in a yield sufficient for chromatographic analysis. Macusine B chloride was also prepared (pages 64 and 117) and both were run on paper in the ethyl acetate/acetone/water solvent in order to determine their positions on chromatograms in relation to compounds in the original mixture (C) of quaternary chlorides. It was

noted that macusine B chlorides (unless in very small amounts just detectable by Dragendorff's reagent or ceric sulphate reagent) gave a double-spot effect with R_f values of 0.54 and 0.35, identical with the values for the two supposed major components of the original mixture. With minimal loading on paper chromatograms, macusine B chloride and mixed chloride C again behaved identically giving a single spot of R_f 0.45.

The product resulting from quaternisation of alkaloid T3 was extracted with chloroform and this extract, run on paper, showed some unchanged tertiary base (R_f 0.99), and a spot of high R_f (0.90) due possibly to a tertiary breakdown product of T3 and a third spot of lower R_f (0.65) probably due to the methochloride of T3 (Q3) which could have been extracted by the ethanol present in the Chloroform B.P. used for extraction.

The water-soluble residue gave on chromatograms three spots; one of R_f 0.12 was not present in the mixed chloride (C) and may be a quaternary compound corresponding to the tertiary breakdown product mentioned above. Of the other two spots, R_f 0.65 and 0.52 (very weak), the former corresponded to a spot on chromatograms of the original chloride mixture on which the other spot, if present, would be masked by macusine B chloride. It could be that, like macusine B chloride, the methochloride Q3 gives a

double spot effect.

Thus far, the three spots in chromatograms of mixed chloride C, from which the tertiary bases normacusine B and T3 (*via* ethanolamide T3A) had been isolated, could be accounted for by the two corresponding methochlorides, macusine B chloride and Q3. Also that fractions which had previously showed only the two main spots (R_f 0.52, 0.35) on paper may contain the single compound macusine B chloride. However, the residues from fraction (C) (Chromax column) and tube fractions -46 to -50 (steady state machine), both of which showed chromatograms identical with pure macusine B chloride, gave infrared spectra with definite ester carbonyl absorption at 1724 $cm.^{-1}$. This is not shown by macusine B salts but is characteristic of the methyl ester tertiary base T3 and, presumably, also its methochloride Q3. Thus both the major quaternaries appear to occur together in all the main unresolved chloride mixtures previously reported.

ISOLATION OF A MINOR QUATERNARY ALKALOID

A deeply coloured residue containing methanol-soluble thiocyanates (residue remaining after separation of macusine B thiocyanate) was chromatographed on hydrochloric acid-washed alumina to remove impurities but the major fraction (TR-1) gave paper chromatograms in ethyl acetate/acetone/water (50:45:17) system which showed major spots at R_f 0.37 and 0.52 resembling the main components of quaternary chloride mixtures; in addition it showed a spot at R_f 0.76 corresponding to a large, very strong spot given by the parent crude thiocyanate mixture. Chemical tests on the highly water-soluble residue revealed the presence of both thiocyanate and chloride showing that the former had been partially converted into the latter on acid-washed alumina, an effect similar to that previously observed on other alkaloid salts[146].

A mixed iodide, precipitated from an aqueous solution of this residue (TR-1), yielded a pure crystalline compound, m.p. 248-250 (decomp.). This quaternary ammonium compound Q2 (iodide) gave on analysis C, 52.77; H, 5.84; N, 5.86; I, 26.92%

$C_{21}H_{27}O_3N_2I$ requires C, 52.29; H, 5.64; N, 5.81; I, 26.31%

$C_{21}H_{27}O_2N_2I$ requires C, 54.06; H, 5.83; N, 6.0; I, 27.2%

From these figures it is difficult to choose between the two formulae although the equivalent weight 469.7, determined

Table 18

Ultraviolet spectra and optical rotations of some alkaloids with a methyleneindoline chromophore.

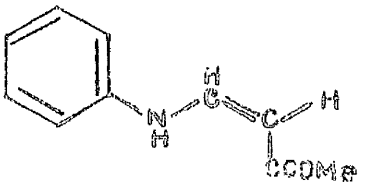
Alkaloid	$\lambda_{max.}^{EtOH}$ mμ (ε)	$[\alpha]_D$	Refs.
Akuammicine	228 (12,100) 297 (11,600) 328 (17,700)	-745°(EtOH)	71, 147.
Condylocarpine	226 (10,350) 296 (9,600) 330 (14,450)	+900 (CHCl$_3$)	71, 139,
Compactinervine	237 (10,050) 297 (8,120) 332 (15,130)	-640° (Pyridine)	54, 71,
Minovincine	227 (10,900) 300 (8,900) 327 (13,950)	+504°(CHCl$_3$)	71, 148,
Minovincinine	224 (9,900) 298 (9,600) 328 (12,600)	-418° (EtOH)	71, 148,
Mossambine	228 (12,250) 298 (10,800) 329 (16,000)	-470°(CHCl$_3$)	71, 149,
Q?? (iodide)	222 (20,440) 295 (11,020) 330 (13,620)	-432° (MeOH)	

by Volhard titration, would favour $C_{21}H_{27}O_3N_2I$ (466.37) rather than $C_{21}H_{27}O_3N_2I$ (482.37).

The ultraviolet absorption spectrum of the iodide (Figure 7) is similar to spectra of the alkaloids figured on page 101) all of which possess the indole nucleus. The high intensity of the absorption at 222 mμ (ε = 20,440) as compared with about ε = 10,000 for these other alkaloids (Table 18) is attributed to the high-intensity absorption of the iodide ion itself at 221 mμ. This is substantiated by the fact that potassium iodide in ethanol was found to have ε = 11,340 at 221 mμ.

The infrared spectrum of Q2 (Figure 8) has bands at 760, 1600, 1660 and 3367 $cm.^{-1}$. The strong absorption at 760 $cm.^{-1}$ is indicative of an ortho-disubstituted benzene ring. The band at 1600 $cm.^{-1}$ is due to aromatic absorption; the unusual intensity of this band, which is normally weak, is probably caused by the adjacent polar (NH) group as observed in the case of the above alkaloids[71].

The absorption at 1660 $cm.^{-1}$ is characteristic of an unsaturated ester of the type below for which the carbonyl absorption was found[150] at 1659 $cm.^{-1}$.



Such a shift of the ester carbonyl absorption towards

FIGURE 7

U.V. SPECTRUM OF COMPOUND Q2
(QUATERNARY IODIDE)

0.001164% IN ETHANOL

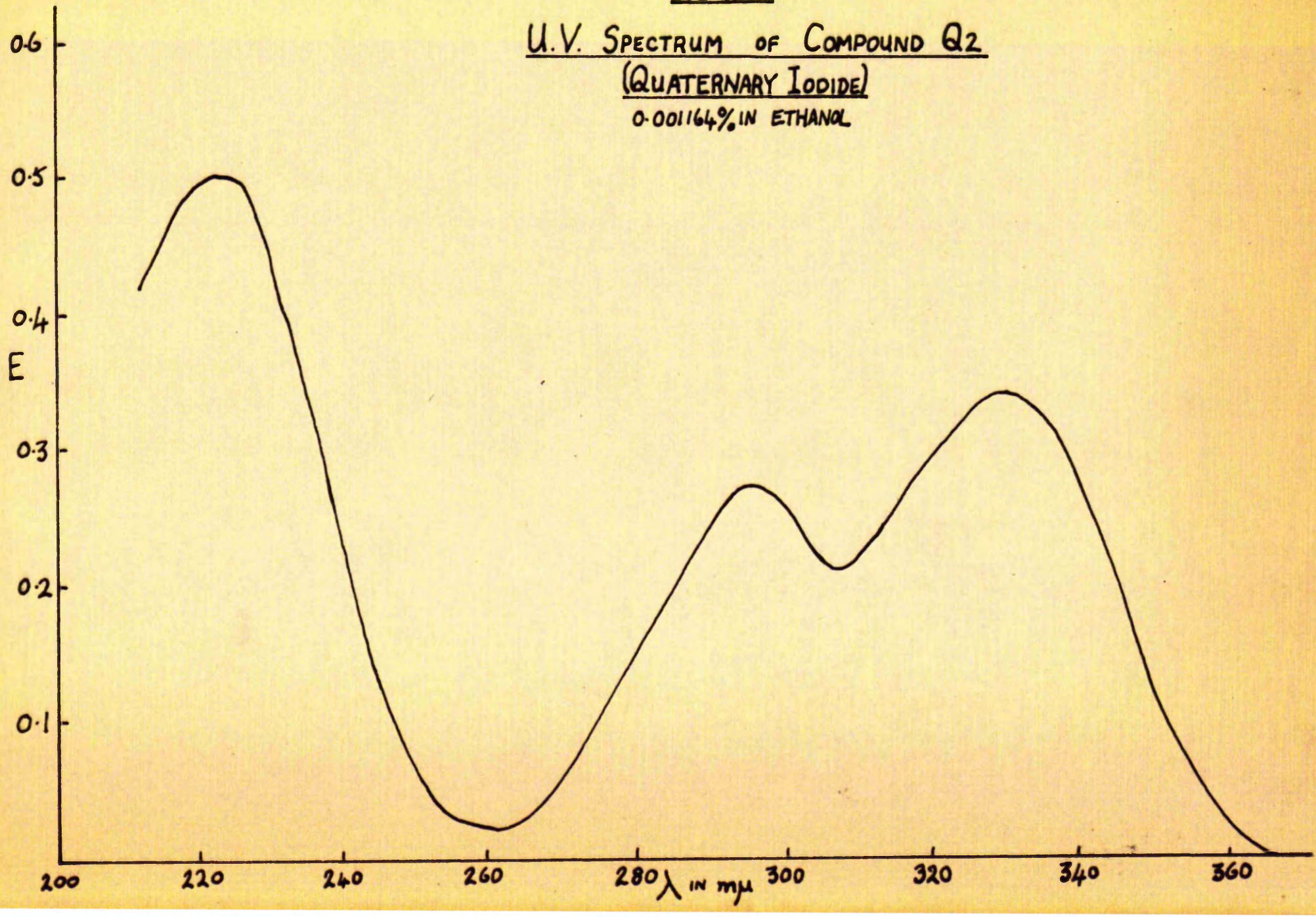
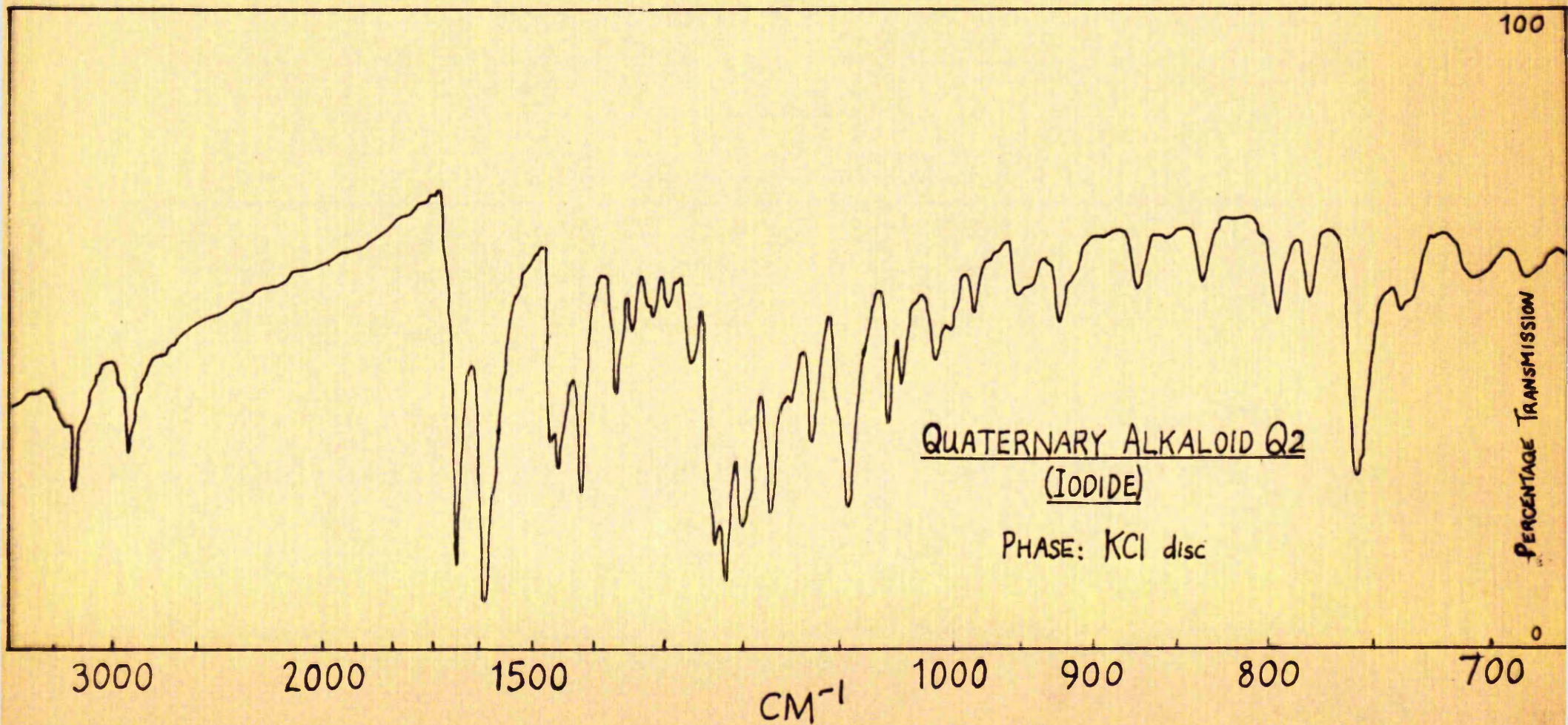
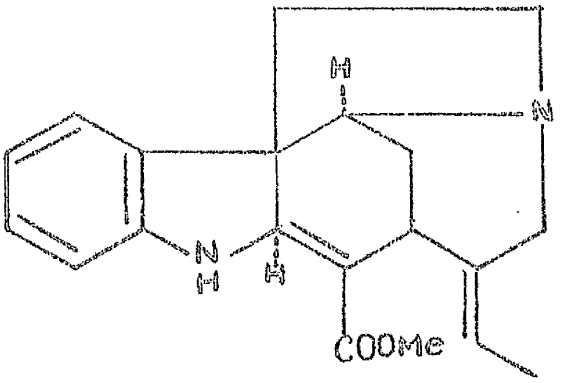


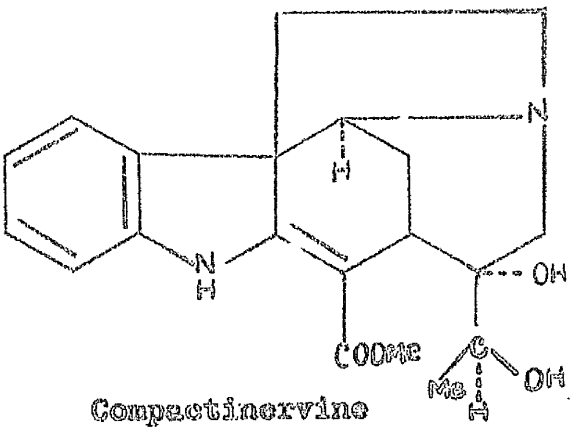
FIGURE 8

I.R. SPECTRUM OF COMPOUND Q2

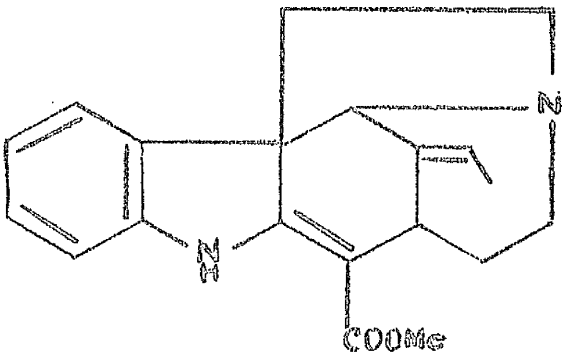




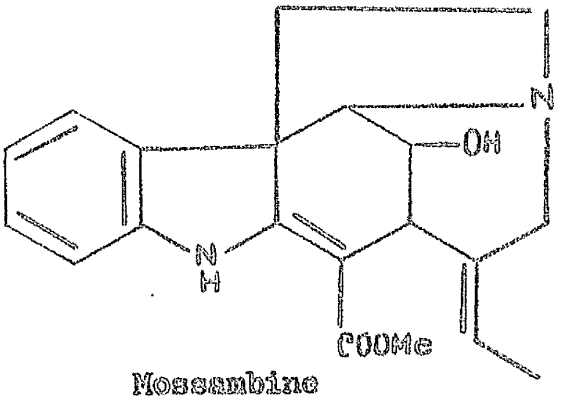
Akuammicine



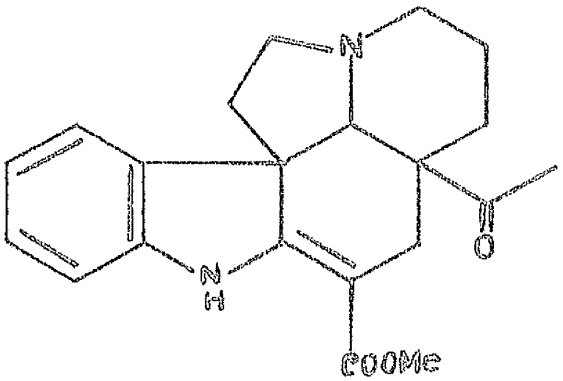
Compactinervine



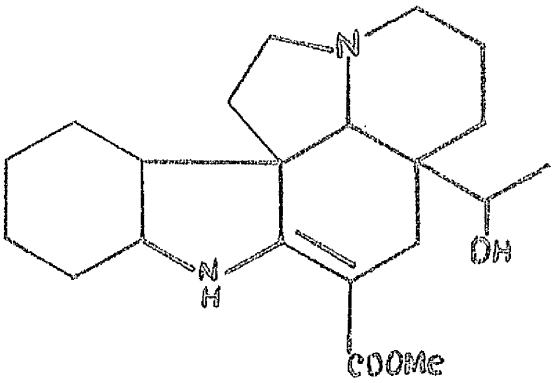
Condylocarpine



Mossambine



Minovincine

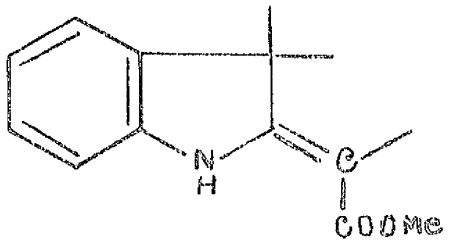


Minovincinine

the lowerfrequency region has been observed in the case of all the alkaloids given above, and reduction of the 2-16-double bond of mossambine[149] and akuammicine[147] shifted the ester carbonyl absorption to its normal position at about 1730 cm.$^{-1}$. Another feature common to Q2 and all the above alkaloids is that the intensity of the aromatic absorption near 1600 cm.$^{-1}$ is higher than that due to the ester carbonyl.

The band at 3367 cm.$^{-1}$ for Q2 is identical with that due to NH absorption in compactinervine[84,71] and minovincinine[71,149]. The absence of absorption in the 3500-3600 cm.$^{-1}$ region for Q2, as distinct from such OH absorption shown by the other two compounds, excludes the possibility of an OH group in Q2 unless there is intermolecular hydrogen bonding, in which case the NH and OH absorptions would interfere with each other.

From the foregoing evidence it is probable that Q2 has the chromophoric system:-



Considering the molecular formula $C_{21}H_{27}O_3N_2I$ it is difficult to account for the third oxygen atom except as hydroxyl, because the presence of a ketonic group is excluded by comparison with minovinicine which shows[71] ketonic absorption

at about 1700 cm.$^{-1}$ The absence of such absorption in the spectrum of Q2 thus lends further support for the formula $C_{21}H_{27}O_2N_2I$.

A further point of similarity between Q2 and the other alkaloids in Table 18 is that they all exhibit very high optical rotatory power.

At this stage the chloride residue from the Chromax column fraction (c), which on paper chromatograms showed only the two major spots (R_f approx. 0.52 and 0.35) was converted to iodide and a very small amount of impure crystalline iodide showing the same infrared spectrum as Q2 was obtained (page 79).

The yield of this compound from both sources was very low, showing Q2 to be a minor alkaloid which, as chloride, must run on paper chromatograms with the two major unresolved mixed chloride fractions.

It is of interest to note that the quaternary compound Q2 is only the third alkaloid having the methyleneindoline chromophore in the Aspidosperma, the other two being the tertiary bases compactinervine and tubotaiwine isolated from A. compactinervium[84] and A. limae, respectively. A quaternary alkaloid of this type, akuammicine methochloride, has been reported in the Apocynaceous plant Hunterum eburnea[121] and several such compounds have been isolated from curare and strychnos species.[109]

PHARMACOLOGICAL ACTIVITY OF MACUSINE B NITRATE

At the outset it was considered that the quaternary alkaloids of A.peroba might prove to be of some pharmacological interest but only one obtained in sufficient quantity for testing was macusine B. A pure sample of the water-soluble nitrate was available and this was tested for neuromuscular blocking activity by measuring the depression of response of the isolated frog rectus abdominis preparation to acetylcholine. Very large doses were required to show activity but there was a linear response over the dose range 0.2 to 2.0 mg. of macusine B nitrate, thus showing that the compound exhibits feeble neuromascular blocking activity.

EXPERIMENTAL

The author wishes to thank Dr. R. Raffauf, Smith Kline and French Laboratories, Philadelphia, for the gift of crude extract used as starting material in this work; Mr. J.L. Paterson for the pharmacological testing of macusine B nitrate, Mr. T. Turnbull of Messrs. Quickfit and Quartz Ltd. for conducting the steaday-state distribution experiment, and Mr. W. McCorkindale for microanalyses.

Melting points are uncorrected.

PRELIMINARY EXTRACTIONS AND SEPARATIONS

Material.

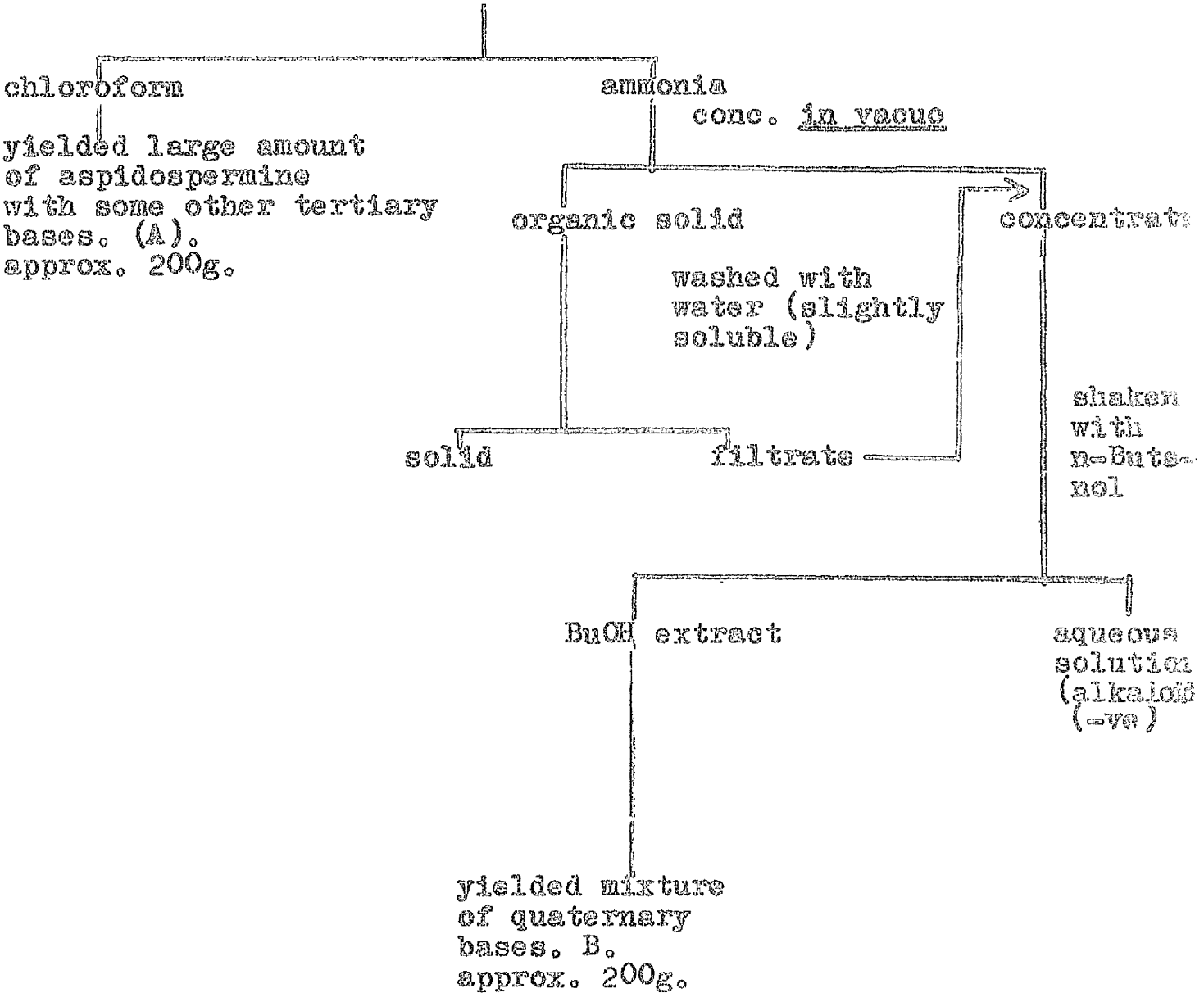
The starting material for this work was a crude dry extract from stem bark of authenticated Aspidosperma peroba F. Allem. obtained according to the extraction scheme shown in Figure 9. Because of solubility differences tertiary and quaternary alkaloids were obtained in separate fractions, A and B respectively, each weighing approximately 200g. The quaternary residue (B), as received, was in the form of a coarse, dry, easily-powderable product which on extraction with water gave a solution strongly positive to Mayer's and Dragendorff's reagents. Solutions in dilute acetic acid (5%) were shown to be alkaloid positive using ceric sulphate[131] solution (1% in 2N H_2SO_4).

Attempt to separate quaternary bases as trichloro-acetates.

The crude dry extract (1g.), finely powdered, was shaken continuously for 24 hours with water (5 ml.), then filtered. To the dark brown filtrate was added, with vigorous stirring, a solution containing trichloroacetic acid (1g.) in water (1 ml.). The solution became slightly turbid and on standing yielded a very small amount of a gummy deposit which was not examined further.

Figure 9

Method of extraction of alkaloids from the dried stem bark of A. peroba.



Precipitation of mixed quaternary bases as reineckates.

The crude extract (20g.) was finely powdered and extracted by trituration with 100 ml. portions of dilute hydrochloric acid, each time decanting off the clear supernatant liquid, until the last extract was very faintly positive to Mayer's reagent and did not give a precipitate with ammonium reineckate solution (total acid extract, 1500 ml.). The clear extract on standing deposited some dirty non-alkaloidal material which was discarded. The acid extract was treated with an excess of saturated aqueous solution of ammonium reineckate and the alkaloidal precipitate was separated by filtration under suction, washed thoroughly with water and dried over sulphuric acid to give 21.3g. of crude reineckate as a chalky, pink powder.

Adsorption chromatography of crude reineckate on alumina.

The crude reineckate (10g.) was dissolved in acetone (200 ml.). Some insoluble black residue (0.9g.) remained undissolved, was filtered off and discarded. The clear solution was passed through a column of alumina (300g., 31x3.5cm., B.D.H., previously treated with dilute hydrochloric acid, washed until neutral and then dried at 100-105°). Development and elution began using acetone and was continued using acetone containing graded increases in

amounts of ethanol, then acetone-ethanol-acetic acid and finally 5% aqueous acetic acid (see Table 3).

Several 100 ml. fractions of eluant were collected and systematically tested to show their behaviour on paper chromatograms. Firstly each fifth fraction was concentrated and tested; subsequently intermediate fractions were concentrated and tested to determine the elution pattern of the alkaloids. According to colour of solution and paper chromatographic results obtained, the fractions from the column were bulked, the solvent removed under vacuum and the residues weighed (Table 3). Small amounts of each residue were re-examined by paper chromatography to check the distribution of alkaloids within each fraction.

Paper chromatography of alkaloid reineckates.

Total reineckate.

Chromatographic separation of the mixed reineckate was attempted using the undernoted solvent systems with upward development on different grades of paper for chromatography.

1. n-Butanol/pyridine/water (1:1:1),
2. Ethyl acetate/pyridine/water (4:2:4),
3. Ethyl acetate/pyridine/water (7.5:3.1:1.65)[131],
4. n-Butanol/glacial acetic acid/water (5:1:4),
5. n-Butanol/citric acid/water (50:1:50),
6. Water-saturated ethyl methyl ketone containing

2% formamide.[131]

After development and drying, the papers were sprayed with modified Dragendorff's reagent[168] to show the presence of alkaloidal compounds.

Reineckate fractions from an alumina column.

Experiments similar to the above were conducted using the major fraction (A) from the alumina column. Whatman No. 1 papers were used and these were equilibrated with the vapours of the aqueous phase from the appropriate solvent before development at laboratory temperature (18-20°).

(a) Ascending chromatography.

Solvents 3 and 6 were used on 57 cm. papers. With the former solvent two spots were separated, one running with the front and the second giving R_f 0.54 but both showing considerable 'tailing'. With the latter solvent results were similar, the first material again running with the front, the second giving R_f 0.81.

(b) Circular chromatography.

On paper discs, 27 cm. diameter, solvents 4 and 5 were used. Both carried most of the material with the solvent front although there was an indication of slight separation with solvent 5, a faint positive being observed at R_f 0.80.

(c) Descending chromatography.

The organic phase from the system acetone/benzene/chloroform/water (60:15:10:20:), to which 3% methanol was added, was used on 57 cm. papers, developing for 3 hours.

Subsequently all bulked fractions A to F from the alumina column, together with the crystals separated from fraction A, were chromatographed using this system and the results are given in Table 4.

Fractions E and F were also chromatographed using downward development with solvent 4 for 16 hours and the results were as follows:-

Fractions	R_f values	Remarks
E	0.86	traces
	0.78	traces
F	0.84	traces
	0.56	traces

Macusine B thiocyanate

Isolation from mixed reineckate.

The crystalline material (0.236g.) which separated from fraction A, eluted from the alumina column, was filtered off, washed with a small amount of acetone and recrystallised twice from hot methanol to give colourless needles (0.166g.), m.p. 283-285° (decomp.), $[\alpha]_D^{25}$ + 33.3° (c., 0.25 in methanol); $\lambda_{max.}^{EtOH}$ 222mμ(log.ε4.55), 273(3.87), 283(3.85), 290(3.72); $\lambda_{min.}^{EtOH}$ 243(3.23), 278(3.84), 288(3.66).

The infrared spectrum (Figure 1) showed a strong sharp band at 2090 cm.$^{-1}$ indicative of -CN streching vibration. A methanolic solution gave a white precipitate with silver nitrate solution; also a red colour on the addition of dilute hydrochloric acid followed by methanolic solution of ferric chloride. The compound gave a bright yellow colour with concentrated nitric acid but gave no colour with solution of p-dimethylaminobenzaldehyde[140].

Found: C,68.65; H,7.0; N,11.2; S,8.8

Calc. for $C_{21}H_{25}N_3OS$, C,68.6, H,6.9; N,11.4; S,8.7%

The equivalent weight was determined by dissolving the thiocyanate (10 mg.) in methanol (5 ml.) and adding dilute nitric acid (3 ml.); a known excess (4 ml.) of N/50 silver nitrate solution was then added and the residual silver nitrate back titrated with N/50 ammonium thiocyanate

solution using ferric alum[153] as indicator. The equivalent weight was found to be 367.1 (calculated molecular weight for $C_{21}H_{25}N_3OS$ = 367.3).

Precipitation from aqueous solution of crude extract.

The crude dry extract (50g.) was finely powdered and extracted with 5x250 ml. portions of distilled water, shaking each time for 6 hours. The combined liquors gave Extract I (1250 ml.). The residue was re-extracted with distilled water, as before, to give Extract II (1250 ml.). Both extracts, on standing, gave a small amount of dark-brown, sticky sediment from which the supernatant liquids were decanted. The sediments, dissolved in methanol, were found to be non-alkaloidal and, after removal of solvent weighed 0.89g. The water-insoluble marc from the initial starting material was dried over sulphuric acid to give 8.65g. of dark brown residue which was also discarded.

The deeply coloured aqueous extracts (I and II) were separately treated as follows. Each was extracted with ether (8x600 ml.) and then concentrated to about 200 ml. in a rotary film evaporator at 55°C. and the aqueous concentrate again extracted with ether (15x200 ml.). The total combined ether extract was evaporated to give a black residue (0.92g.) containing traces of alkaloidal material, probably tertiary bases, which were not further examined.

A. Extract I.

To the 200 ml. of concentrate, saturated ammonium thiocyanate solution (20 ml.) was added gradually, with vigorous stirring, until no further precipitation occurred. After allowing to stand overnight, the supernatant liquid was decanted off from the brown gummy deposit. The precipitate was washed with water (4x10 ml.) to remove any traces of ammonium thiocyanate and the washings were added to the separated liquid. The precipitate was dried over phosphorus pentoxide to give a powderable solid (12.673g.).

The powder was washed thrice with 20 ml. portions of aqueous methanol (20%) and twice with 10 ml. portions of methanol to remove most of the colouring matter and the fawn-colouredresidue was finally suspended in 10 ml. of methanol and the mixture filtered. The precipitate was again washed with methanol (10 ml.) and then dried to give an almost colourless powdery solid (4.9g.). The washings were combined and kept.

B. Extract II.

This was treated in the same way, as before, and required 6 ml. of saturated ammonium thiocyanate solution for complete precipitation. The precipitate, obtained in the form of a fine suspension, was separated by filtration. The residue was washed with water and then with methanol, as before and

then dried to give a pale coloured solid (0.85g.). The washings were combined with the corresponding washings of the first precipitate. The combined aqueous methanol and methanol washings were taken to dryness to give mixed thiocyanates (7.4g.). This was reserved and subsequently worked to yield compound Q2. The initial supernatant liquid combined with the water washings was reserved and subsequently used for the preparation of reineckates then chlorides on which a chromatographic separation was attempted (page 122).

The precipitates from A (4.9g.) and B (0.85g.) were combined on the basis of their infrared spectra (Figure 1) and melting points (276-280°, 275-279° respectively) and the material was recrystallised from boiling methanol to give macusine B thiocyanate (2.18g.) as colourless needles, m.p. 283-285° (decomp.). From the mother liquor another batch (2.06g.), with the same melting point, was recovered.

Macusine B nitrate.

Macusine B thiocyanate (0.5g.) in 50% aqueous methanol (80 ml.) was titrated with the calculated amount of silver nitrate solution (13.61 ml., $\frac{N}{10}$ $AgNO_3$), the precipitate of silver thiocyanate filtered off and washed with water. The combined filtrate and washings were evaporated to dryness to give macusine B nitrate (0.506g.) as a colourless glassy solid. This was crystallised twice from hot water as clustered needles, (0.352g.) m.p. 142-147° (decomp.) $[\alpha]_D^{21.5}$ + 16° (c., 0.5 in methanol) (Found: N,11.3 $C_{20}H_{28}N_8O_4$ requires N,11.3%). From the mother liquor another 0.05g. was recovered.

Macusine B iodide.

Macusine B nitrate (0.3g.) in water (6 ml.) was treated with an excess of saturated aqueous solution of potassium iodide. The resulting precipitate was allowed to stand overnight, collected, washed with water and dried over phosphorus pentoxide to give a colourless solid (0.323g.). This was recrystallised twice from methanol-ether (dissolved in warm methanol and ether added to give a faint cloudiness) to give macusine B iodide as prisms m.p. 274-276° (decomp., after darkening from 250° and sintering from 261°),

$[\alpha]_D^{21.5}$ + 17° (c., 0.5 in methanol) (Found: C,55.0; H,5.8; N,6.3; I,29.4. Calculated for $C_{20}H_{28}IN_2O$; C,55.05; H,5.8; N,6.4; I,29.1%) Lit.,[128] m.p. 280-281° (decomp., after darkening from 250°); (Lit.[130] m.p. 275-277° (decomp.,) $[\alpha]_D^{20}$ + 14 ± 2° (c., 0.51 in water).

From the mother liquor another 0.040g. was recovered.

Macusine B picrate.

Macusine B nitrate (0.05g.) in water (2 ml.) was treated with excess of saturated sodium picrate solution, and the precipitate washed with water then dried to give a yellow powder (0.058g.). This, after two successive recrystallisations from aqueous ethanol (80%), gave macusine B picrate (0.030g.) as slender needles, m.p. 224-226° (Found C,57.8; H,5.15; N,12.7 $C_{26}H_{27}N_5O_8$ requires C,58.1, H,5.1, N,12.7%).

The equivalent weight of the picrate was determined spectrophotometrically[128]. The picrate (1.548mg.) was dissolved in N/10 sodium hydroxide solution (50 ml.) and the extinction measured at wavelength 355mμ on a Hilger ultra-violet spectrophotometer. The equivalent weight was calculated from the expression,

$$\text{Equivalent weight} = \frac{1.445 \times 10^4 \times 10 \times \%\text{strength}}{\text{Extinction}}$$

(where 1.445×10^4 is the ε_{355} for the picrate ion in the Hilger spectrophotometer)

The equivalent weight of macusine B picrate was found to be 539.4 (molecular weight for $C_{26}H_{27}N_8O_8$ = 537.5).

Macusine B chloride.

Macusine B nitrate (0.04g.) in deionised water (10 ml.) was added to a column (8x1.5 cm.) of Deacidite FF, in the chloride form, and the column washed with water (60 ml.) until the effluent became negative to Meyer's reagent. The clear colourless solution was taken to dryness *in vacuo* at 55°C to give a colourless solid (0.032g.). This was crystallised from ethanol-ether to give colourless prisms (0.025g.), m.p. 247-249° (decomp., after darkening from 225°) (Lit.[122] m.p. 248-249° (decomp., after darkening from 230°), $[\alpha]_D^{20}$ + 17.3° (c., 0.15 in water) (Lit[122], + 15.6±1.7° in water). A few crystals dissolved in concentrated nitric acid gave an immediate green colour which quickly became greenish-yellow[122]. A small amount of the compound moistened with concentrated sulphuric acid gave a pale grey colour on the addition of ceric sulphate solution[122]. A few crystals were dissolved in concentrated sulphuric acid and a crystal of anhydrous ferric chloride was added[122]; a blue colour graduallydeepeningwas obtained. No colour was given by the compound with solutions of *p*-dimethylaminobenzaldehyde[140].

NORMACUSINE B

Conversion of macusine B thioxyanate to normacusine B

Macusine B thiocyanate (0.75g.) was refluxed with ethanolamine (10 ml.) at 170-175° for 45 minutes. After cooling the reaction mixture was treated with 1.5g. of potassium hydroxide in water (25 ml.) and extracted to completion with ether (15x50 ml.). The combined ether extract was washed with water, dried over sodium sulphate and then evaporated to give a fawn coloured solid (0.59g.) which crystallised readily from acetone as colourless needles (0.237g.), m.p. 271-273°, $[\alpha]_D^{22.5}$ + 33.1° (C, 0.98 in methanol); $\lambda_{max.}^{EtOH}$ 227mμ(log ε 4.49), 281(3.98), 290(3.88), $\lambda_{min.}^{EtOH}$ 246(3.47) and 288(3.87) (Found: C, 77.5; H, 7.5, N, 9.3. Calculated for $C_{19}H_{22}N_2O$; C, 77.5, H, 7.5; N, 9.5%. The equivalent weight of normacusine B was determined by non-aqueous titration[164]. The dried, material (approx. 10mg., accurately weighed) was dissolved in glacial acetic acid and titrated with N/50 acetous perchloric acid using oracet blue B as indicator. The equivalent weight was found to be 298.1 (calculated molecular weight for $C_{19}H_{22}N_2O$ = 294.3).

From the mother liquor a second batch of crystals (0.163g.) was obtained. This consisted of two types of crystals, needles, m.p. 271-273° and prisms, m.p. 243-245°, the

former type being most abundant. (In another experiment, normacusine B, obtained as above, crystallised from methanol as prisms, m.p. 243-245°). The common identity of these two types of crystals was established by comparison of their infrared spectra and also be their similar behaviour on thin layer chromatograms.

Using a Camag applicator, glass plates (20 x 10 cm: were spread with a suspension of aluminium oxide G for thin layer chromatography (Merk, according to Stahl, 40g. in 100 ml. water) to give a layer 0.30 mm. deep. The plates were dried at 120° for 4 hours and used immediately after cooling or, alternatively, stored in a dessicated atmosphere for up to 3 days until required for use. The compounds to be tested were applied in methanol or acetone solution using a fine capillary tube and the chromatograms developed with chloroform/ethanol (9:1) to give a 10 cm. run. After drying, the plates were sprayed with modified Dragendorff's reagent to detect the alkaloids.

The mother liquor remaining after removal of normacusine B became deep brown in colour. This solution when tested on thin layer chromatograms, as before, gave four spots of R_S values 0.14, 0.74, 1.0 and 1.2 (S = normacusine B, R_s = 1). These compounds were present in trace amounts only.

Normacusine B acetate.

Normacusine B (0.18g.), acetic anhydride (3 ml.) and pyridine (6 ml.) were kept at room temperature for 60 hours with occasional shaking. The clear solution was concentrated, as far as possible, under reduced pressure at 60° to give a syrup (0.278g.) which was recrystallised twice from ether to yield normacusine B acetate (0.113g.) as colourless needles, m.p. 221-223°, $[\alpha]_D^{25}$ + 11.8° (c, 0.5 in methanol) (Found: C, 74.8; H, 7.2; N, 8.5. Cal. for $C_{24}H_{34}N_2O_8$; C, 74.9; H, 7.2; N, 8.3%).

From the mother liquor another 0.035g. was recovered after two recrystallisations.

Normacusine B hydrochloride.

Normacusine B (0.026g.) in ethanol (1 ml.) was treated with ethanolic hydrochloric acid (0.08 ml., 1.33N) and the resulting hydrochloride precipitated with ether. The colourless precipitate was washed twice with ether in a centrifuge tube to leave normacusine B hydrochloride (0.025g.) which could not be crystallised.

Normacusine B picrate.

Amorphous normacusine B chloride (0.025g.) was dissolved in water (1 ml.) and to the solution a saturated solution of picric acid was added until no more precipitate was obtained. The precipitate was filtered, washed with water to give normacusine B picrate (0.033g.) which, after two recrystallisation

from ethanol, gave yellow prisms (0.021g.), m.p. 239-245° (decomp.). (Found C,57.2; H,4.7; N,13.15; Equiv. wt., 531.3 (spectrophotometric, see page 116). $C_{28}H_{28}N_8O_9$ requires C,57.35, H,4.8, N,13.4%. Mol. wt., 523.5).

ATTEMPTED SEPARATION OF OTHER QUATERNARY BASES

Precipitation of reineckates from the residual solution after separation of thiocyanates.

The combined aqueous filtrate and washings from A and B (about 450 ml.) was acidified to congo-red with dilute hydrochloric acid and a small amount of a non-alkaloidal gummy precipitate was removed by filtration and discarded. The clear filtrate was treated with an excess of saturated aqueous solution of ammonium reineckate, stirring constantly. After allowing to stand for 24 hours, the precipitate was collected, thoroughly washed with water and then dried in a vacuum desiccator to give the crude alkaloid reineckate as a pink solid (12.76g.)

Conversion of reineckates to chlorides.

The crude mixed reineckate (12.7g.) obtained as above, was dissolved in dry acetone (240 ml.). A small amount of dirty, gummy material was filtered off and discarded. The clear acetone solution was titrated with silver sulphate solution (0.5%) until no further precipitation of silver reineckate occurred. An amount of barium chloride solution equivalent to the silver sulphate used was then added, the solution filtered, and the precipitates washed several times with aqueous acetone (25%). The combined filtrate and washings were taken to dryness under reduced pressure at 55°.

The brown residue obtained was dissolved in warm dry methanol (15 ml.) and filtered free from a small amount of barium chloride. The filtrate was evaporated to dryness to give a pale-brown, crude mixed-alkaloid chloride (6.34 g.) (chloride A).

Preparation of chloride from the whole reineckate obtained from an acid extract of the crude material.

The whole reineckate (7 g.) (page 107) was dissolved in dry acetone (140 ml.) and filtered to remove a small amount of insoluble dirty material. The clear solution was converted to chloride by the method described above to give the mixed chloride (3.92 g.) as a dark brown solid (chloride B).

Preparation of chloride from a reineckate fraction purified on alumina.

The reineckate (4 g.), obtained from eluant-fraction A removed from an alumina column (page 58 , that is the fraction which had yielded mascusine B thiocyanate), was dissolved in dry acetone (80 ml.) and converted to chloride, as above, to give a pale-brown solid with a faint green tinge (chloride C, 1.8 g.).

Paper chromatography of crude chlorides.

Paper chromatographic separations of the crude mixed chlorides A and B, applied in methanol, were attempted on 57 cm. Whatman No. 1 papersusing both descending and ascending techniques with the following solvents:-[101]

1. Ethyl acetate/pyridine/water (7.5:2.3:1.65),
2. Water-saturated ethyl methyl ketone with 1-3% methanol,
3. Ethyl methyl ketone/water/cellosolve (300:70:15),
4. Ethyl acetate/acetic acid/water (7.5:0.9:0.9),
5. Ethyl acetate/pyridine/water (200:90:200).

Running times were such as to give approximately 46cm. development. After development the papers were dried and sprayed with modified Dragendorff's reagent.

Purification of crude chloride A.

The crude chloride mixture (6.24g.) was dissolved in ethanol (110 ml.). Some light brown non-alkaloidal material remained undissolved and was filtered off. The clear solution was adsorbed on an acid-washed alumina column (B.D.H., 60g., 22 x 2 cm.). Elution from the column was started with ethanol and continued until the eluant was only faintly positive to alkaloidal reagents; further elution continued with methanol, and finally with 50% aqueous

methanol. Fractions were taken to dryness under reduced pressure at 55°.

The fractions were tested on paper with solvents 1 and 2 above and, similarly, with a solvent system(No.6) consisting of acetone with 8% water[1a1]. In addition, the solvent system (No. 7) ethyl acetate/acetone/water (50:45:17) [1a1] was used with upward development for 3 hours on Whatman No. 1 paper and the results are given in Table 9 (page 72); the type of separation achieved with this solvent is illustrated in Figure 10.

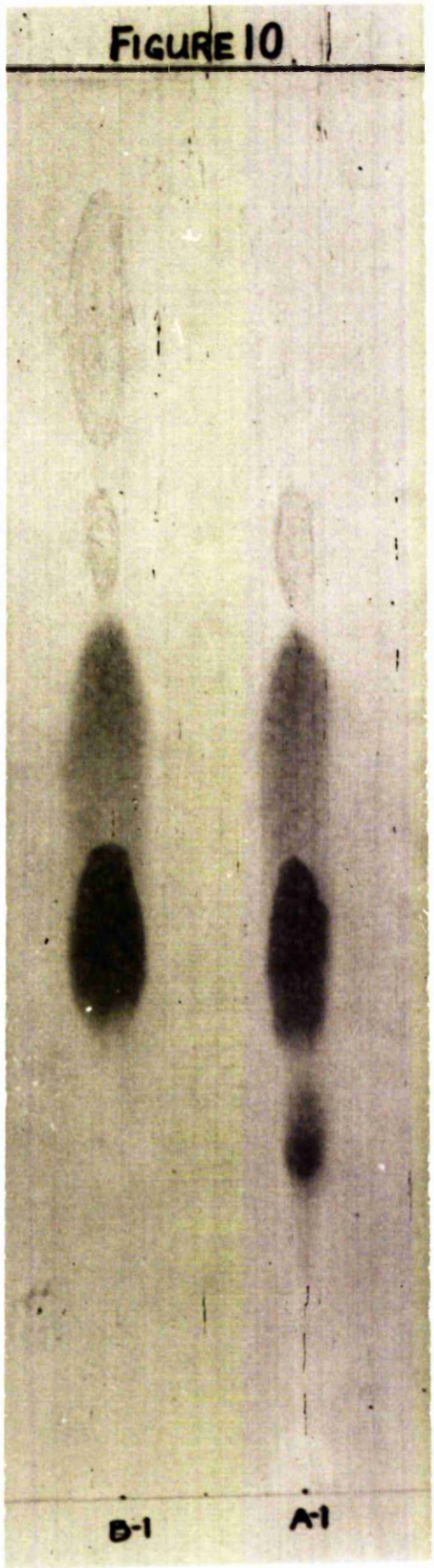
Thin-layer chromatography of mixed chloride (A-1).

Using a technique similar to that described for chromatography of normacusine B (page 119) a solution of the residue from fraction A-1, in methanol, was examined on alumina plates, previously heated to 300° for 3 hours, using acetone/ethyl acetate/ethanol (1:1:1) as developing solvent. After drying, the plates were sprayed with modified Dragendorff's reagent.

Purification of crude chloride B.

The whole chloride (2 g.) was dissolved in ethanol (30ml.) and a small amount of dirty, insoluble material filtered off. The clear solution was passed through an acid-washed alumina column (30 g., 11 x 2 cm.) eluting first with

FIGURE 10



ethanol, then methanol and finally aqueous methanol.

The fractions (Table 10, page 73) were taken to dryness under reduced pressure and then tested by paper chromatography using solvent system 7. The results are given in Table 11.

Attempted fractionation of residue B-1 on alumina.

Residue B-1 (0.2 g.) was dissolved in ethanol (3 ml.) and chromatographed on a column of acid-washed alumina (15 g., 9.5 x 1.5 cm.). The column was developed with ethanol and 12 positive fractions, each of 5 ml., were collected. Each fraction was tested by paper chromatography, as before (solvent 7), and found to contain the same components as the starting material (B-1).

Paper chromatography of mixed chloride C.

The sample C was chromatographed directly on paper using the above technique (solvent 7) and the results indicated the presence of three compounds with R_f values 0.35 (very strong), 0.54 (strong) and 0.65 (weak).

Partition chromatography of mixed chlorides on cellulose columns.

(i) Cellulose powder.

A column (18 x 2 cm.) of cellulose powder (25 g.), packed dry, was washed first with a solution of 8-hydroxyquinoline dissolved in the solvent water-saturated ethyl methyl ketone with 2% methanol, then with the pure solvent, initially until free from 8-hydroxyquinoline and finally for a further 18 hours.[120] A solution of the mixed chloride C (0.1 g.) in methanol (1.5 ml.) was mixed with powdered cellulose (1 g.) and the mixture was dried in a vacuum desiccator then equilibrated with the vapour of the solvent overnight. The impregnated powder was added to the prepared column and developed with the above solvent at a flow rate of 1 ml./3 minutes. Positive fractions (17 x 5 ml.) were collected, concentrated, then run on paper chromatograms in the above solvent and also in ethyl acetate/acetone/water (solvent 7). The first five positive fractions (containing only a trace of the front-moving component) were combined to give a brown residue (2.5 mg.) which was not examined. The remaining fractions gave chromatograms identical with those of the starting material.

A similar experiment was carried out using the solvent system 8% water in acetone on a column (375 g., 30 x 4 cm.) packed wet in acetone, washed with a solution of 8-hydroxyquinoline in the acetone/water solvent[121] and then with the

solvent for 10 hours. The chloride mixture C(0.5 g.) was applied to the column in about 15 ml. of solvent and development proceeded at a flow rate of 2 ml. per minute. Positive fractions (45 x 50 ml.) were collected and tested by paper chromatography as above. The first ten positive fractions, when combined, gave a small amount (6 mg.) of a dark brown residue containing the front-moving component. The later fractions all gave chromatograms identical with those of the original mixture.

(ii) Paper roll chromatography.

A ChroMax paper column (930 g., 75 x 5.8 cm.) was inserted in a pressure mantle according to the instructions given in the Operation Manual (LKB), polythene plugs being used for both inlet and outlet. The apparatus was assembled in a constant temperature room and the solvent, ethyl acetate/acetone/water (50:45:17), was delivered from a 2 l. Mariotte flask. An initial experiment, passing methyl red (10 mg. in 10 ml. solvent) through the column, was conducted to determine the correct operating pressure for the mantle.

Mixed chloride A-1 (1.5 g.), dissolved in the solvent (30 ml.), was applied to the column and development proceeded to give continous elutions at a rate of 12-14 ml. per hour. After discarding 650 ml. of non-alkaloidal eluant, 712 positive fractions were collected at hourly intervals.

An aliquot portion (1-2ml.) of every 10th fraction was

An aliquot portion (1-2ml.) of every 10th fraction was

taken to dryness and the residue, in methanol, run on paper chromatograms using the above solvent.

On the basis of paper chromatography results, fractions were bulked, concentrated to remove the organic solvents and then shaken with a volume of petroleum ether (b.p. 40-60°) equal to that of the aqueous concentrate. The clear aqueous layer was separated and taken to dryness under reduced pressure and the residues weighed (Table 12). The residues from the combined fractions and the original mixture were chromatographed together on paper; results are given in Table 13.

Attempted fractional crystallisation of quaternary chlorides.

Attempts were made to crystallise the residues (c) and (g) successively from ethanol/ethyl acetate, ethanol/ether, methanol/ethyl acetate, methanol/ether.

Conversion of mixed chlorides to iodide.

Residue (c) (0.05 g.) was dissolved in water (0.5 ml.) and excess saturated solution of potassium iodide was added. The precipitate was washed with water and dried to give a pale brown residue. Crystallisation from ethanol/ether gave a small amount of brown crystalline material (2-3 mg.). The infrared spectrum was determined.

Residue (g) (0.03 g.) was treated with potassium iodide solution as above. The gummy precipitate could not be crystallised.

Conversion of mixed chlorides to picrates.

Residue (c) (0.05 g.) in water (0.5 ml.) was treated with excess aqueous solution of picric acid. The precipitate was washed with water and dried to give a yellow solid (0.055 g.) which could not be crystallised from ethanol, aqueous ethanol, aqueous acetone, or ethanol/ether.

The above experiment was repeated with residue (g) (0.025 g.); no crystalline picrate was obtained.

Counter-current distribution of mixed chlorides.

The partition coefficients of the mixed chloride B were found by s aking 20 mg. quantities in 2 ml. each of the corresponding organic and aqueous phases from the following solvent mixtures:-

1. Ethyl methyl ketone/water (15:10),
2. Ethyl methyl ketone/ethylacetate/water (75:5:54),
3. n-Butanol/water (1:1).

Craig apparatus.

The mixed chloride B (500 mg.) was dissolved in 25 ml. of the aqueous phase from solvent 1 and subjected to counter-current separation using this solvent system in a Craig machine giving a shaking period of 2 minutes and a settling

time, initially, of 15 minutes. The settling time was extended, eventually, up to 5 hours.

Steady-state machine.

Mixed chloride B-1 (800 mg.) was divided amongst the three centre tubes of the machine, filled with solvent 3 above, and distribution started using an initial programme of 1:1 upper/lower. The distribution of compounds in the tubes was checked at intervals by thin-layer chromatography (as below) and the programme adjusted according to the results of these analyses.

A shaking period of 2 minutes and a settling time of 15 minutes were used. At the end of 330 transfers, the total effluents (upper and lower phase) were separately concentrated in a rotary evaporator at 50°. At intervals, thereafter, further batches of the effluents were separately concentrated until a total of 448 transfers had been made. The two-phase liquid remaining in each tube, after the final transfer, was evaporated and each concentrate kept separate.

Thin-layer chromatography of steady-state solvent fractions.

Effluent concentrates and tube concentrates were examined by thin-layer chromatography using the following technique.

Plates were spread with a 0.02 in. thick layer of Kieselgel G, dried, then activated at 105° for 30 minutes. The solvent, n-butanol saturated with water, was allowed

to ascend 15 cm., the plates dried and then sprayed with a 1% solution of ceric ammonium nitrate in 10% sulphuric acid. Results are shown on page 87 .

(The above work relating to steady-state distribution was performed by Messrs. Quickfit and Quartz Ltd. at Abingdon. Berkshire.)

Paper chromatography of steady-state solvent fractions.

All the concentrates were taken to dryness under reduced pressure and the residues weighed (Table 14).

Selected residues from both effluent and tube fractions were re-dissolved in small volumes of methanol and chromatographed on paper as previously described for the initial mixture (page126) using the ethyl acetate/acetone/water solvent. Results are given in Table 15.

Examination of a single-compound fraction from the steady-state machine.

The residues (total 37.1 mg.) from the concentrates plus-21-25 to U 369-388 were dissolved in methanol and the solution mixed then concentrated under reduced pressure. The precipitate was filtered off and washed and the filtrate tested for alkaloids. Attempts were made to crystallise an alkaloid chloride from the filtrate.

CONVERSION OF MIXED QUATERNARY CHLORIDES TO TERTIARY BASES

N-Demethylation of quaternary compounds in chloride (C).

The mixed chloride C(1 g.) was refluxed with ethanolamine (15 ml.) at 170-175° for 45 minutes. The reaction mixture, after cooling, was treated with potassium hydroxide (2 g.) in water (30 ml.) and the solution extracted with chloroform (10 x 100 ml.) The combined chloroform extract was washed with water, dried over sodium sulphate and evaporated to give an orange-yellow solid (0.76 g.)

Adsorption chromatography of the tertiary base mixture.

The residue above, dissolved in chloroform (50 ml.) was added to an alumina column and developed successively with chloroform, 10% ethanol in chloroform, and ethanol. Fractions (77 x 25 ml.) were collected and suitably combined after testing on chromatoplates using the technique described on page 119. Details of the combined fractions (1-7) are summarised in Table 16.

The combined fractions were tested on chromatoplates using normacusine B as a reference compound and R_S values were calculated. The results are given in Table 17.

Crystallisation of normacusine B.

The residue from fraction 1 was crystallised from acetone to yield colourless crystals (0.12 g.) which were identified as normacusine B (see page 118).

Crystallisation of ethanolamide T3A.

(i) The crystalline deposit (m.p. 245-247°) obtained

on concentration of fraction 4 from the alumina column, was separated and recrystallised from acetone to give colourless needles (0.084 g.), m.p. 247-249°, unchanged after further recrystallisation, $[\alpha]_D^{21}$ + 39° (c., 0.20 in methanol); λ_{max}^{EtOH} 226 mμ (log ε 4.53), 284(3.88), 293(3.79), $\lambda_{min.}^{EtOH}$ 250(3.44) and 290(3.78) (Found: C,71.5; H,7.09; N,11.95. $C_{21}H_{28}N_2O_2$ requires C,71.76; H,7.17; N,11.95%. The mother liquor, combined with the residue (0.097 g.) from fraction 4, yielded the same compound (0.08 g.). The infrared spectrum for each sample was determined (Figure 3).

(ii) The residue from fraction 3, crystallised twice from acetone yielded colourless crystals (0.02 g.), m.p. 247-249°. The infrared spectrum was determined.

Conversion of the ethanolamide (T3A) to the corresponding methyl ester (T3).

The ethanolamide (0.1 g.) was dissolved in dry methanol (5 ml.) and refluxed in a constant supply of dry hydrochloric acid for 4 hours. The reaction mixture was taken to dryness, dissolved in water (10 ml.) and basified with solution of ammonia. The basified solution was extracted with chloroform (5 x 20 ml.), and the extract washed with water then taken to dryness to give a colourless glassy residue (0.09 g.). The residue was crystallied from a aqueous ethanol to give

colourless needles (0.052 g.), m.p. 227-230° (decomp.). Two further recrystallisations from aqueous ethanol gave needles (0.029 g.), m.p. 232-234° (decomp.), $[\alpha]_D^{23}$ + 37.7° (c., 0.13 in methanol); $\lambda_{max.}^{EtOH}$ 225 mμ (log ε 4.46), 283(3.86), 291(3.79), $\lambda_{min.}^{EtOH}$ 248(3.42) and 289(3.78) (ε values were calculated using a molecular weight derived from $C_{30}H_{38}N_2O_2$ which would be the molecular formula for the methyl ester corresponding to the ethanolamide $C_{31}H_{38}N_2O_2$). From the mother liquor another 0.02 g. was recovered.

The crystals were checked for homogeneity on chromatoplates and the infrared spectrum determined (Figure 5).

Quaternisation of tertiary base T3

The base T3 (0.015 g.) in dry methanol was heated with excess of methyl chloride in a sealed metal bomb for 4 hours. The reaction mixture was taken to dryness and the brown sticky residue was triturated with chloroform (5 x 3 ml.) to remove any traces of unchanged tertiary base. The chloroform solution was taken to dryness to give a small amount of pale-brown residue. Both the chloroform-soluble and chloroform-insoluble (water-soluble) residues were dissolved in methanol and run on paper chromatograms together with the tertiary base (T3) and chloride mixture (C) from which the ethanolamide (T3A) was made; subsequently also with macusine B chloride, in both weak and strong solutions.

ISOLATION OF A MINOR QUATERNARY ALKALOID (Q2)

Adsorption chromatography of methanol-soluble quaternary thiocyanates.

The crude mixed thiocyanates TR (6.0 g.) (residue remaining after removal of solvent from the methanol and aqueous-methanol washings of the mixed thiocyanates from which macusine B thiocyanate was obtained, page 114) were dissolved in methanol (20 ml.) and passed through a column of acid-washed alumina (B.D.H., 60 g., 13.5 x 2.5 cm.). Development and elution was continued with methanol until the eluant became alkaloid-negative. Three fractions were collected and taken to dryness; results are given below.

Fraction	Eluant		Residues	
	Volume ml.	Colour	Weight (g.)	Remarks
TR-1	100	pale yellow	4.0	pale yellow solid
TR-2	130	deep yellow	0.27	grey-brown solid
TR-3	500	brownish yellow	0.24	grey-brown solid

The residue were run on paper chromatograms using the ethyl acetate/acetone/ water solvent. Subsequently residue TR-1 was run alongside a sample of the initial thiocyanate residue (TR) and a mixed chloride (A-1).

R_f values and relative intensities obtained are shown below.

Fraction	R_f values and intensity of spot on paper					
	0.16	0.23–0.25	0.34 0.36	0.50 0.52	0.63 0.65	0.76
TR-1			+++++	++++		++++
TR-2			+	++	+	
TR-3			+	++	+	
TR						+++++
Chloride (A-1)	+	++	+++++	++++	+	+

The residue TR-1 dissolved in water, gave a pale reddish colour with ferric chloride solution and a heavy precipitate with silver nitrate after heating with sodium nitrite and nitric acid to remove thiocyanate.

The small, deeply coloured fractions TR-2 and TR-3 were not examined.

Preparation of quaternary iodide

The residue from fraction TR-1 (0.75 g.) was dissolved in water (1 ml.) and treated with an excess of saturated aqueous solution of potassium iodide. The precipitate was

washed with water, dried, and crystallised from ethanol to give buff coloured prisms (0.065 g.), m.p. 242-245° (decomp.), then recrystallised twice from methanol/ether to give cream coloured prisms (0.046 g.), m.p. 248-250° (decomp.), $[\alpha]_D^{22.5}$ -432° (C., 0.25 in methanol), $\lambda_{max.}^{EtOH}$ 222 mμ (log ε 4.31), 295(4.04), 330(413), $\lambda_{min.}^{EtOH}$ 261(3.03) and 308(3.93). The infrared spectrum was determined (Figure 8) and the equivalent weight (469.7) found by Volhard titration. Analysis gave C,52.77; H,5.84; N,5.86; I,26.92%.

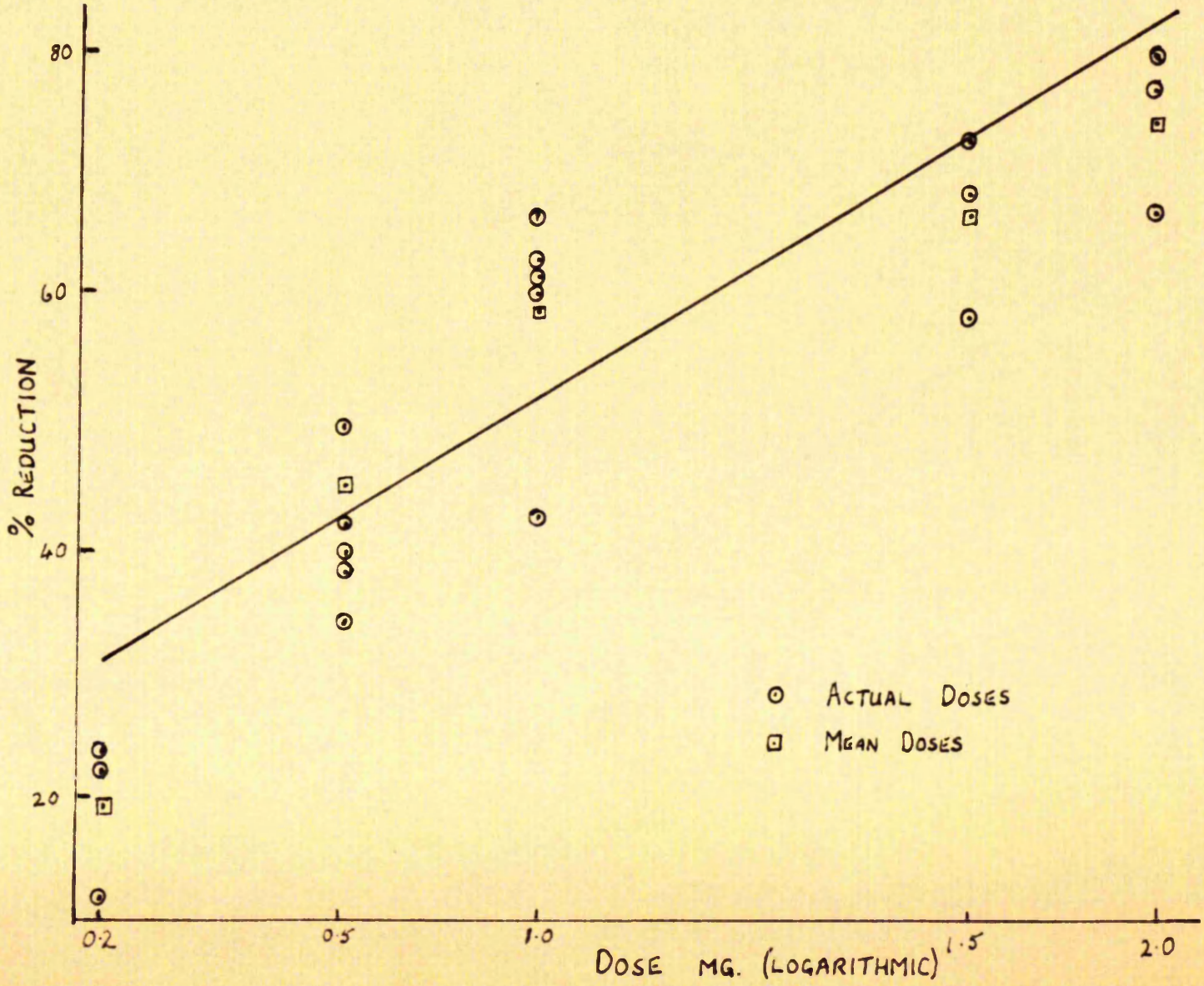
The ultraviolet spectrum of potassium iodide (0.0012% in ethanol) was determined.

PRELIMINARY PHARMACOLOGOCAL INVESTIGATION OF MACUSINE B

The isolated frog rectus abdominis preparation was made and its response to a 10 γg. dose of acetylcholine was measured. Macusine B nitrate, dissolved in frog Ringer solution was applied and after two minutes the response to a 10 γg. dose of acetylcholine was measured.

The reduction of response due to macusine B nitrate using doses ranging from 0.2 to 2.0 mg., was measured on 18 preparations and the results are shown in Figure 11.

FIGURE 11


80
60
40
20
% REDUCTION
0·2
0·5
1·0
1·5
2·0
DOSE MG. (LOGARITHMIC)
ACTUAL DOSES
MEAN DOSES

REFERENCES

REFERENCES

1. Trease, A Textbook of Pharmacognosy, Bailliere Tindall and Cox, London, 8th ed., 1960, p. 460.
2. Pichon, Bull. Mus. Hist. Nat. Paris, 1947, ser. 2, 19, 362.
3. Woodson, Jr., Ann. Missouri Bot. Gard., 1951, 38, 119.
4. Ducke, Anais Acad. brasil. Ciene., 1955, 27, 381.
5. Standely, Tropical Woods, 1933, No. 36, 13.
6. Bisset, Ann. bogor., 1958, 2, 105.
7. Br. Guiana Forest Bull., 1948, No. 2 (n.s.), 39.
8. Dominguez, Rev. farm. (Buenos Aires), 1932, 73, 82. (per Chem. Abs., 1932, 26, 4102).
9. Floriani, ibid., 1938, 80, 135. (per Chem. Abs., 1938, 32, 9394).
10. Schmutz, Pharm. Acta Helv., 1961, 36, 103.
11. Schmutz and Lehmer, Helv. Chim. Acta, 1959, 42, 674.
12. Orazi, Corral, Holker and Djarassi, J. Org. Chem., 1956, 21, 979.
13. Penzoldt, Berl. klin. Wschr., 1879, 60, 268, quoted by Schmutz, Pharm. Acta Helv., 1961, 36, 103.
14. Wood, Jr., Univ. Penn. Med. Bull., 1910, 23, 1. (per Chem. Abs., 1910, 4, 2151).
15. Cow, J. Pharmacol., 1913-14, 5, 341. (per Chem. Abs., 1914, 8, 1617).
16. Sollmann, A. Manual of Pharmacology, W.B. Saunders Company, London, 7th ed., 1948, p. 494

17. Floriania, Rev. farm. (Buenos Aires), 1930, 72, 70. (per Chem. Abs., 1930, 24, 3279).

18. Floriani, Rev. centro. estud. farm. bioquim., 1935, 25, 373, (per Chem. Abs. 1936, 30, 1415).

19. Floriani, Anales farm. bioquim., (Buenos Aries). 1931, 2, 215. (per Chem. Abs., 1932, 26, 2541).

20. Raymond-Hamet, Compt. rend., 1930, 191, 157.

21. Banerjee and Lewis, Nature, 1953, 171, 802.

22. Idem., J. Pharm. Pharmacol., 1954, 6, 246.

23. Idem., ibid., 1954, 6, 660.

24. Idem., ibid., 1954, 6, 466.

25. Idem., ibid., 1955, 7, 42.

26. Raymond-Hamet, Compt. rend. Soc. Biol., 1956, 150, 967.

27. Idem., ibid., 1957, 151, 74.

28. Malone and Roth, J. Pharm. Sci., 1962, 51, 345.

29. Becker, Iowa State Coll. J. Sci., 1949, 23, 189.

30. Palmer, M. Pharm, thesis University of Nottingham, 1954.

31. Schmutz, Hunziker and Hirt, Helv. Chim. Acta, 1957, 40, 1189.

32. Orazi, Corral, Holker and Djarassi, Anales Soc. quim. argentina, 1956, 44, 177, (per Chem. Abs., 1957, 51, 13313).

33. Fraude, Ber., 1878, 11, 2189.

34. Hesse, Annalen, 1882, 211, 249.

35. Rothlin, Trab. Inst. Bot. Farmacol., 1918, No. 38, quoted by Bisset, Ann. begor., 1958, 1, 105.

36. Ewins, J. Chem. Soc., 1914, 2738.

37. Field, Ibid., 1924, 1444.

38. Orazi, Anales Asoc. quim. argentina, 1946, 34, 158. (per Chem. Abs., 1948, 42, 2326).

39. Henry, The Plant Alkaloids, J. and A. Churchill Ltd., London, 4th ed., 1949, p. 511.

40. Marion, in Manske and Holmes, The Alkaloids, Academic Press Inc., New York. 1952, Vol. II, p. 422.

41. Fish, Qaisuddin and Stenlake, Chem. and Ind., 1964, 319.

42. Saxton, in Manske, The Alkaloids, Academic Press, London, 1960, Vol. VII, pp. 4-199.

43. Finch, Taylor and Ulshafer, Experientia, 1963, 19, 296.

44. Boit, Ergebnisse der Alkaloid-Chemie bis 1960, Akademie-Verlag, Berlin, 1961, pp. 499-557.

45. Bartlett and Taylor, J. Amer. Chem. Soc., 1960, 82, 5941.

46. Schnoes, Burlingame and Biemann, Tetrahedron Letters, 1962, No. 22, 993.

47. Withop and Patrick, J. Amer. Chem. Soc., 1954, 76, 5603.

48. Djerassi, Gilbert, Shoolery, Johnson and Biemann, Experientia, 1961, 17, 162.

49. McLean, Palmer and Marion, Canad. J. Chem., 1960, 38, 1547.

50. Biemann, Friedmann Spiteller and Spiteller, Tetrahedron Letters, 1961, No. 14, 485.

51. Vamvacas, von Philipsborn, Schlittler, Schmid and Karrer, Helv. Chim. Acta, 1957, 40, 1793.

52. Spitellerand Spiteller-Friedmann, Monatsh., 1963, 94, 779.

53. Biemann, Spiteller-Friedmann and Spiteller, J. Amer. Chem. Soc., 1963, 85, 631.

54. Djerassi, Nakagawa, Wilson, Budziklewicz, Gilbert and Antonaccio, Experientia, 1963, 19, 467.

55. Pinar and Schmid, Annalen, 1963, 668, 97.

56. Gilbert, Ferreira, Owellen, Swanholm, Budziklewicz, Durham, Djerassi, Tetrahedron Letters, 1962, No. 2, 59.

57. Djerassi, Antonaccio, Budziklewicz, Wilson and Gilbert, ibid.,1962, No. 22, 1001.

58. Woodward, Angew. Chem., 1956, 68, 13.

59. Djerassi, Budziklewicz, Owellen, Wilson, Kump, Le Count, Battersby and Schmid, Helv. Chim. Acta.,1963, 46, 742.

60. Burnell, Medina and Ayer, Chem. and Ind., 1964, 33.

61. Gorman, Burlingame and Biemann, Tetrahedron Letters, 1963, No. 1, 39.

62. Antonaccio and Budziklewicz, Monatsh., 1962, 93, 962.

63. Tunmann and Rachor, Naturwiss., 1960, 47, 471.

64. Van Tamelen, Aldrich and Katz, J. Amer. Chem. Soc., 1957, 79, 6426.

65. Gilbert, Antonaccio and Djarassi, J. Org. Chem., 1962, 27, 4702.

66. Dastoor and Schmid, Experientia, 1963, 19, 297.

67. Ferreira, Gilbert, Owellen and Djarassi, ibid., 1963, 19, 585.

68. Biemann and Spiteller, Tetrahedron Letters, 1961, No. 9, 299.

69. Poisson, Le Men and Janot, Bull. Soc. Chim. France, 1957, 610.

70. Antonaccio, Pereira, Gilbert, Vorbrueggen, Budzikiewicz, Wilson, Durham and Djerassi, J. Amer. Chem. Soc., 1962, 84, 2161.

71. Neuss, Physical Data of Indole and Dihydroindole Alkaloids, Lilly Research Laboratories, Indianapolis 6, Indiana, U.S.A. 1962 - 1963, Vol. II.

72. Arnold, Berlage, Bernauer, Schmid and Karrer, Helv. Chim. Acta, 1958, 41, 1585.

73. Battersby and Yeowell, Proc. Chem. Soc., 1961, 17.

74. Ondetti and Deulofeu, Tetrahedron, 1961, 15, 160.

75. Gilbert, Brissolese, Finch, Taylor, Budzikiewicz, Wilson, Djerassi, J. Amer. Chem. Soc., 1963, 85, 1523.

76. Peckolt, Ber. deut. pharm. Ges., 1909, 19, 529.

77. Antonaccio, Rev. quim. ind. (Rio de Janeiro), 1957, 26, 149, (per Chem. Abs., 1958, 52, 14081).

78. Boit, Ergebnisse der Alkaloid-Chemie bis 1960., Akademie-Verlag, Berlin, 1961, p. 643.

79. Mills and Nyburg, J. Chem. Soc., 1960, 1458.

80. Ferrari, McLean, Marion and Palmer, Canad. J. Chem., 1963, 41, 1531.

81. Gilbert, Brissolese, Wilson, Budzikiewicz, Durham and Djerassi, Chem. and Ind., 1962, 1949.

82. Pinar and Schmid, Helv. Chim. Acta, 1962, 45, 1283.

83. Pinar, von Philipsborn, Vetter and Schmid, ibid., 1962, 45, 2260.

84. Djerassi, Archer, George, Gilbert and Antonaccio, Tetrahedron, 1961, 16, 212.

85. Djerassi, Archer, George, Gilbert, Shoolery and Johnson, Experientia, 1960, 16, 532.

86. Djerassi, Brewer, Budzikiewicz, Orazi and Corral, J. Amer. Chem. Soc., 1962, 84, 3480.

87. Idem., Experientia, 1962, 18, 113.

88. Djerassi, George, Finch, Lodish, Bedzikiewicz, Gilbert, J. Amer. Chem. Soc., 1962, 84, 1499.

89. Antonaccio, J. Org. Chem., 1960, 25, 1262.

90. Djerassi, Owellen, Ferreira and Antonaccio, Experientia, 1962, 18, 397.

91. Neuss and Boaz, J. Org. Chem., 1957, 22, 1001.

92. Buchi, Mayo and Hochstein, Tetrahedron, 1961, 15, 167.

93. Boehm, Abhandl. Kgl. Sächs. Ges. Wissensch., 1895, 22, 201; 1897, 24, 1.

94. King, J. Chem. Soc., 1935, 1381.

95. D'Arcy and Taylor, J. Pharm. Pharmacol., 1962, 14, 129.

96. Shamma and Slusarchyk, Chem. Rev., 1964, 64, 59.

97. Tomita and Kugu, J. Pharm. Soc. Japan, 1959, 79, 317.

98. Boit, Engebnisse der Alkaloid-Chemie bis 1960, Akademie-Verlag, Berlin, 1961, pp. 330 - 346.

99. King, J. Chem. Soc., 1948, 265.

100. Dutcher, Ann. N.Y. Acad. Sci., 1951, 54, 326.

101. Bodendorf and Scheibe, Arch. Pharm., 1954, 287, 555.

102. McIntyre, Curare, Its History, Nature and Clinical Use. University of Chicago Press, Chicago, 1947, pp. 36 - 46.

103. Santos, Arch. Pharm., 1951, 284, 360.

104. Knabe, Chem. Ber., 1958, 91, 1612.

105a. Boit, Ergebnisse der Alkaloid-Chemie bis 1960, Akademie-Verlag, Berlin, 1961, pp. 216 - 229.

105b. Idem., ibid., pp. 361-369.

106. Crow and Hodgkin, Tetrahedron Letters, 1963, No. 2, 85.

107. Ewing, Hughes, Ritchie and Taylor, Austral. J. Chem., 1953, 6, 78.

108. Bernauer, Forschr. Chem. Org. Naturstoffe, 1959, 17, 184.

109. Battersby and Hodson, Quart. Rev., 1960, 14, 75.

110. Hesse, Hiltebrand, Weissmann, von Philipsborn, Bernauer, Schmid and Karrer, Helv. Chim. Acta, 1961, 44, 2211.

111. Nagyvary, Arnold, von Philipsborn, Schmid and Karrer, Tetrahedron, 1961, 14, 138.

112. Fritz, Besch and Wieland, Annalen, 1963, 663, 150.

113. Bernauer, Helv. Chim. Acta, 1963, 46, 197.

114. Battersby, Yeowell and Jackmann, Proc. Chem. Soc., 1961, 413.

115. Casinovi, Ciasca, Lis and Marini-Bettolo, Sci. Repts. Inst. Super Sanita, 1961, 1, 51.
(per Chem Abs., 1962, 56, 8841).

116. Gerrup-Besanez, Annalen, 1875, 176, 88, quoted by Goodson and Henry, J. Chem. Soc., 1925, 1640.

117. Goodson and Henry, J. Chem. Soc., 1925, 1640.

118. Goodson, ibid., 1932, 2626.

119. Sharp, ibid., 1934, 1227.

120. Hamilton, Hamor, Robertson and Sim, ibid., 1962, 5061.

121. Bartlett, Korzun, Sklar, Smith and Taylor, J. Org. Chem., 1963, 28, 1445.

122. Battersby, Binks, Hodson and Yeowell, J. Chem. Soc., 1960, 1848.

123. Buchi, Manning and Hochstein, J. Amer. Chem. Soc., 1962, 84, 3393.

124. Janot, Laine and Goutarel, Ann. pharm. franc., 1960, 18, 673.

125. Calderwood and Fish, Personal Communication.

126. Patel, Ph.D. thesis, London University, 1963.

127. Evans and Partridge, Quart. J. Pharm., 1948, 21, 126.; J. Pharm. Pharmacol., 1949, 1, 593.

128. Chilton and Partridge, J. Pharm. Pharmacol., 1950, 2, 784.

129. Schmid, Kebrle and Karrer, Helv. Chim. Acta, 1952, 35, 1864.

130. Wieland and Merz, Chem. Ber., 1952, 85, 731.

131. Marini-Bettolo and Casinovi, in Lederer, Chromatographic Reviews, Elsevier Publishing Co., London, 1959, Vol. 1, p. 75.

132. Casinovi, ibid., 1962, Vol. 5, p. 161.

133. Raffauf, Personal Communication.

134. German Patent, 1036859/1956.

135. Dutcher, J. Amer. Chem. Soc., 1946, 68, 419.

136. King, J. Chem. Soc., 1949, 3263.

137. Karrer and Schmid, Helv. Chim. Acta, 1946, 29, 1862.

138. Wieland, Konz and Sonderoff, Annalen, 1937, 527, 160.

139. Staufacher, Helv. Chim. Acta, 1961, 44, 2006.

140. Feigl, Spot Tests in Organic Analysis, Elsevier Publishing Co. London, 6th ed., 1960, p.290.

141. Humig and Baron, Chem Ber., 1957, 90, 403.

142. Tomita and Takano, J. Pharm. Soc. Japan, 1960, 80, 301.

143. Levy, Le Men and Janot, Compt. rend., 1961, 253, 131

144. Zurcher, Ceder and Boekelheide, J. Amer. Chem. Soc., 1958, 80, 1500.

145. Hagdahl and Danielson, Nature, 1954, 174, 1962.

146. Lederer and Lederer, Chromatography, Elsevier Publishing Co., London, 2nd ed., 1957, p. 65.

147. Levy, Le Men and Janot, Bull Soc. Chim. France, 1960, 979.

148. Plat, Le Men, Janot, Budzikiewicz, Wilson, Durham and Djerassi, ibid., 1962, 2237.

149. Goutarel, Le Men, Wilson, Budzikiewicz, and Djerassi, ibid., 1962, 1088.

150. Aghoramurthy and Robinson, Tetrahedron, 1957, 1, 172.

151. Chromatography, E. Merck, Darmstadt, Germany, p. 140.

152. Vogel, A Textbook of Quantitative Inorganic Analysis, Longmans, Green and Co., London, 2nd ed., 1951, p. 257.

153. Cunningham, Dawson and Spring, J. Chem Soc. 1951, 2305.

154. Beckett and Stenlake, Practical Pharmaceutical Chemistry, University of London, The Athlone Press, 1962, p. 114.