Quadrigemines-A and -B, Two Minor Alkaloids of *Hodgkinsonia frutescens* F. Muell

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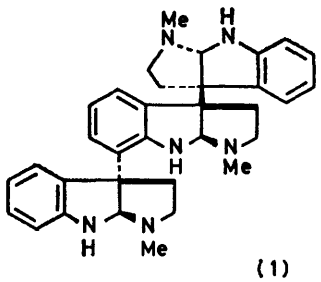
The structures of two minor alkaloids of *Hodgkinsonia frutescens* F. Muell., quadrigemines-A and -B, have been shown by chemical degradation to be (2) (as a mixture of diastereoisomers) and (14) respectively (no stereochemistry). A tentative stereochemical assignment, based mainly on optical rotations, can however be made for the quadrigemine-A mixture, which can be seen as an approximately 1 : 1 mixture of diastereoisomer (5) and one (or a mixture of both) of the *meso*-diastereoisomers. It is noteworthy that these are the first examples of alkaloid structures made up of four tryptamine units.

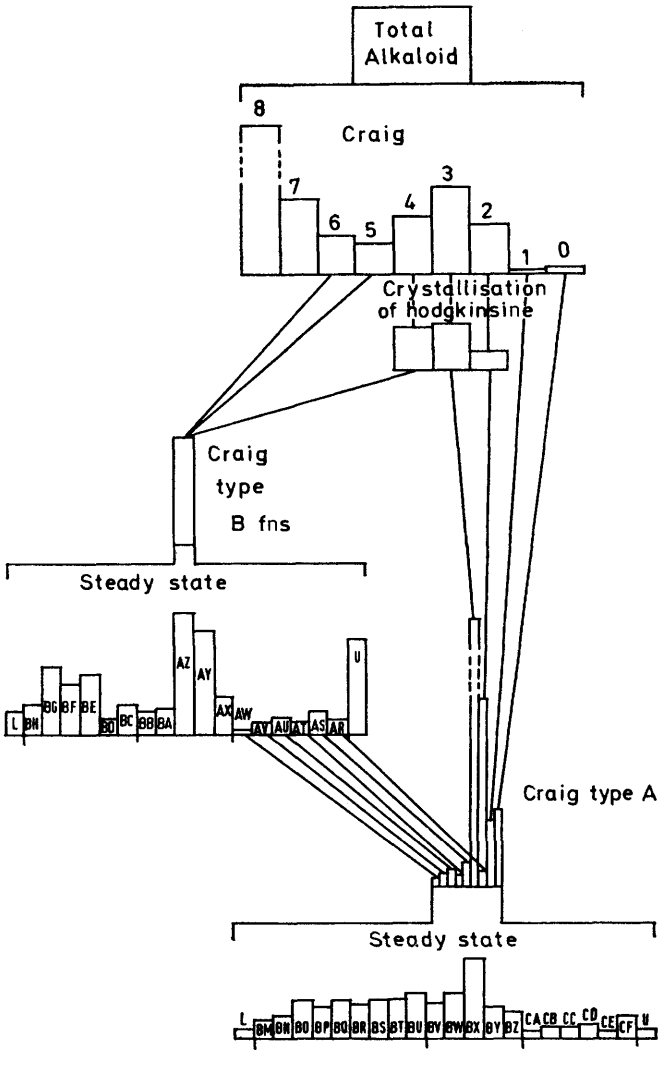
THE leaves of *Hodgkinsonia frutescens* F. Muell. contain a complex mixture of alkaloids, the main crystalline component of which, hodgkinsine, was first isolated in the late 1940s by Anet, Hughes, and Ritchie.[1] Hodgkinsine was shown to have structure (1) (excluding stereochemistry) by chemical degradative studies in these laboratories,[2] and the absolute and relative stereochemistry was determined by *X*-ray crystallographic structure determination.[3]



(1)

The existence of tetrameric bases in the alkaloids of the leaves of *Hodgkinsonia frutescens* F. Muell. was revealed by the presence of a significant peak at *m/e* 690 in the mass spectrum of the mixture of bases obtained after removal by crystallisation of the main alkaloid, hodgkinsine.[4] A long counter-current fractionation, mostly monitored by mass spectrometry, followed by preparative t.l.c., led to the isolation of amorphous quadrigemine-A (18 mg, 0.001% of dry leaf weight) and of crystalline quadrigemine-B (198 mg, 0.01%): the bases are isomeric, and have a molecular formula $C_{44}H_{50}N_8$. The instability of aqueous solutions of salts of alkaloids in this group, even at pH values near to neutrality, precluded the use of a buffer system for the separation, and appropriate aqueous–organic two-phase systems had to be used. The Figure gives an indication of the procedure adopted, and full details are given in the Experimental section. This fractionation led to the isolation of three other unknown bases, which will be described in a subsequent publication.

Structure of Quadrigemine-A.—Quadrigemine-A, though noncrystalline, runs as a well-formed single spot



in six different solvent systems. The peak at highest mass in the mass spectrum is at *m/e* 690.415 6 ($C_{44}H_{50}N_8$;

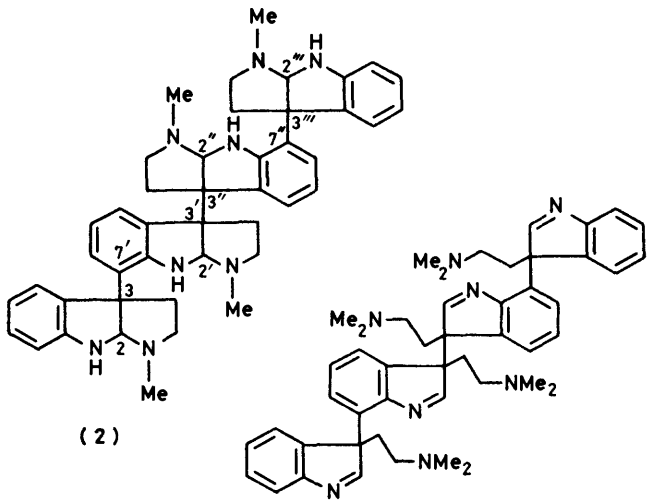
[1] E. F. L. J. Anet, G. K. Hughes, and E. Ritchie, *Austral. J. Chem.*, 1961, **14**, 173; E. F. L. J. Anet, Ph.D. Thesis, Sydney University, 1949.

[2] R. Atitullah, Ph.D. Thesis, Manchester University, 1966; paper in preparation.

[3] J. Fridrichsons, M. F. Mackay, and A. McL. Mathieson, *Tetrahedron Letters*, 1967, 3521; *Tetrahedron*, 1974, **30**, 85.

[4] The work described in this paper was carried out in 1967—1968 (K. P. Parry, Ph.D. Thesis, Manchester University, 1968).

molecular formula confirmed by combustion analysis): that this peak is the actual parent is seen in its increase in intensity from 0.5 to 1.5% on decreasing the electron-beam energy from 70 to 15 eV. Of considerable interest is the great similarity of the mass spectrum between *m/e* 200 and 350 with the corresponding region of the mass spectrum of hodgkinsine; the electronic and vibrational spectra are also very similar to those of hodgkinsine.[2] The n.m.r. spectrum was not very informative, the only recognisable absorptions being those of the benzene region (τ 2.9—3.6) and of the *N*-methyl groups, which form a broad singlet at τ 7.64; the ratio of the integral of the *N*-methyl and aminoethyl side-chain CH_2 region (τ 6.8—8.2) and of that of the benzene region is compatible with the proposed structure. Unfortunately, the C-2 hydrogen (N–CH–N) and the *N*-hydrogen absorptions are complex, probably due to the presence of configurational and conformational isomers: diffuse absorptions at τ 6.0 and 4.4, and partially overlain by the benzene CH region around τ 2.8 and 3.8 disappear on addition of D_2O and must correspond to *N*-hydrogen; a diffuse absorption at around τ 5.14, corresponding to between 2.2 and 2.5 H, sharpens up somewhat on D_2O addition and must represent some of the four C-2 hydrogens. These data point to a hodgkinsine-like structure. Since the peak in the mass spectrum corresponding to a halving of the molecule is both the first fragment and the base peak, the four tryptamine units must be symmetrically disposed, as expressed in structure (2). This structure, with stereochemistry not

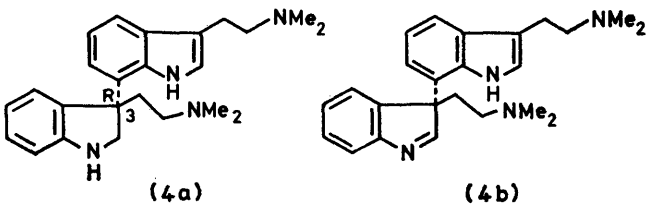


(2)

(3)

defined, was confirmed by the following simple chemical degradation.

Quadrigemine-A reacts rapidly with an excess of methyl iodide to yield an amorphous tetramethiodide which is converted by aqueous NaOH into a tetramethine base (3) (M^+ 746, 1%): the n.m.r. spectrum of this base shows four one-proton singlets at τ 1.32, 1.56, 1.82, and 1.92 attributable to 3*H*-indole 2-hydrogens, and four six-proton singlets at τ 8.00, 8.02, 8.04, and 8.08 corresponding to four NMe_2 groups (see Experimental section for a discussion of these signals). Potassium borohydride reduction of this base yields only one product, a dimeric indolylindoline (73% yield) identical with the indolylindoline (4b) derived from hodgkinsine[2] (by n.m.r.,



(4a) (4b)

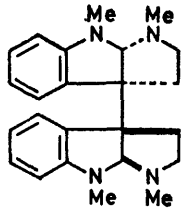
Note—When derived from quadrigemine-A, (4a) is admixed with the racemate; when derived from quadrigemine-B, the stereochemistry is unknown.

mass, and electronic spectroscopy, and by t.l.c. behaviour in six different systems) except for a very appreciable difference in the specific rotation values: the quadrigemine-A-derived product shows $[\alpha]_D^{23}$ +32° (EtOH), which is only about half that observed for the hodgkinsine-derived product (+58°).[2]

Stereochemistry of Quadrigemine-A.—The much reduced optical activity in the indolylindoline derived from quadrigemine-A suggests that the alkaloid is a mixture of diastereoisomers: this follows if one makes the reasonable assumption that the chiral centre in (4a) is not racemisable [the corresponding centre in a possible precursor 3*H*-indolylindole (4b) could conceivably racemise by a reverse Plancher rearrangement, but this is considered to be unlikely under the reaction conditions]. Quadrigemine-A then is a mixture of diastereoisomers in which the chiral centres at C-3 and C-3‴ (2) are in an *R* : *S* ratio of about 3 : 1.

It is possible to arrive at a tentative conclusion concerning the overall stereochemistry of the quadrigemine-A diastereoisomer mixture by assuming an approximate additivity of the optical activity of the chiral centres in the molecule: these centres are of two types, the β,β′-type as in chimonanthine involving two asymmetric carbons and the C-3, C-7′-type as at C-3 in (4a).

In chimonanthine, calycanthidine, and folicanthine



(4c)

(4c) the β,β′-quaternary chiral centres have an *RR* configuration,[5] and the alkaloids all have a specific rotation

[5] S. F. Mason and G. W. Vane, *J. Chem. Soc.* (*B*), 1966, 370; I. J. Grant, T. A. Hamor, J. M. Robertson, and G. A. Sim, *J. Chem. Soc.*, 1965, 5678.

of the order of −320°: one can thus assume a β,β′-centre to contribute −320° if *RR*, +320° if *SS*, and of course 0° if *RS* as in mesochimonanthine.

The indolylindoline (4a) from hodgkinsine, with an *R* configuration for C-3, has a specific rotation of *ca.* +60°; with an *S* configuration this would be −60°.

Table 1 gives the predicted specific rotations of the possible quadrigemine-A diastereoisomers.

TABLE 1

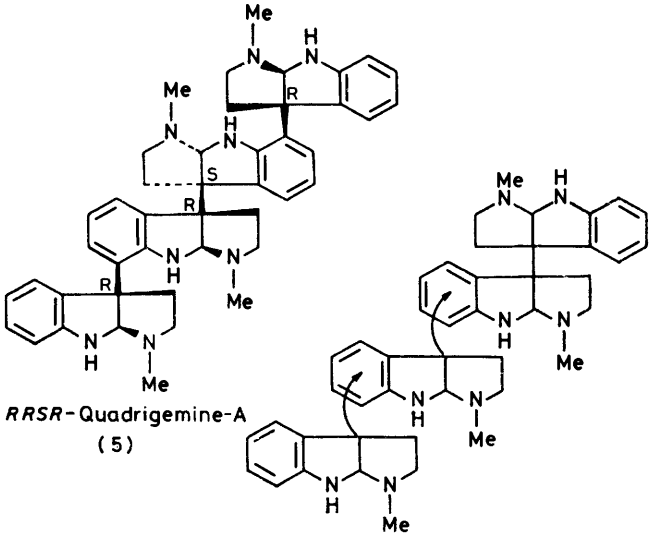
Diastereoisomers with an optically active β,β′-centre bond

C-3‴	*R*	*S*	*R*	*S*	*R*	*S*
C-3″	*R*	*S*	*R*	*S*	*S*	*R*
C-3′	*R*	*S*	*R*	*S*	*S*	*R*
C-3	*R*	*S*	*S*	*R*	*R*	*S*
$[\alpha]_D$	−100°	+100°	−160°	+160°	+220°	−220°
$[\alpha]_D$ of derived (3)	+60°	−60°	0°	0°	+60°	−60°

Diastereoisomers with a *meso*-β,β′-centre bond

C-3‴	*R*	*S*	*R*	*R*
C-3″	*R*	*S*	*R*	*S*
C-3′	*S*	*R*	*S*	*R*
C-3	*R*	*S*	*S*	*S*
$[\alpha]_D$	+60°	−60°	0°	0°
$[\alpha]_D$ of derived (3)	+60°	−60°	0°	0°

From the above figures it seems reasonable to conclude that, with an observed specific rotation of +32° for quadrigemine-A and +32° for the indolylindoline (4a) derived from it, the alkaloid is an approximately 1 : 1 mixture of optically active *RRSR* diastereoisomer, (5), and of *RRSS*, or *RSRS*, *or* a mixture of these two *meso*-diastereoisomers.



RRSR-Quadrigemine-A

(5)

(6)

This would allow a reasonable interpretation of the n.m.r. spectrum of the tetramethine base (3), in which, of the four 3*H*-indole C-2 hydrogen signals, two would be due to the *RRSR* diastereoisomer and two to the *meso*-diastereoisomer; a similar interpretation would apply to the four NMe_2 signals.

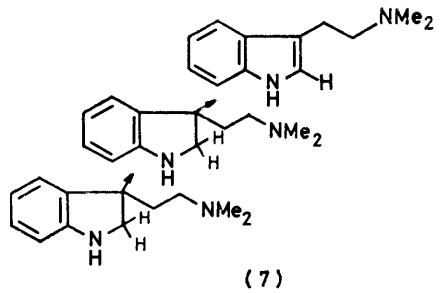
Structure of Quadrigemine-B.—The molecular formula $C_{44}H_{50}N_8$ for quadrigemine-B was firmly established by high-resolution mass spectroscopy, combustion analysis, and molecular-weight determination by Barger's vapour-pressure method. Further evidence for the peak at *m/e* 690 being the molecular ion was obtained by changing the energy of the electron beam from 70 to 17 eV, when the intensity of the peak increased from 12 to 34%.

The n.m.r. spectrum of quadrigemine-B is very similar to that of hodgkinsine except for an extra absorption in the form of a well resolved one-proton doublet at τ 5.1 (J = 3 Hz) which must correspond to one of the C-2 hydrogens, since it becomes a sharp singlet on addition of D_2O: conformational movements are probably responsible for the diffuse nature of the absorption due to the remaining three C-2 hydrogens. The electronic and vibrational spectra of quadrigemine-B and hodgkinsine are very similar indeed, the main difference being an extra NH stretching band at 3 370 cm^{-1} in the tetrameric base.

The two main fragment ions in the mass spectrum correspond to fission into moieties containing one and three tryptamine residues, which strongly suggests that the β,β′-bond links one tryptamine unit to a group of three, as expressed in structure (6). The following degradative work leads to the definition of the inter-unit links, but does not determine the relative stereochemistry.

Quadrigemine-B reacts with an excess of methyl iodide to give a crystalline tetramethiodide, which is readily converted by aqueous NaOH into the amorphous tetramethine base, $C_{48}H_{58}N_8$. The electronic spectrum of this base is compatible with the presence of four 3*H*-indole chromophores, and undergoes the expected reversible shift in acid: that no rearrangement to indole occurs under mild conditions is evidence that all four 3*H*-indole systems have a quaternary C-3. The n.m.r. spectrum of the base shows four sharp one-proton singlets at τ 1.26, 1.36, 1.90, and 1.98, showing the presence of four 3*H*-indole C-2 hydrogens, and singlets corresponding to NMe_2 groups at τ 7.98, 8.08, and 8.10 in an estimated ratio of 2 : 1 : 1, in a complex region (τ 6.8—8.7) containing the four aminoethyl side-chains, and a complex aromatic region at τ 2.3—3.5.

Reductive fission of the β,β′-bond in the tetramethine base with borohydride, under the conditions which lead to the corresponding fission of hodgkinsine trimethine,[2] leads to a mixture which can be resolved by preparative



(7)

t.l.c. into 3-(2-dimethylaminoethyl)indole (22% by weight; 88% yield) and a trimeric base, $C_{36}H_{48}N_6$ (61%

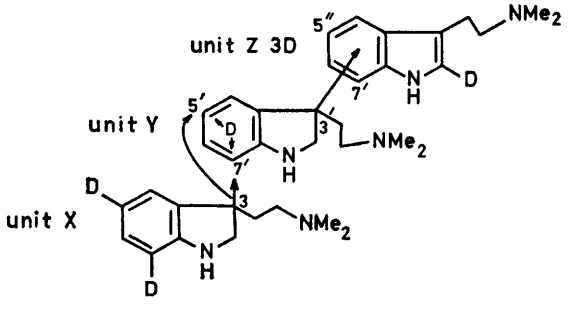
by weight; 81% yield). This trimeric base can be given part structure (7) on the following evidence.

The electronic absorption is similar to that of a 2 : 1 mixture of 3-(2-dimethylaminoethyl)indoline and 3-(2-dimethylaminoethyl)indole; the n.m.r. spectrum shows two indoline *N*-hydrogens at τ 5.8 and τ 6.0—6.6 (obscured) exchanging with D_2O, one indole *N*-hydrogen at τ 1.6 (exchanges with D_2O), an indole α-hydrogen as a broad singlet at τ 3.52 which is sharpened by the addition of D_2O, a 2*H* AB quartet at τ 6.22, 6.40 (J = 9 Hz) and a 2*H* singlet at τ 6.46 corresponding to two isolated indoline CH_2 groups.

The mass spectrum (M^+ 564.395 2) shows intense peaks at *m/e* 492, 493, and 506, corresponding to loss of $CH_2CH_2NMe_2$ and CH_2=CH–NMe_2, characteristic of indolines in this group of compounds, and to loss of CH_2NMe_2, characteristic of tryptamines; a strong peak at *m/e* 189 corresponds to ionisation of the terminal indoline unit, which is in good agreement with the fact that whereas the mass spectrum of the bi-indoline from hodgkinsine shows a strong *m/e* 189 peak, that of the corresponding indolylindoline does not.

It has already been argued that in the tetramethine base all the 3*H*-indole units have quaternary C-3 positions: this means that the two indoline units in the indolylbi-indoline (7) have quaternary C-3 positions which must each form one end of the two inter-unit bonds. The position of the other end of the two inter-unit bonds can be argued from the following observations, based largely on deuteriation experiments. It is known from previous work on simple indolines and indoles and on hodgkinsine degradation products [2] that acid-catalysed deuteriation of indoles leads to deuterium exchange at all available aromatic positions, whereas indolines undergo deuteriation only at two of the four benzene positions, *ortho* and *para* to the nitrogen. Deuteriation of the indolylbi-indoline (7) under the same conditions was complicated by fission into dimeric and monomeric products, but the surviving indolylbi-indoline was found to have incorporated seven deuterium atoms quite cleanly (*N*-deuteriums were washed out with H_2O).

If none of the aromatic positions had been involved in



(8)

inter-unit bonding, then an incorporation of nine deuterium atoms would have been expected; that only seven are taken up means that *two* aromatic positions are involved and since the indole α-position is known to be free, two benzene positions are involved; furthermore, this result means that the central indoline unit (unit Y) is linked by way of a position *ortho* or *para* to the ring nitrogen, for linkage by a *meta*-position would have required an uptake of eight deuterium atoms. The situation is summed up in structure (8).

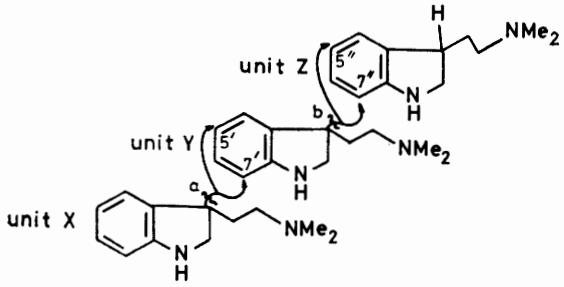
A reaction which throws further light on the problem is acetylation: the action of acetic anhydride at 100 °C or at room temperature on the indolylbi-indoline results in monoacetylation, only after several hours' refluxing is a small proportion, *ca.* 5%, of a diacetyl derivative produced. That this is a question of steric hindrance is demonstrated by the very easy formylation of the monoacetyl derivative to a formyl acetyl compound. The only structure, based on (8), which has a hindered indoline NH is that with a C(3)–C(7′): this then becomes a reasonable hypothesis.

Reduction of the indolylbi-indoline with zinc and aqueous H_2SO_4 gives a moderate yield of a ter-indoline (M^+ 566): this base proved to be very reactive and quickly deteriorated, especially in the presence of chloroform. The n.m.r. spectrum was not very informative, but was consistent with the proposed structure: a 6*H*-absorption at τ 6.3—6.5 corresponds to the indoline α-CH_2 groups—this overlies a diffuse NH absorption in the same region; most of the remaining aliphatic CH absorption lie in a large peak, τ 7.6—7.8, with two higher field much smaller complex bands at around τ 8.2 and 8.7. The latter band may be due to paraffin impurity, or to deshielded resonances belonging to conformers in which an aminoethyl side-chain finds itself in the deshielding region above a benzene ring. All attempts to obtain a straightforward deuteriation led to fission into a product which was analysed mass spectrally and shown to be a mixture of deuteriated 3-(2-dimethylaminoethyl)indole, 3-(2-dimethylaminoethyl)indoline, and indolylindoline dimeric base. At this stage this fission provided evidence for a link from C-3′ to C-5″ or C-7″ between units Y and Z. This can be argued as follows. Complete H^+-catalysed fission of the ter-indoline involves two bond breakages, a and b, and whichever the order these occur in, units X and Y each must be converted into the indole (11; R = R′ = H) and unit Z must become the indoline (10; R = R′ = R″ = H); that indoline (10; R = R′ = R″ = H) is produced is proof that the YZ inter-unit bond is being broken; since it has been demonstrated above that indolines only undergo H–D exchange at C-5 and C-7, and since the inter-unit bond fission must involve protonation of the aromatic carbon involved, it follows that the link between units Y and Z must involve carbons C-5″ *or* C-7″ and not C-4″ *or* C-6″. With the previous argument about the nature of the XY inter-unit link, partial structure (9) may be written for the ter-indoline.

The indolylindoline must arise by fission at b; a t.l.c. comparison with the indolylindoline derived from hodgkinsine was not made because of shortage of

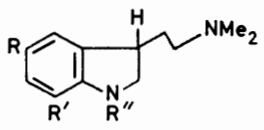
material, for each exploratory reaction was carried out on just enough material to allow good mass spectral analysis.

The final decision between 5′ and 7′, and 5″ and 7″ as terminals for the inter-unit bonds was achieved by hydrolytic fission of the trinitro-derivative of the ter-indoline.



(9)

The ter-indoline reacts readily with acetic anhydride at room temperature to give a monoacetyl derivative [which provides preliminary evidence for a C(3′)–C(7″) link between units Y and Z as well as C(3)–C(7) between X and Y]; formylation however leads as expected to the triformyl derivative. The region between τ 1.0 and 2.3 contains the absorptions ascribable to the *N*-formyl hydrogens: the situation here is complicated a little by the presence of one or possibly two pairs of conformational isomers. Nitration of the triformylter-indoline gives a mixture of products, from which a trinitro-derivative can be isolated by preparative t.l.c. The characterisation of this compound is not complete: it moves as a single spot on t.l.c.; it does not give a mass spectrum; not enough of it was available for an n.m.r. spectrum, nor could any be spared for a microanalysis. The main information on this compound comes from electronic spectra and from the products of hydrolytic fission: the electronic spectrum of the compound is almost identical with that of 3-(2-dimethylaminoethyl)-1-formyl-5-nitroindoline (10; R = NO_2R′ = H, R″ = CHO) and different from that of the isomeric indoline (10; R = H, R′ = NO_2, R″ = CHO); the electronic spectrum of the deformylated base is also almost identical with that of the indoline (10; R = NO_2, R′ = R″ = H) and quite different from that of the isomeric indoline (10; R = R″ = H, R′ = NO_2); that



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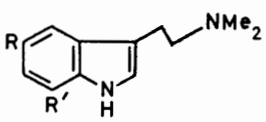
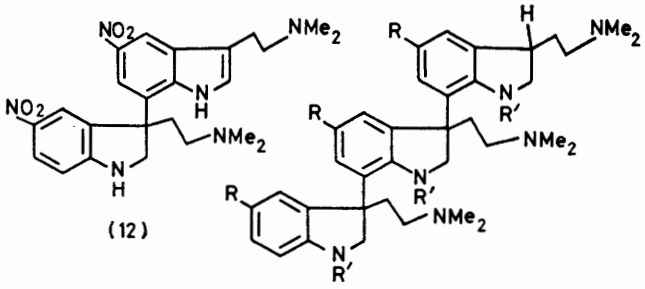


(11)

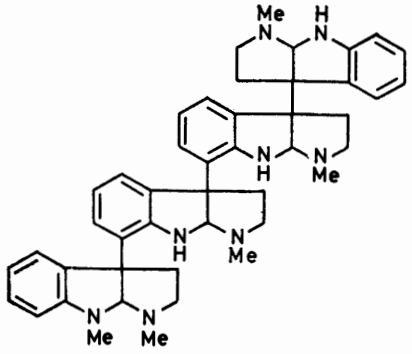
the nitration product is trinitro, with one nitro-group in each benzene ring, emerges from the total absence of compounds with an un-nitrated benzene ring among the products of acid-catalysed fission. This fission was carried out in 3M-aqueous HCl at 95 °C in a sealed tube. Preparative t.l.c. of the total basic product gave three well separated bands which together accounted for just over 90% of material put on: the band at R_F 0.62 (41%) was 3-(2-dimethylaminoethyl)-5-nitroindoline (10; R = NO_2, R′ = R″ = H); that at R_F 0.41 (25%) was 3-(2-dimethylaminoethyl)-5-nitroindole (11; R = NO_2, R′ = H); that at R_F 0.28 was the dinitroindolylindoline (12) (26%) as evidenced from the mass spectrum and the electronic spectrum, which was very similar to that of an equimolar mixture of (10; R = NO_2, R′ = R″ = H) and (11; R = NO_2, R′ = H), and quite different from those of equimolar mixtures of the two 7-nitro-compounds, the 5-nitroindoline, and the 7-nitroindole, and the 7-nitroindoline and the 5-nitroindole.



(12)

(13)

These data quite conclusively prove that the triformyltrinitroter-indoline has structure (13; R = NO_2, R′ = CHO), from which follows that the structure of quadrigemine-B is correctly represented by (14).

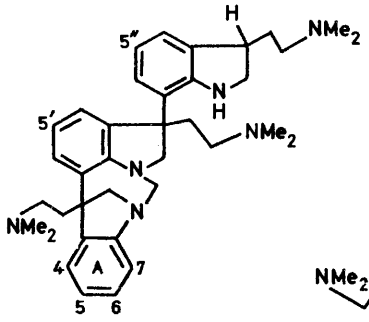


(14)

One of the difficulties encountered in the handling of the ter-indoline (13; R = R′ = H) involved reaction with adventitious formaldehyde in the atmosphere to give a product $C_{37}H_{50}N_6$ (M^+ 578.409 7): the reaction is a condensation of two indoline NH groups with one mole of CH_2O with the formation of a cyclic geminal diaminomethane system, a reaction with very many precedents. Possible reaction involving C–C bonding at an aromatic position is rendered very unlikely by the smooth conversion of the compound into the triformylter-indoline (13; R = H, R′ = CHO) with HCO_2H–Ac_2O.

There are two possible structures for this product (15; R = H) and (16; R = H). Structure (15) is

strongly favoured on the following grounds: *a priori* one would expect initial reaction with the least hindered NH to give a methylene ammonium ion which then could react intramolecularly with the hindered NH of the central indoline unit; 1-acetylter-indoline does not react with formaldehyde at room temperature, but reacts at 70 °C to give $C_{39}H_{52}N_6O$ to which structure (16; R = Ac) can be given, since the 1-acetyl group does not



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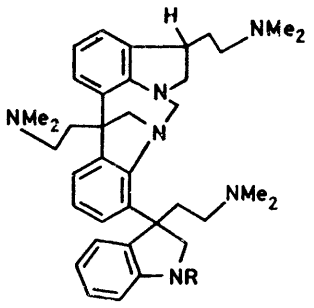
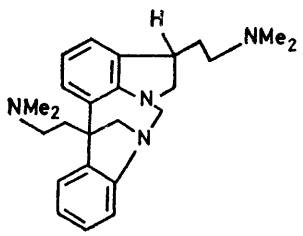


(16)

migrate easily (the corresponding 1-acetylbi-indoline derived from hodgkinsine is largely unchanged after prolonged reflux in Ac_2O); the bi-indoline from hodgkinsine reacts rapidly with CH_2O at room temperature to give a condensation product, $C_{25}H_{34}N_4$, to which structure (17) can be given.



(17)

It is interesting to note that the base (15) exchanges 6 deuterium atoms in 0.3M-DCl–D_3PO_4–D_2O, where only 4 might have been expected to exchange (at C-7, C-5, C-5′, and C-5″): the extra two deuteriums are almost certainly at C-4 and C-6 in ring A.

The nitrogen atom attached to this benzene ring is now at a bridgehead because of the methylene bridge between it and the corresponding nitrogen in the middle unit: thus this nitrogen atom is nowhere near as involved mesomerically with the benzene ring, and the *meta*-positions are no longer very much less reactive than the *ortho*- and *para*-positions.

Indirect proof that the methylene bridge does not carry any deuterium comes from a study of the analogous hodgkinsine–formaldehyde compound (17). This exchanges five deuterium atoms quite smoothly: when this deuteriated product is made to react with formic acid–acetic anhydride, loss of the methylene bridge occurs to give a diformylbi-indoline which still contains five deuterium atoms. This loss of the methylene bridge also occurs in base (15), which with formic acid–acetic anhydride yielded the triformylter-indoline (13; R = H, R′ = CHO). Shortage of material prevented us from carrying out this experiment on hexadeuterio-(15).

Some six years after completion of this work Johns, Lamberton, and their co-workers reported the isolation of psychotridine, $C_{55}H_{62}N_{10}$, a pentameric analogue of the quadrigemines.[6] The structure they proposed was based on spectral data and analogy with hodgkinsine.

EXPERIMENTAL

Mass spectra were run at 70 eV, unless otherwise stated. In all the spectra of quadrigemine degradation products, ions at *m/e* 72, 58, and 45, corresponding to $CH_2CH_2NMe_2$, CH_2NMe_2, and $NHMe_2$, were so intense, that the next most intense ion was taken as base peak.

Merck silica F_{254} (MSiF) and alumina F_{254} (MAlF) plates were used. Solvent systems were:

A	CH_2Cl_2, MeOH, NEt_3 (8 : 1 : 1)	MSiF
B	Et_2O, NEt_3, MeOH (100 : 10 : 1)	MAlF
C	Et_2O, $NHEt_2$ (10 : 1)	MAlF
D	B run twice	MAlF
E	$CHCl_3$, NEt_3 (100 : 1 run twice)	MAlF
F	C_6H_6, NEt_3, MeOH (100 : 1 : 1)	MAlF
G	Et_2O, NEt_3, MeOH (10 : 1 : 1)	MAlF
H	$CHCl_3$, NEt_3, MeOH (30 : 1 : 1 run twice)	MAlF
I	C_6H_6, NEt_3, MeOH (10 : 1 : 1)	MAlF

Isolation of the Alkaloids.—Extraction of Hodgkinsonia frutescens *leaves.* In a typical extraction, finely milled leaves (745 g) were extracted in a Soxhlet apparatus initially with ether (2.5 l) and then with a 3% (v/v) solution of triethylamine in benzene (2.5 l).

After filtration, each extraction was concentrated *in vacuo* to give a viscous green oil. A solution of the oil in methanol was poured into a mixture of ethyl acetate (250 ml) and aqueous 5% tartaric acid (200 ml). After separation the organic phase was washed with further aqueous 5% tartaric acid (2 × 100 ml). The combined aqueous acidic extracts were washed with small amounts of ethyl acetate until these washings were colourless. After basification of the aqueous layers with potassium carbonate, ethyl acetate extraction (3 × 400 ml) yielded no alkaloids from the initial ether extraction of the leaves, and a pale brown frothy solid from the benzene–triethylamine extract (6.99 g, 0.94%): seeding a solution of the benzene extract in a minimal quantity of benzene yielded hodgkinsine (1.5 g, 21%); the combined mother-liquor materials were then fractionated.

Separation of the bases. The course of the fractionation of the bases is presented schematically in the Figure. The code numbers refer to Tables 2, 3, and 4.

(1) Craig separation. The Craig machine had 115 tubes, each with a capacity of 40 ml for upper phase and 40 ml for lower phase. The solvent system used was an equilibrated two-phase mixture made up by volume from n-hexane

[6] N. K. Hart, S. R. Johns, J. A. Lamberton, and R. E. Summons, *Austral. J. Chem.*, 1974, **27**, 639.

(40 parts), n-propyl acetate (60 parts). formamide (50 parts), methanol (50 parts), and ammonia (*d*, 0.880; 1 part).

minute for 2 min, followed by 1 min settling, allowed efficient phase mixing and separation to occur.

Material was isolated from the fractions as follows.

TABLE 2

Craig separation

Fraction number	Tubes combined	Wt. of fraction (mg)	Wt. of hodgkinsine removed by crystallisation (mg)	U.v. spectra	Mass spectra	Type of material	Future use
0	115—180 upper phase moved out of instrument	96.9	0	Pure PhNCN $\lambda_{max.}$ 304 nm; H^+, 294 nm		A	Steady-state Craig type A material section (b)
1	90—114	54.8	0	ditto	Trimeric and tetrameric hodgkinsine types	A	ditto
2	84—89	632.0	403	ditto	ditto	A	ditto
3	76—83	1 140.0	559	ditto	ditto	A	ditto
4	66—75	762.0	200	Essentially PhNCN $\lambda_{max.}$ 302 nm; H^+, 289 nm	As above but significant peaks at *m/e* 214, 386, 172	B	Steady-state Craig type B material section (6)
5	56—65	405	0	ditto	ditto	B	ditto
6	46—55	522	0	ditto	Shows intense peaks at *m/e* 214, 172, 386, and 540	B	ditto
7	31—45	1 040	0	not PhNCN	Intense peaks at *m/e* 386, 214, 172, 173. Hardly any peaks associated with hodgkinsine type materials	C	Retained
8	0—30	6 980.0	0	ditto	Intense peaks at *m/e* 172, 173, 214, 344, 386, 387, 429, 430, and 602	C	Retained
	Total	11.63 g (86% recovery)	1.162 g				

Note—fractions are classed as type A, B, or C on the basis of the u.v. spectra and mass spectra: type C show no PhNCN u.v. behaviour, and no trimeric or tetrameric peaks in the mass spectrum; type A show pure PhNCN u.v. behaviour, and trimeric and tetrameric peaks in the mass spectrum; type B are intermediate in type.

The total basic material after removal of crystallised hodgkinsine (13.51 g) was placed in tube 0 and the machine given 190 transfers (5 min agitation time, 5 min settling time); after 120 transfers, introduction of fresh upper phase into the machine ceased. The contents of the tubes were combined into eight fractions on the basis of a u.v. and mass spectrum assay (see Table 2) and each fraction isolated as follows. The solvents were removed *in vacuo* to give an oil which after dilution with an equal volume of water was extracted with ethyl acetate (× 3). After drying and concentrating to a small volume, the layer was washed with small amounts of water (× 3), dried, and boiled down *in vacuo*.

Where possible, hodgkinsine was removed by crystallisation from a concentrated benzene solution.

The results of this preliminary separation are given in Table 2.

(2) Steady-state separations. Further resolution of the basic material required two steady-state liquid–liquid separations, and subsequent purification of the fractions was effected by preparative t.l.c. and crystallisation.

A Quickfit and Quartz steady-state instrument fitted with 120 tubes with a capacity of 10 ml for each phase was used.

The solvent system was obtained by equilibration of a mixture by volume of cyclohexane (60 parts), methanol (66 parts), ethyl acetate (40 parts), and water (34 parts). Instrumental agitation at the rate of 20 oscillations a

Fractions containing only upper phase solvent were dried and boiled down *in vacuo*.

Fractions containing lower-phase solvent were concentrated *in vacuo* at 30 °C. The resultant aqueous concentrate was basified with solid potassium carbonate and extracted with ethyl acetate (× 3). After drying, the combined ethyl acetate layers were boiled down *in vacuo*.

(*a*) Steady-state separation of Craig type B material. The Craig fractions 4, 5, and 6 (1.5 g) were placed in tube 0 and subjected to the following programme of transfers based on u.v. the assays at intermediate stages.

(*b*) Steady-state separation of Craig type A material.

Programmed		Actual No. of transfers executed		
No. of transfers	Ratio upper : lower	upper	lower	Total
100	24 : 16	60	40	100
200	24 : 19	172	128	300
200	24 : 19	284	216	500
500	24 : 19	563	437	1 000

The results of the fractionation are given in Table 3.

Material isolated from fractions 0, 1, 2, and 3 of the Craig separation and fractions AR-AW from the upper phase

collector in (*a*) (see Figure) were placed in the steady-state machine:

Tube no.	Fraction from (a) (steady state)	Fraction from (1) Craig separation
−5	AW	
−4	AV	
−3	AU	
−2	AT	
−1	AS	
0		3
1	AR	2
2		1
3		0

The machine was programmed as follows, the progress of the separation being observed by a u.v. t.l.c. assay.

TABLE 3

Steady-state separation of Craig type B material

Fraction number	Tubes in fraction	Weight (mg)	Use
Upper-phase fraction collector			
	0—167	154.1	Retained
AR	168—212	18.4	Combined with type A Material from Craig separation and further fractionated (see Table 3)
AS	213—262	31.1	
AT	263—312	13.8	
AU	313—407	19.9	
AV	408—512	15.8	
AW	513—563	6.2	
Instrument			
AX	+45 to +60	52.5	T.l.c. system A R_F 0.28 35.9 mg Quad. B 0.45 3.4 mg unknown 0.58 7.0 mg alkaloid V
AY	+15 to +44	136.9	T.l.c. system A R_F 0.28 71.4 mg Quad. B 0.58 46.8 mg alkaloid III
AZ	−30 to +14	158.3	T.l.c. system A R_F 0.28 21.9 mg Quad. B 0.56 99.2 mg alkaloid III
BA	−45 to −31	33.0	Retained
BB	−60 to −46	30.0	Retained
Lower-phase fraction collector			
BC	371—437	43.0	Combined with BD
BD	336—370	21.2	T.l.c. BC and BD system B R_F 0.16 9.4 mg unknown 0.23 10.8 mg unknown 0.47 43.2 mg mesochimonanthine
BE	281—335	77.8	Retained
BF	231—280	66.3	Retained
BG	181—230	84.3	T.l.c. system C R_F 0.37 alkaloid I
BH	126—180	41.4	T.l.c. system C R_F 0.38 alkaloid I
	0—125	30.1	Retained
	Total	1.04 g	70%

Note—The centre tube of the instrument was designated zero and tubes on the upper-phase exit side as positive and those on the lower-phase exit side as negative. Fractions emerging from the instrument were collected in the appropriate fraction collectors and are designated by the number of the upper or lower phase transfer at which they emerged from the instrument, *i.e.* those with the largest number emerged last.

Quadrigemine-A (2).—Fraction BY (41.5 mg) was chromatographed by t.l.c. on system D; the band at R_F 0.43 (32 mg) was rechromatographed on system E to give a band at R_F 0.51 (24.8 mg), which on further rechromatography on system F gave material at R_F 0.52 (18.0 mg) as a white amorphous resinous froth which moved as a single well-formed spot; $[\alpha]_D^{23}$ +32° (EtOH) (Found: C, 76.6; H, 7.6; N, 15.5%; M^+, 690.415 6. $C_{44}H_{50}N_8$ requires C, 76.5; H, 7.3; N, 16.2%; M^+, 690.415 8), $\nu_{max.}$(CCl_4) 3 270 and 3 420 cm^{-1}; $\lambda_{max.}$(EtOH) 244 (ε 19 800) and 304 nm (ε 10 900); $\lambda_{max.}$(EtOH–HCl) 234 (ε 19 300) and 294 nm (ε 9 150); n.m.r. spectrum (CCl_4): τ 2.9—3.6 (well resolved complex signals, aromatic CH), 2.6—4.5 (low broad unresolved signals, probably 2 NH and 2 N-*CH*-N), 5.14 (broad singlet, 2 N-*CH*-N), 6.02 (broad singlet, 2 NH), and 6.8—8.2 (broad absorption with an overlying broad singlet at 7.65 *ca.* 28 H); the combined aromatic and other signals between τ 2.6 and 4.5 integrated to between 17 H and 18 H. Mass spectrum (260 °C): 690 (0.5%), 657 (0.3), 345 (22), 344 (100), 314 (23), 302 (23), 301 (36), 287 (15), 271 (13), 259 (15), 257 (10), 245 (12), 172 (4), and 130 (5); (260 °C, 15 eV) 690 (1.5), 657 (0.9), 345 (26), 344 (100), 314 (5), and 302 (4).

Programmed		Actual No. of transfers executed		
No. of transfers	Ratio upper : lower	upper	lower	Total
110	24 : 24	55	55	110
300	12 : 24	143	232	375
500	19 : 24	366	513	879

Analysis of the fractions obtained is given in Table 4.

Quadrigemine-A Hofmann Base (2).—Quadrigemine-A (8.8 mg) in acetone–methanol–benzene (1 : 2 : 5; 0.5 ml) was treated with methyl iodide (0.5 ml) and the reaction left at 0 °C in the dark for 15 h, when the amorphous crude methiodide separated out. The mother liquor was decanted, the methiodide dissolved in water (5 ml), and this aqueous solution thoroughly extracted with ether. The aqueous phase was treated with aqueous 10% NaOH (0.5

ml), and the Hofmann base extracted with ethyl acetate (3 × 5 ml). This gave a colourless gum (7.7 mg, 81%). Attempted t.l.c. chromatography failed, the material remaining at the origin, presumably because of cyclisation to a *gem*-diamino-cation; $\lambda_{infl.}$(EtOH) 283 nm (ε 7 500); $\lambda_{max.}$(EtOH–HCl) 294 nm (ε 7 800); τ(CCl_4) 1.32 (s, 1 H), 1.56 (s, 1 H), 1.82 (s, 1 H), 1.92 (s, 1 H), 2.3—3.2 (complex, 14 H), and 8.00, 8.02, 8.04, and 8.08 (peaks corresponding to *N*-methyls). Mass spectrum: 746 (1%, M^+, $C_{48}H_{58}N_8$), 688 (9), 675 (1), 643 (2), 620 (1), 617 (3), 604 (2), 373 (12), 316 (100), 301 (9), 271 (17), 257 (28), 245 (31), and 243 (18).

Potassium Borohydride Cleavage of Quadrigemine-A Hofmann Base.—A solution of the Hofmann base (6.9 mg) in methanol (1 ml) was treated with 10% aqueous NaOH (0.02 ml) and KBH_4 (200 mg), and the reaction refluxed for 3 h. The resulting ether-soluble basic material (6.6 mg) was chromatographed on a MAlF plate with $CHCl_3$–MeOH–NEt_3 (75 : 1 : 1) to give the indolylindoline (4a) (5.5 mg) at R_F 0.42: this was rechromatographed on a MAlF plate with Et_2O–MeOH–NEt_3 (20 : 1 : 1) to give the pure indolylindoline (4a) (4.8 mg, 73%), $[\alpha]_D^{23}$ +32° (EtOH); $\lambda_{max.}$(EtOH) 284 and 292 (ε 6 230 and 6 140); $\lambda_{infl.}$ 276 nm (ε 5 370); $\lambda_{max.}$(EtOH–HCl) 264, 269, 282, and 293 nm (ε 4 400, 4 730, 4 730, and 3 980); τ(CCl_4) 1.08br (s, 1 H), 2.6—3.5 (complex), 6.19 and 6.35 (AB system, J = 10 Hz, 2 H), 6.4br (s, 1 H, removed by addition of D_2O); peaks at 7.79 and 7.83 corresponding to *N*-methyls in a complex region 7.0—8.5. Mass spectrum: 376 (53%), 318 (85), 304 (47), 273 (100), and 245 (42); (140 °C, 18 eV): 376 (97), 318 (100), 304 (94), and 273 (27).

Quadrigemine-B (14).—All the fractions designated Quad B in the steady-state and preparative t.l.c. separations were combined and crystallised. By a combination of t.l.c. and recrystallisation, Quadrigemine-B was isolated as a white crystalline compound, m.p. 229—234 °C, after softening from 226 °C (198 mg) from MeOH, R_F 0.33 on system B; $[\alpha]_D^{23}$ +263° (EtOH) (Found: C, 75.0; H, 7.1; N, 15.7%; M^+, 690.414 4. $C_{44}H_{50}N_8$·MeOH requires C, 74.9; H, 7.3; N, 15.6%; M^+, 690.415 8), $\nu_{max.}$(CCl_4) 3 250, 3 370, and 3 410 cm^{-1}; $\lambda_{max.}$(EtOH) 245 and 304 nm (ε 19 850 and 10 030); $\lambda_{max.}$(EtOH–HCl) 236 and 295 nm (ε 19 300, and 8 250); τ(CCl_4) 2.9—3.8 (complex and well resolved *ca.* 14 H), 3.8—4.3br (1 H), 4.5—5.5 (complex, 3 H), 5.10 (d, J = 3 Hz, 1 H, s with D_2O), 5.5—6.5br (3 H), and 6.8—8.2 (complex, *ca.* 28 H). Mass spectrum: 690 (12%), 517 (80), 516 (100), 486 (21), 474 (17), 473 (16), 459 (10), 455 (8), 443 (14), 429 (20), 416 (10), 400 (11), 388 (8), 386 (11), 173 (64), 172 (91), and 130 (27); (265 °C, 17 eV): 690 (34), 517 (80), 516 (100), and 172 (60).

Quadrigemine-B Tetra-methiodide.—A solution of quadrigemine-B (53.6 mg) in acetone–methanol–benzene, 1 : 1 : 3 (0.2 ml) and methyl iodide (0.2 ml) was left at 0 °C in the dark for 15 h. The crystalline methiodide (103.5 mg) had m.p. 235—240 °C (decomp.).

Quadrigemine-B Hofmann Base.—A solution of the above methiodide (103.5 mg) in water (10 ml) was treated with aqueous 10% NaOH (5 ml), and the mixture extracted with ether (4 × 7 ml): the ether-soluble Hofmann base was obtained as a white amorphous resin (47.2 mg, 85%) (M^+, 746.477 9. $C_{48}H_{58}N_8$ requires 746.478 4), $\lambda_{infl.}$(EtOH) 283 nm (ε 7 790); $\lambda_{max.}$(EtOH–HCl) 230 and 292 nm (ε 22 000 and 8 000); $\lambda_{max.}$(EtOH–NaOH) 260 nm (ε 16 300);

TABLE 4

Steady-state separation of combined type A material (see Figure)

Fraction number	Tubes combined	Tubes weight (mg)	T.l.c. analysis	Mass spectral analysis
Upper-phase fraction collector				
	0—81	164.5	Retained	Not run
CF	82—111	33.4	ditto	Complex mixture
CE	112—131	9.7	ditto	Not run
CD	132—206	20.6	ditto	Complex mixture
CC	207—256	16.2	ditto	Not run
CB	257—311	15.7	ditto	Not run
CA	312—366	12.8	ditto	Not run
Instrument				
BZ	+46 to +60	35.8	Retained	Trimeric and tetrameric alkaloids present
BY	+25 to +45	41.3	T.l.c. system D. R_F 0.43 32.0 mg Quad. A	Primarily Quad. A
BX	−10 to +24	103.4	Seeding yielded almost pure hodgkinsine	Almost pure trimeric material
BW	−35 to −11	61.3	T.l.c. system D. R_F 0.45 2.2 mg Quad. B 0.66 48.7 mg hodgkinsine	Not run
BV	−60 to −36	46.6	T.l.c. system D. R_F 0.43 6.0 mg Quad. B 0.67 28.6 mg hodgkinsine	Mainly trimeric material Tetrameric compounds present
Lower-phase fraction collector				
BU	427—513	59.1	T.l.c. system D. R_F 0.50 46.2 mg Quad. B 0.55 3.0 mg hodgkinsine	Not run
BT	369—426	48.3	T.l.c. system D. R_F 0.50 41.2 mg Quad. B	Shows peaks associated with tetrameric and trimeric material though former dominant
BS	319—368	53.4	T.l.c. system D. R_F 0.50 44.7 mg Quad. B	Not run
BR	292—318	43.1	Not chromatographed	Similar to BT
BQ	265—291	49.0	Not chromatographed	Not run
BP	236—264	42.2	T.l.c. system D. R_F 0.50 33.7 mg Quad. B	Similar to BT
BO	194—235	48.2	T.l.c. system D. R_F 0.50 38.2 mg Quad. B	Not run
BN	159—193	27.6	T.l.c. system B. R_F 0.34 11.5 mg Quad. B	Similar to BT but tetrameric material more dominant
BM	128—158	26.4	T.l.c. system B. R_F 0.32 12.7 mg Quad B 0.56 5.7 mg unknown	Not run
	0—127	34.0	Retained	Not run
	Total	993 mg 92%		

τ(CCl_4) 1.26 (s, 1 H), 1.36 (s, 1 H), 1.90 (s, 1 H), 1.98 (s, 1 H), 2.2—3.5 (m, 14 H), and 7.98, 8.08, and 8.10 (peaks corresponding to *N*-methyls) in the region 6.9—8.7 which also includes the aminoethyl CH_2 absorptions (complex *ca.* 40 H). Mass spectrum: 746 (17%), 688 (67), 674 (26), 643 (19), 559 (20). 502 (100), 457 (20), 431 (19), 173 (47), 143 (17), and 130 (30).

Potassium Borohydride Cleavage of Quadrigemine-B Hofmann Base.—A solution of the Hofmann base (47.2 mg) in methanol (1 ml) was treated with 10% aqueous NaOH (0.04 ml) and KBH_4 (300 mg), and the reaction refluxed for 3 h. The resulting ether-soluble basic material (45.6 mg) was chromatographed on a MAlF plate with Et_2O–MeOH–NEt_3 (10 : 1 : 1).

Material on the base-line (3.5 mg, 7%) was uncleaved reduction product (mono-demethylated tetrahydro-base) (Found: M^+, 736.494 9. $C_{47}H_{60}N_8$ requires 736.494 1), $\lambda_{max.}$(EtOH) 240 and 296 nm (ε 10 800 and 4 790); $\lambda_{max.}$(EtOH–HCl) 238 and 294 nm (ε 9 300 and 4 200). Mass spectrum: 736 (6%), 691 (6), 678 (50), 665 (32), 664 (32), 633 (34), 506 (60), 492 (80), 476 (18), 461 (18), 416 (20), 388 (22), 360 (23), 318 (31), 273 (32), 189 (60), and 144 (100).

Material at R_F 0.3—1.0 was rechromatographed on the same system to give two products: (*a*) at R_F 0.63, *NN*-dimethyltryptamine (10.6 mg, 22%) and (*b*) at R_F 0.43, indolylbi-indoline (8) (28.0 mg, 61%) (Found: M^+, 564.395 2. $C_{36}H_{48}N_6$ requires M^+, 564.394 0), $\nu_{max.}$($CHCl_3$) 3 200, 3 400, and 3 450 cm^{-1}; $\lambda_{max.}$(EtOH) 293; $\lambda_{infl.}$ 245 and 289 nm (ε 9 100, 9 890, and 8 560); $\lambda_{max.}$(EtOH–HCl) 270, 282, and 291; $\lambda_{infl.}$ 263 nm (ε 6 650, 6 250, 4 440, and 6 050); τ(CCl_4) 1.58br (1 H, removed by D_2O), 2.6—3.6 (complex, *ca.* 10 H), 3.52br (s, 1 H, sharpens on D_2O addition), 5.80br (s, 1 H, removed by D_2O), a complex region composed of an AB system 6.22 and 6.40 (2 H, J = 9 Hz) partly overlaid by a fairly sharp absorption at 6.46 (s, 2 H), and a broad absorption at 6.30 (1 H, removed by D_2O); peaks at 7.82 and 7.88 corresponding to *N*-methyls in a complex region 7.0—7.9 (*ca.* 30 H). Mass spectrum: 564 (30%), 506 (85), 493 (87), 492 (100), 461 (32), 416 (20), 388 (24), 360 (28), 318 (47), 305 (16), 273 (30), 189 (57), and 144 (44).

Deuteriation of the Indolylbi-indoline (8).—The interaction of pure PCl_5 (647 mg) and D_2O (2.6 ml) yielded 5N-DCl–3N-D_3PO = 8N mineral acid (for the sake of simplicity 1 mol l^{-1} H_3PO_4 is here taken to be 3N): 0.5 and 0.15 ml of the above solution each diluted to 4 ml with D_2O gave N-acid and 0.3N-acid respectively.

The indolylbi-indoline (in lots of 0.5 mg) was heated in evacuated sealed tubes with the 0.3N-acid for 4 h and with the N- and 8N-acids for 10.5 h. The contents of each of the tubes were then worked up in the usual manner for basic material.

The reaction in 0.3N-acid led to incomplete deuteriation and some fission.

The reaction in N-acid led to complete fission to *NN*-dimethyltryptamine and the indolylindoline (4a): in the mass spectrum, the parent peaks of the latter were 383 (12%), 382 (37), 381 (39), and 380 (15) indicating incomplete deuteriation.

The reaction in 8N-acid led to partial fission, with survival of fully deuteriated indolylbi-indoline. Mass spectrum: 571 (24%), 513 (80), 500 (100), 499 (98), 468 (40), 323 (45), 191 (75), and 146 (58). Note that the indolylindoline had been completely volatilised when the above mass spectrum was recorded.

Monoacetyl Indolylbi-indoline.—A solution of the indolylbi-indoline (5.4 mg) in acetic anhydride (1 ml) was left at 20 °C for 30 min, the excess acetic anhydride then hydrolysed with water, and the reaction worked up for ether-soluble base. The resulting amorphous material (5.5 mg, 97%) was homogeneous by t.l.c. The unit mass spectrum showed the highest peak at mass 606, which corresponds to $C_{38}H_{50}N_6O$; $\lambda_{max.}$(EtOH) 253, 283, and 291 nm (ε undetermined); $\lambda_{max.}$(EtOH–HCl) 254 and 281; $\lambda_{infl.}$ 291. Mass spectrum: 606 (5%), 548 (30), 535 (100), 534 (64), 503 (28), 458 (18), and 430 (18).

Monoacetyl(monoformyl)indolylbi-indoline.—A solution of monoacetylindolylbi-indoline (0.5 mg) in formic acid–acetic anhydride (1 : 1) (0.5 ml) was heated on a steam-bath for 30 min. After hydrolysis of the acetic anhydride with water, the reaction was worked up for basic material. The observed parent peak in the mass spectrum, 634, corresponds to $C_{39}H_{50}N_6O_2$. Mass spectrum: 634 (12%), 576 (100), 563 (30), 562 (34), 546 (18), 531 (20), 503 (20), 458 (14), and 430 (10).

The Ter-indoline (13; R = R′ = H).—A solution of the indolylbi-indoline (44.3 mg) in 50% (v/v) aqueous sulphuric acid (15 ml) was treated with zinc dust (1 g) and shaken for 15 min. Further amounts of zinc dust (4 × 1 g) and 50% (v/v) sulphuric acid (4 × 5 ml) were added during 90 min after which shaking was continued for a further 2 h; the reaction mixture was then diluted with sufficient water to cause the zinc to coagulate. The zinc was filtered off, washed with N-aqueous sulphuric acid and then with water, and the combined filtrates poured slowly into an efficiently stirred and cooled mixture of concentrated aqueous NaOH (100 g NaOH in 100 ml H_2O) and ether (100 ml). The ether layer was run off, and the aqueous extracted further with two 50-ml quantities of ether. The combined ether extracts yielded 26.1 mg (59%) of a colourless resin. This was chromatographed on MAlF with 40 : 1 : 3 ethyl acetate–methanol–triethylamine. The band at R_F 0.35 was pure ter-indoline (19.2 mg). N.m.r. spectrum: see theoretical section. Peak at highest mass in the mass spectrum, 566, corresponding to $C_{36}H_{50}N_6$; $\lambda_{max.}$(EtOH) 246 and 300 nm (ε 14 300, 7 630); $\lambda_{max.}$(18N-H_2SO_4) 260; $\lambda_{infl.}$ 269 and 275 nm (low ε). Mass spectrum: 566 (23%), 506 (15), 495 (76), 494 (100), 449 (12), 421 (15), 377 (44), 348 (25), 307 (32), 306 (38), 305 (20), 304 (20), 259 (26), 245 (18), 233 (14), 189 (100), 144 (50), and 130 (27).

Triformylter-indoline (13; R = H, R′ = CHO).—A solution of the ter-indoline (26.1 mg) in 1 : 1 (v/v) formic acid–acetic anhydride (10 ml) was heated on a steam-bath for 30 min, the excess acetic anhydride was hydrolysed with water, and the mixture worked up for basic material. The product was chromatographed on MAlF and developed three times with Et_2O–MeOH–NEt_3 (30 : 4 : 3): the band at R_F 0.6 yielded the pure triformylter-indoline (19.4 mg) (Found: M^+, 650.390 6. $C_{39}H_{50}N_6O_3$ requires M^+, 650.394 4), $\nu_{max.}$($CHCl_3$) 1 670 cm^{-1}; $\lambda_{max.}$ 252 and 284 nm (ε 29 000 and 6 600); τ(CCl_4) 1.10, 1.58, 1.85, 1.95, and 2.30 (singlets corresponding to 3 H), 2.2—3.3 (complex 9 H), 5.4—7.1 (complex 6 H), and 7.82 and 7.93 corresponding to *N*-methyls in a complex region 7.1—8.5 containing the aminoethyl side-chains. Mass spectrum: 650 (5%), 621 (2), 592 (9), 579 (48), 578 (100), 560 (15), 550 (22), 505 (15), 487 (10), and 477 (11).

Nitration of Triformylter-indoline (13; R = H, R′ = CHO).—Triformylter-indoline (17.1 mg) was cooled to 0 °C, treated with swirling with conc. HNO_3–conc. H_2SO_4 (3 : 2,

v/v; 2 ml) at 0 °C, and was then left at 20 °C for 30 min. The reaction mixture was added to water at 0 °C with cooling, and the resulting solution worked up for ether-soluble basic material; care was taken to work as rapidly as possible the temperature being kept as near 0 °C as possible to avoid partial hydrolysis of the formyl groups. The basic material was an orange resinous froth (18.4 mg), and was chromatographed on MAlF with EtOAc–MeOH (15 : 4) to yield two main bands (a) and (b). (a) At R_F 0.73, consisted of partially nitrated triformylter-indoline (12 mg, 63%); peak at highest mass in the mass spectrum 695, $C_{39}H_{49}N_7O_5$ requires 695; $\lambda_{max.}$(EtOH) 245 and 330 nm (ε 17 000 and 17 000), $\lambda_{min.}$ 275 (ε 8 000); after mild acid hydrolysis $\lambda_{max.}$(EtOH) 300 and 388 nm. Mass spectrum (290 °C, 30 eV) 695 (30%), 667 (80), 666 (100), 460 (50), 622 (40), 620 (40), 595 (75), and 583 (50). (b) At R_F 0.85, which yielded the triformyl(trinitro)ter-indoline (13; R = NO_2, R′ = CHO) (3.6 mg, 18%), $\lambda_{max.}$(EtOH) 327 nm (ε 27 500); after mild acid hydrolysis $\lambda_{max.}$ 392 and 322. A mass spectrum could not be obtained.

Acid Cleavage of Triformyl(trinitro)ter-indoline (13; R = NO_2, R′ = CHO).—The trinitro-compound (3.4 mg) was heated in 3N-aqueous HCl (0.2 ml) in an evacuated sealed tube for 13 h at 95 °C. The total basic ether-soluble product was chromatographed on MAlF with $CHCl_3$–MeOH–NEt_3 (30 : 1 : 1) to yield four bands: (a) at R_F 0—8% of unidentified material. (b) At R_F 0.28 the dinitroindolylindoline (12) (0.52 mg, 26%); peak at highest mass in the mass spectrum 466 ($C_{24}H_{30}N_6O_4$ requires 466); $\lambda_{max.}$(EtOH) 268, 323, and 375 nm; mass spectrum (200 °C, 15 eV) 466 (50%), 464 (10), 436 (38), 434 (60), 420 (18), 406 (15), 394 (30), 378 (18), and 376 (35). (c) At R_F 0.41 the 5-nitrodimethyltryptamine (11; R = NO_2, R′ = H) (25%); $\lambda_{max.}$(EtOH) 269 and 323; $\lambda_{infl.}$ 255 nm. (d) At R_F 0.62 the 5-nitrodimethyldihydrotryptamine (10; R = NO_2, R′,R″ = H) (41%); peak at highest mass in the mass spectrum 235 ($C_{12}H_{17}N_3O_2$ requires 235); $\lambda_{max.}$(EtOH) 323 and 391 nm; mass spectrum (130 °C, 15 eV): 235 (100%), 218 (10), 207 (4), 162 (5), and 146 (12).

T.l.c. Analysis of Cleavage Products.—Two systems on MAlF were found to differentiate clearly between the 5- and 7-nitrodimethyltryptamines and the two corresponding indolines.

System I	$CHCl_3$–MeOH–NEt_3	30 : 1 : 1	run twice.
System II	C_6H_6–MeOH–NEt_3	10 : 1 : 1	run twice.

	Synthetic compounds		Cleavage products	
	System I	System II	System I	System II
5-Nitrodimethyltryptamine (11; R = NO_2, R′ = H)	0.61	0.43	0.61	0.43
7-Nitrodimethyltryptamine (11; R = H, R′ = NO_2)	0.78	0.74		
5-Nitrodihydrodimethyltryptamine (10; R = NO_2, R′ = R″ = H)	0.74	0.61	0.74	0.61
7-Nitrodihydrodimethyltryptamine (10; R = R″ = H, R′ = NO_2)	0.82	0.78		
Dinitrobi-indoline (2′,3′-dihydro-12)			0.57	
Dinitroindolylindoline (12)			0.41	0.22

Attempted Deuteriation of Ter-indoline (13; R = R′ = R″ = H).—The ter-indoline (in 0.3 mg lots) was heated in 0.3N-, N-, and 8N-D_2O–DCl–D_3PO_4 (prepared as above) in evacuated sealed tubes at 95 °C for 20 h. Total basic material was in each case used directly without further treatment for mass-spectrometric analysis. (a) Material from treatment with 0.3N-acid shows pronounced cleavage to the indolylindoline (4a): 381 (32%), 323 (67), 309 (40), 278 (100), 263 (60), 262 (60), and 250 (84); there was also the deuteriated methyleneter-indoline (15) most of which came off the probe after the indolylindoline, 584 (20), 526 (14), 512 (45), 394 (18), 368 (32), and 323 (67). (b) and (c) Material from treatment with N- and 8N-acid both showed complete cleavage to the indolylindoline (4a): 383 (14%), 382 (32), 381 (12), 380 (16), 379 (10), 324 (75), 310 (40), 279 (100), 264 (40), 251 (80), and 236 (44).

Methyleneter-indoline (15).—A solution of ter-indoline (0.1 mg) in methanol (1 ml) was treated with formalin (0.1 ml of a 10% solution). After 30 min at 20 °C, the solvent was removed under reduced pressure and a part of the total residue subjected to mass spectral analysis (Found: M^+, 578.409 7. $C_{37}H_{50}N_6$ requires M^+ 578.410 0). Mass spectrum at 200 °C, 70 eV: 578 (42%), 576 (10), 520 (20), 518 (20), 507 (60), 506 (100), 461 (10), 435 (11), 389 (18), 362 (16), 318 (20), and 273 (20).

Conversion of the Methyleneter-indoline (15) *into the Triformylter-indoline* (13; R = H, R′ = CHO).—A solution of the methyleneter-indoline (0.1 mg) in formic acid–acetic anhydride (1 : 1) (0.5 ml) was heated at 95—100 °C for 1 h. The total ether-soluble basic material from this reaction gave a mass spectrum identical with that of the triformylter-indoline (13; R = H, R′ = CHO) (see above).

Methylenebi-indoline (17). A solution of the bi-indoline 2′,3′-dihydro-(4a) (0.1 mg) in MeOH (2.5 ml) containing one drop of 10% aqueous formalin was left for 5 min at 20 °C, after which the solvent was removed under reduced pressure, and the residue worked up for basic material (using 0.1N-aqueous HCl). The mass spectrum of the total base showed no trace of a peak corresponding to the original bi-indoline, and a peak at highest mass which is also the base peak at 390, corresponding to $C_{25}H_{34}N_4$, $\lambda_{max.}$(EtOH) 250 and 300 nm (ε 14 500 and 6 500). Mass spectrum (130 °C, 70 eV): 390 (100%), 332 (15), 319 (33), 318 (92), 287 (16), 273 (22), 259 (32), 245 (20), 144 (10), and 130 (25).

Deuteriation of the Methylenebi-indoline (17).—The methylenebi-indoline (in two 0.05 mg lots) was heated in evacuated sealed ampoules in 0.3N- and N-DCl–D_3PO_4–D_2O (prepared as above) to 100 °C for 24 h. Total basic material was in each case used directly without further treatment for mass spectrometric analysis.

(a) Material from treatment with N-acid showed complete fission into monomeric products. (b) Material from treatment with 0.3N-acid showed monomeric products, but also pentadeuteriated methylenebi-indoline: 395 (90%), 337 (12), 324 (48), 323 (100), 292 (12), 278 (30), 264 (22), 263 (22), and 250 (22) and also extra peaks at 410 (30%) and 341 (30) (unexplained).

The ' contaminated ' pentadeuteriomethylenebi-indoline was treated with 1 : 1 (v/v) formic acid–acetic anhydride (0.5 ml) at 95—110 °C for 30 min. Total basic product showed a mass spectrum corresponding to diformylbi-indoline: 439 (42%), 381 (18), 367 (100), 349 (20), and 340 (45).

Mono-acetylbi-indoline. A solution of the bi-indoline

2′,3′-dihydro-(4a) (0.2 mg) in acetic anhydride (0.5 ml) was left at 20 °C for 30 min, and the mixture then worked up for basic material. The peak at highest mass in the mass spectrum, 420, corresponds to $C_{26}H_{34}N_4O$; $\lambda_{max.}$(EtOH) 251, 281, and 291; $\lambda_{infl.}$ 300 nm (qualitative spectrum). Mass spectrum (130 °C, 70 eV): 420 (24%), 349 (100), 348 (75), 276 (9), 259 (11), 245 (11), 234 (27), and 189 (18).

Monoacetylter-indoline. The ter-indoline was acetylated under the above conditions. The peak at highest mass in the mass spectrum, 608, corresponds to $C_{38}H_{52}N_6O$. Mass spectrum: 608 (12%), 537 (90), and 536 (100).

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