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THE STRUCTURE OF ROXBURGHINES A–E, NEW INDOLE ALKALOIDS FROM AN *UNCARIA* Sp*

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Abstract—The structural elucidation of roxburghines A–E, new diastereoisomeric indole alkaloids $C_{31}H_{32}N_4O_2$, isolated from the leaves and stems of an *Uncaria* Sp., is reported. The derivation of the skeleton (**4**) from two tryptamine and one monoterpenoid C_{10} units is consistent with the current biogenetic theory. Tetrahydroalstonine, one isomer, and dihydrocorynantheine were also isolated.

THE plant *Uncaria gambier* Roxb. (*Ourouparia gambier* Baillon) belongs to the family Rubiaceae. It grows in Malaysia and is extensively used to produce the commercial tannin Gambir. This tannin contains fluorescent alkaloids, previously investigated by Pavolini *et* al.,[1] and recently shown to have an aromatic ring E yohimbine type structure.[2,3] These results prompted further investigation of *Uncaria gambier* itself, from which an unknown base with sympatholitic activity has been reported.[4] Dihydrocorynantheine, its 9-OH derivative (gambirine[5]) and three oxindole alkaloids with a corynantheine skeleton, rhynchophylline, isorhynchophylline and rotundifoline[6] have been isolated from the leaves and stems of the plant. Chan recently found the heteroyohimbine-type oxindoles gambirdine and isogambirdine in the same plant.[7]

Different batches of leaves and stems of *U. gambier*, supplied by the Botanic Gardens of Singapore, appeared, however, to contain a completely different set of tertiary alkaloids. These leaves are morphologically similar but of a slightly different colour. We have not been able to establish whether these new batches belong to a variety of *U. gambier*, or whether season and place of collection account for the difference.

None of the alkaloids was found in the methanolic extract of the new leaves, but the presence of five new bases was clearly indicated by the red reaction with ceric sulfate on TLC plates. Silica gel chromatography afforded the pure compounds, which we propose to call roxburghine A (**1**), B (**2**), C (**3**), D (**4**) and E (**5**). Two of them, roxburghine D and E, are also present in the stems, together with tetrahydroalstonine, one isomer, and dihydrocorynantheine.

Some physical constants and the amounts isolated are reported in Table 1. All five alkaloids have the molecular formula $C_{31}H_{32}N_4O_2$ (high resolution mass spectra), and show very similar spectral properties. The UV spectra of **1–5** in neutral (95% EtOH) solution show differences only in the absorption intensity. They show no shift

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TABLE 1

Roxburghine	No.	mg*	m.p.	$[\alpha]_D^{20}$ in MeOH	$Ce(IV)(SO_4)_2$ reaction	R_D†
A	1	3–5	290–295°	−264°	brown	1·61
B	2	34	255°	−350°	red	1·39
C	3	10	245–250°	−221°	red-violet	1·26
D	4	53	197–200°	+160°	red	1·00
E	5	18	234–236°	−105°	red	0·15

* amount isolated from 1 kg of leaves.

† on TLC (SiO_2 Merck HF_{254}) in ether; R_f value of roxburghine D = 1·00.

in acidic (N HCl) and basic (N NaOH) media. The IR spectra also are very similar: the prominent bands are NH at 2·96 and 3·08 μ and C=C—CO at 6·02 and 6·20 μ (**4** in KBr). Differences in the chemical shift of some signals and coupling constants are observed in NMR, while mass spectral fragmentations are not completely alike. This information, together with the optical activity, indicates that the roxburghines are probably diastereoisomers.

The formula $C_{31}H_{32}N_4O_2$ is unusually intermediate between a "monomeric" and a "dimeric" indole alkaloid.[8] The only known alkaloids of this type are cincophyllamine and isocincophyllamine,[9] for which the proposed structures have tryptamine combined with a quinuclidine indole alkaloid.

As the amount of material available was limited, due to difficulty in obtaining supplies, the chemical degradation reactions were almost exclusively made on roxburghine D (**4**), although for some reactions **2** and **5** were used. The NMR spectrum at 100 MHz of roxburghine D (acetone-d_6) shows (Fig. 4) two NH at 9·72 and 10·08 δ (two unsubstituted indole nuclei), one $\diagdown$C=CHN— or $\diagdown$C=CHO— vinylic proton at low field (7·53 δ), one OMe (3·64 δ), one tertiary Me (singlet) at high field (1·21 δ), a $-CH_2-CH-N$ at 4·31 δ, (shifted 1 ppm to lower fields by addition of acid), and 14 other protons between 1·25 and 3·7 δ. The UV absorption (Fig. 1) was not consistent with the simple summation of two unsubstituted indole chromophores, but indicated the superposition of a strongly conjugated group. As already mentioned, the IR suggested an α,β-unsaturated CO. The conjugated double bond appeared fairly resistant to catalytic hydrogenation, that succeeded only in low yields with PtO_2 in acetic acid, to give a dihydroderivative $C_{31}H_{34}N_4O_2$ (**6**). Similar results were obtained with Zn and acetic acid. The saturation of the double bond was shown by the new CO band in IR (5·80 μ), by the absence of the vinylic proton in NMR, and by the change in UV, which was now a typical indole spectrum (Fig. 1). Subtraction of the absorption of **6** from that of **4** (Fig. 1) gave a chromophore with λ_{max} at 290 nm (ε 25500). The only possible explanation of such a high absorption was an enamino–carbonyl system $\diagdown$N—CH=C—C=O, which was also consistent with the low-field shift of the vinylic CH in the NMR spectrum of **4**. This interpretation directed our attention towards vallesiachotamine (**7**), the only known alkaloid with such a chromophore,

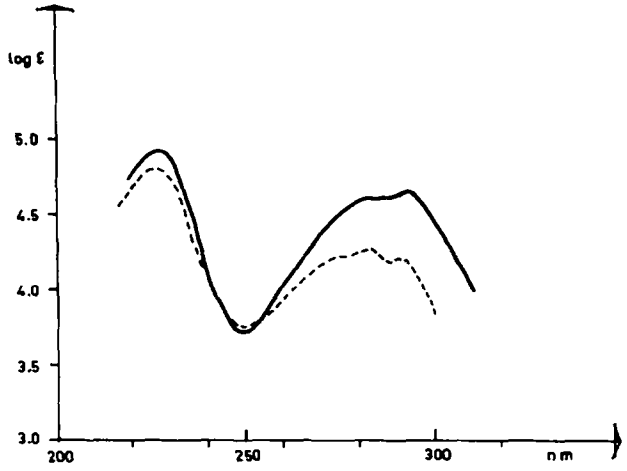
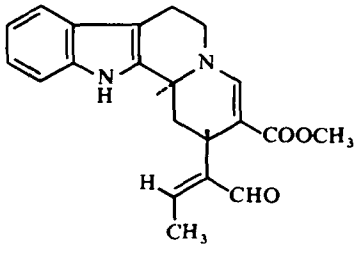


FIG. 1 UV spectrum of ——— roxburghine D (4) and - - - - dihydroroxburghine D (6).

the structure[10] and stereochemistry[11] of which has recently been established. Subtraction of the absorption of tetrahydroharman* from that of 4 (Fig. 2) gave the spectrum of vallesiachotamine,[10] thus providing a clue to the presence in 4 of three



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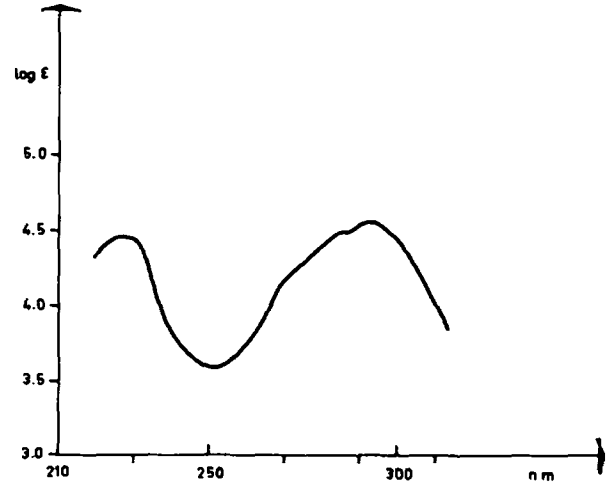


FIG. 2 Subtraction of the UV spectrum of tetrahydroharman from that of 4.

* Prepared according to P. Gray, *J. Am. Chem. Soc.* 76, 2796 (1954); UV: 225·5, 287, 292·5 nm (log ε 4·52, 3·87, 3·75 in 95% EtOH).

independent chromophores, two of the tetrahydro-β-carboline type, and one of the enamino–carbonyl type. The third chromophore in **4** could then be extended to $\diagup\!\!\diagdown N-CH=\overset{|}{C}-COOCH_3$. Thus, while the ester group was highly resistant to warm methanolic NaOH, treatment with N HCl[12] in methanol gave the decarbomethoxy-derivative **8**, $C_{29}H_{30}N_4$. It is an unstable compound, but the disappearance of the 290 nm chromophore in the UV and of the CO band in the IR could be observed. The NMR spectrum differs from that of **4** by the absence of OMe, and furthermore shows at 6·01 δ a doublet of 7 Hz with further small splitting (ca. 1 Hz), which collapses to a singlet on irradiation at 4·28 δ. The pattern in the 4·2–4·4 region integrates for two superimposed protons, one of which is the $-CH_2-\overset{|}{C}H-N\diagup\!\!\diagdown$ (4·37 δ), and the other, not analysed, must be the H_β of the sequence $N-CH_\alpha=CH_\beta-R$, coupled to H_α (6·01 δ). Here, as in the spectrum of **4** (Fig. 4), absence of coupling with NH indicates that the nitrogen is tertiary. The vinylogous urethane nature of the chromophoric group is in agreement with the value of pK^*_{MCS} of **4** (6·39, as is usual for tetrahydro-β-carbolines). Compound **4** behaved as a monoacidic base when titrated with 0·1 N HCl, as the enaminoester is too feeble a base to be titrated under these conditions.† Moreover, following the indications of Djerassi,[10] **4** could be now reduced quantitatively to **6** with $NaBH_4$ in acetic acid.[13]

The tertiary nature of the third and fourth N atoms was established by (i) the mass spectrum of the monomethiodide **9**, (ii) the failure of acetylation, and (iii) treatment with D_2O in anhydrous dioxan, which exchanged (mass spectrometric determination) the two indolic N_a-H.

The following experiments were directed to determine the skeleton of roxburghine D. Dehydrogenation with Se at 300° gave a mixture from which only 3-ethylindole and harman could be isolated. With Pd black a complex mixture was obtained, from which a very small amount of a compound of mass 428, with an extensively conjugated chromophore and still containing the tertiary Me was extracted. As the yield was very low, work in this direction was discontinued. Hofmann degradation of **4** methiodide or **6** methiodide with alcoholic N KOH, or 20% NaOH aq, or Ag_2O, yielded only a derivative of **8**. Also reduction of **4** with Na in NH_3 reduced only the double bond and the ester function, affording the dihydrocarbinol **10**. Intractable products were obtained from $Pb(AcO)_4$ or $Hg(AcO)_2$ or BrCN treatment. Cleavage of **5** with phenyl chloroformate[14] gave an urethane in which the chlorine had been exchanged by ethoxyl (**11**) or hydroxyl (**12**), depending on the solvent. The reagent must have attacked the enamine moiety, as the dihydroderivative **6** was unaffected, but no further information on the structure could be obtained.

The attack of iodine and sodium acetate in methanol[15] on **4** yielded a yellow optically active compound (**13**), probably an iodide. Its mass spectrum shows prominent peaks at *m/e* 486, 471, 428 and 413, which may be explained by a thermal Hofmann reaction,[16] with elimination of HI, followed by loss of CH_3^*, $COOCH_2$, and CH_3^* + $COOCH_2$. The absence of other fragments indicates a stable policyclic aromatic ion.

† Heterocyclic analogues of this compound have *pK* between 1 and -1 (unpublished results from this laboratory).

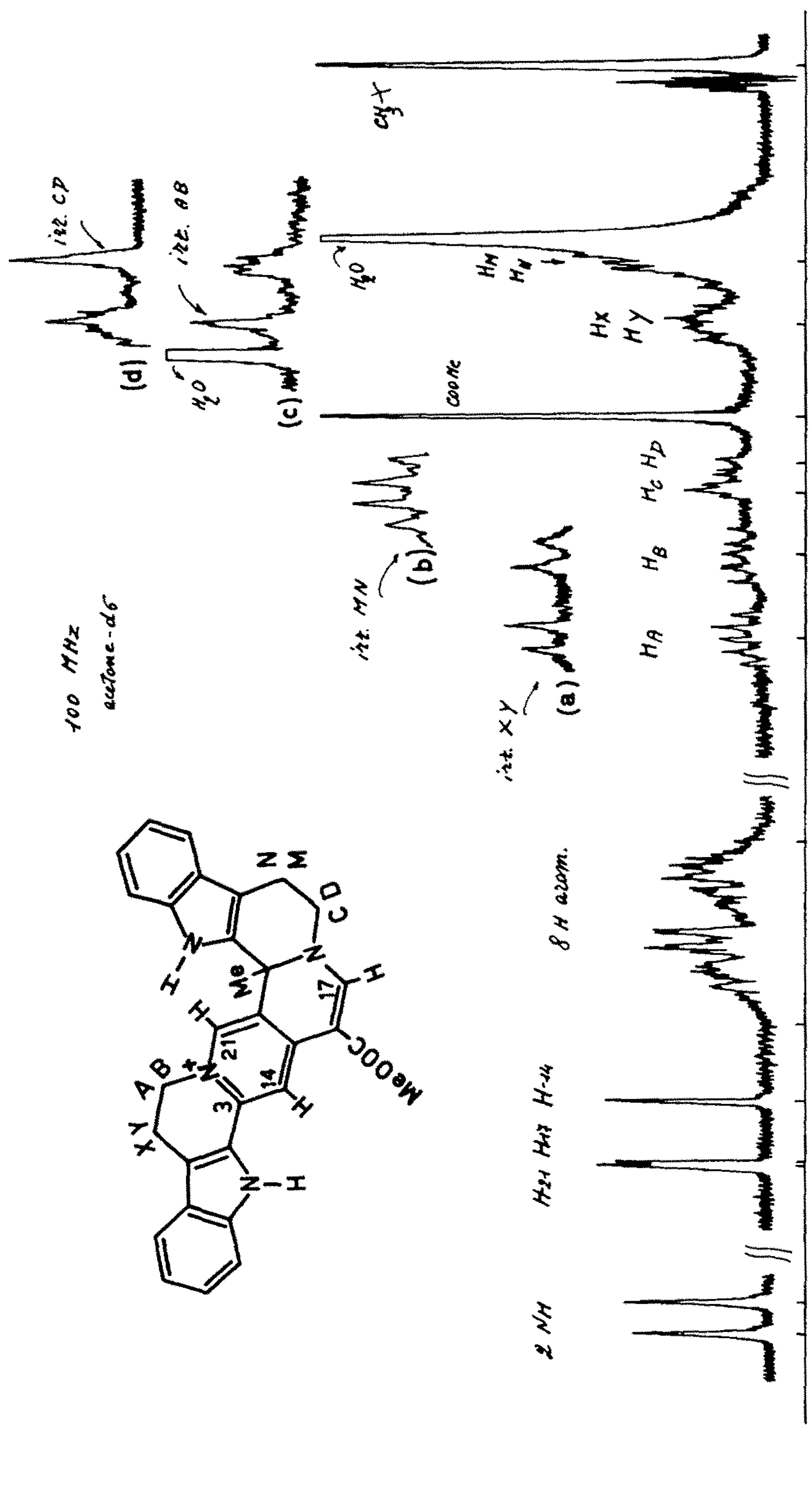
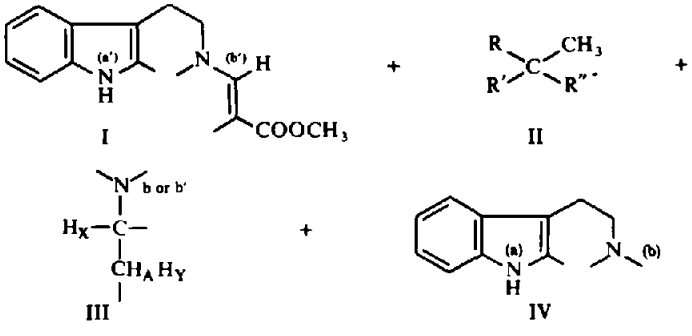


FIG. 3 NMR spectrum of dehydroroxburghine D (13).

These data would point to a formula $C_{31}H_{27}N_4O_2I$, which is consistent with the total proton count in the NMR spectrum of **13** (100 MHz, acetone-d_6). Fig. 3 shows: 2 indole NH, 8 aromatic protons, three singlets of one H each at low field, one OMe, and the C—Me. The other 8 protons appear as two ABXY systems (named in Fig. 3 ABXY and CDMN). The irradiation at the center of the XY multiplet transforms the AB portion into a system of two doublets with J = 13·5 Hz (Fig. 3a), leaving all the other signals unchanged. Irradiation of the MN protons, partially masked by H_2O absorption, changes only the low field multiplet (H_C and H_D) into two doublets with J_{CD} = 12·5 Hz (Fig. 3b). The decoupling of H_M and H_N results from irradiation at 4·2 δ (Fig. 3c), whereas strong irradiation of the AB pattern produces only a perturbation (Fig. 3d) of the X and Y protons, because of the larger δ_{AB}. The AB multiplets can be interpreted as a 5- and 7-line pattern, with $|J_{AX} + J_{AY}|$ = 13·0 Hz and $|J_{BX} + J_{BY}|$ = 14·0 Hz respectively. The XY and the CDMN patterns are too complicated to be analysed. J_{AB} and J_{CD} are therefore geminal couplings. The only possible assignments for the protons are according to the two different indole—CH_2—CH_2—$N_{(b)}$ sequences. Consequently it can be assumed that these two sequences are also present in roxburghine D. If the two couples of C atoms had been involved in a rearrangement, though this is unlikely under the mild conditions of the reaction **4** → **13**, they would not remain saturated. To account for even a remote possibility of a skeletal rearrangement, no other deduction based on **13** will be made here. The discussion of its formula and chemical shift values is below (page 2268).

The following partial structure containing 28 of the 32 H atoms of roxburghine D is deduced from the experiments outlined:



At this point an accurate analysis of the 100 MHz NMR spectrum of roxburghine D itself, with extensive double and triple resonance experiments (Fig. 4, detailed discussion below) gave the complete sequence of the remaining protons, named $H_{C,B,M,N}$. H_X (4·31 δ) must be the hydrogen at C-3 in a tetrahydro-β-carboline, i.e. adjacent to a nitrogen $N_{(b)}$ (or $N_{(b')}$), and must be equatorial,[17] because of its chemical shift, displacement with acid and values of the coupling constants.

The two protons H_A and H_Y must be geminal since both are coupled with each other and with H_X. As H_A and H_B show diaxial interaction, they must be axial. H_C is strongly coupled with H_B and since it does not show any interaction with H_A and H_Y, it must be vicinal to H_B and axial. Moreover, H_C is coupled with two other protons (H_M and H_N), whose chemical shift is in agreement with a position α to a N atom. The whole sequence can be represented in partial structure **V**:

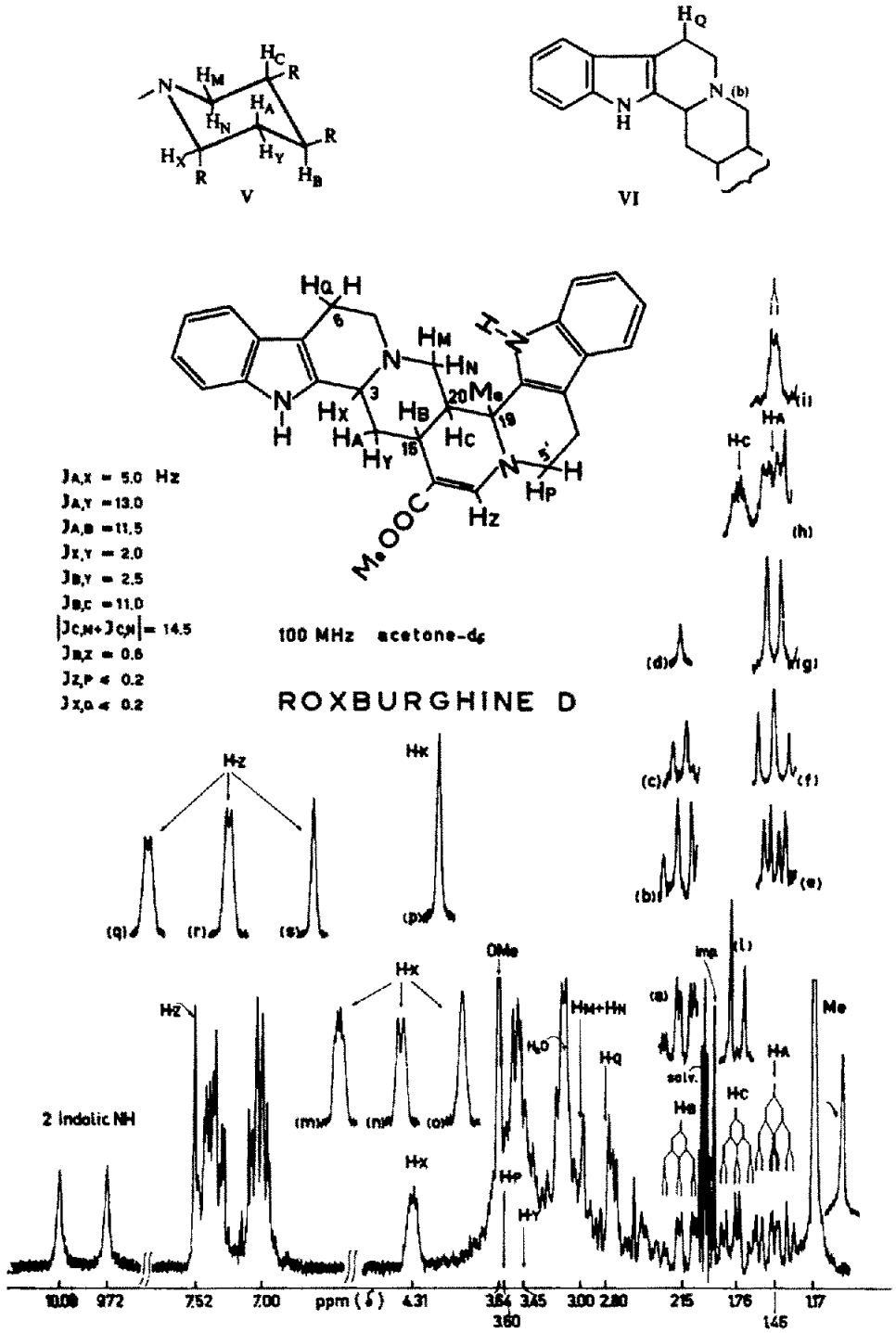
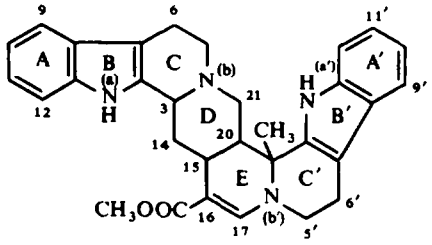


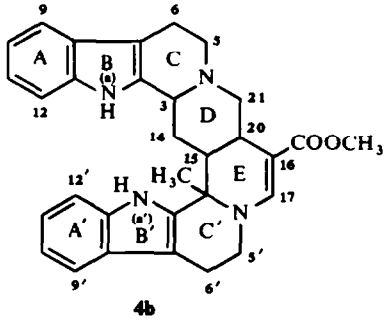
FIG. 4 NMR spectrum of roxburghine D (4).

Chemical shift values and effects on them of non-bonding interactions are discussed later. Small long-range couplings were also observed between H_B and the vinylic proton H_Z, between this latter and a proton at 3·60 δ (H_P), and between H_X and a proton at 2·80 δ (H_Q) probably at position C-6.

The partial formula V requires that the N atom in V must be the same as in IV, and combination of IV and V gives the partial formula VI. Combination of partial formulae I, II and VI accounts for all the atoms of roxburghine D, and indicates only two possible formulae, **4a** and **4b**:



4a

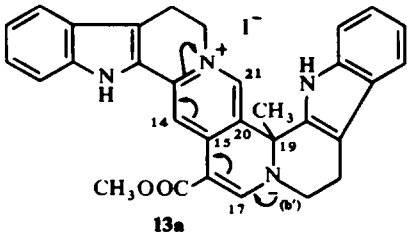


4b

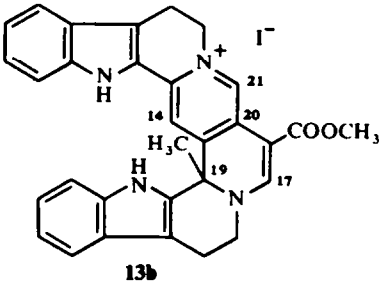
Careful analysis of mass spectra of roxburghine D, d_2-roxburghine D, **6**, **8** and **10** all support the proposed formulae **4a** and **4b**. The detailed discussion and interpretation of fragmentation is postponed (see Schemes 1–4), but the main points indicating the presence in **4a** and **4b** of groups I–V are the following: (i) the much higher percentage of M^+-CH_3 peak in respect to the ubiquitous M^+-1 is in agreement with the tertiary and near-to-nitrogen nature of the Me group; (ii) the cleavages of **4**, d_2-**4** and **6** into a series of couples of ions, e.g. 362–130, 336–156, 321–171, 307–184 for **4**. The former ion contains the tetracyclic "vallesiachotamine" C_{17} unit, the latter a tricyclic tetrahydro-β-carboline C_{11} unit or part of it. This is again proof of the presence of two tetrahydro-β-carboline moieties in **4**, i.e. of the structure of fragment IV; (iii) the fragmentation into the couple 362–130 requires an interpretation (see below) which supports the presence of an eighth ring (ring E). It must be noted that mass spectral results do not give evidence in favour of **4a** in respect to **4b**.

By reexamining the structure of the dehydrogenation product **13**, we can see that all the data are consistent with formula **13a** obtained by dehydrogenation of **4a**, or with formula **13b**, deriving from **4b**. The extended conjugated UV spectrum and the absence of any side chain (NMR) are evidence of a policyclic highly conjugated skeleton. In particular, the three singlets at low field in the NMR spectrum (Fig. 3)

exclude all 10 possible combinations of the "vallesiachotamine-type" tetracyclic C_{17} unit with the tetrahydro-β-carboline C_{11} unit, as all would give rise to a dehydrogenated product with two aromatic *ortho* protons.



13a



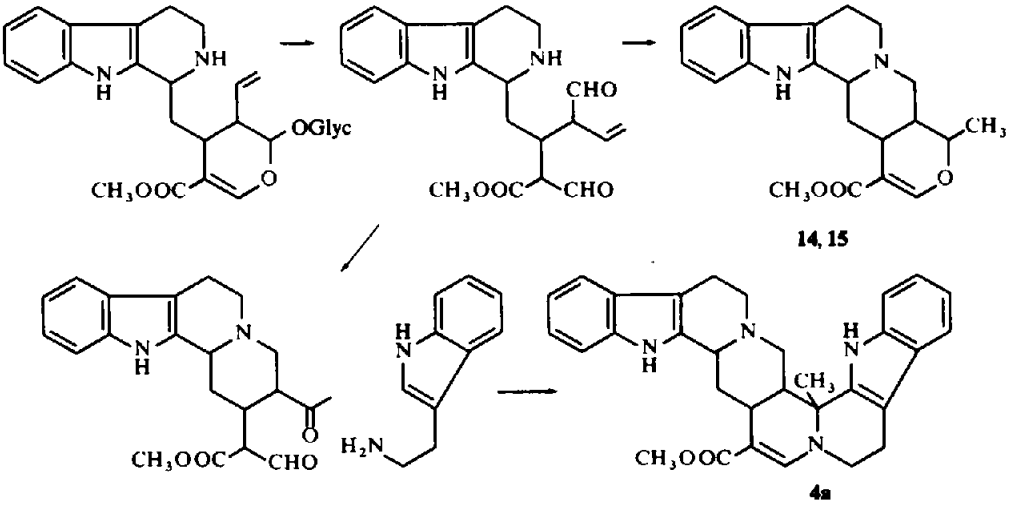
13b

The possibility of positive charge delocalization on both the N atoms $N_{(b)}$ in **13a** could explain the very low field shift of the two protons at C-17 and C-21 (8·78 and 8·76 δ), and, together with the deshielding effect of the coplanar *peri* carbonyl group at C-16, also of C-14 (8·43 δ). The 8 protons of the two indole—CH_2—CH_2—$N_{(b)}$ sequences are differently deshielded as a consequence of the distribution of the N^+ positive charge (which should be prevailing on $N_{(b)}$) and also of the different environmental effects (mainly indole rings). For probably the same reasons the C—Me signal goes downfield from **4** (1·21 δ) to **13** (1·99 δ). The N^+ charge delocalization is not possible in the corresponding formula **13b**.

In compound **13** the carbon 19, carrying the Me group, is the only one left which is chiral. As a result, **13** appears to be strongly levorotatory ($[\alpha]_D = -520°$). The presence of the asymmetric center may be another, though weak, argument against the possibility of a rearrangement during the dehydrogenation. The same reaction with I_2 and AcONa was carried out on roxburghines B, C and E, to give three dehydroderivatives. They have identical TLC behaviour, mass and UV spectra (and also NMR for the compound from **2**). The optical rotations were resp. $[\alpha]_D = +580°$, $-670°$ and $+430°$. These determinations were made on very small amounts of not highly purified material. The samples were poorly soluble and iodine was an expecially difficult contaminant to remove. In our opinion the differences are due to impurities, dehydroroxburghines B and E are identical, and antipodes at center 19 to dehydroroxburghines C and D, which are identical too. The conversion of roxburghine E (**5**) into roxburghine B (**2**) by heating with Zn and AcOH is in agreement with the results. These conditions are known[18] to induce epimerization at carbon 3. Thus if **5** and **2** are epimers at C-3, they must have the same configuration at C-19, and give the same dehydroderivative, as indeed happens. Similar epimerization

reactions on **3** and **4** gave only dihydroderivatives. This all confirms that at least four roxburghines are diastereoisomers.

The only remaining point is the choice between the two structures **4a** and **4b** of roxburghines. The long-range coupling between H_B and H_Z in the NMR spectrum of roxburghine D (Fig. 4) favours **4a**. This coupling is much more reasonable in **4a**, where H_B (H_{15}) is in an allylic position in respect to H_Z (H_{17}). H_B (H_{15}) is one bond farther from H_Z (H_{17}) in **4b**, and the stereochemical conditions for such a coupling are not realized. Moreover the chemical shift values of H_B (2·15 δ) and H_C (1·76 δ) are much more in agreement with an allylic position and resp. a position linked only to saturated carbons, as occurs in **4a**, but not in **4b**. The strongest argument in favour of **4a** is the consistency of its structure with the current theory of the biogenesis of indole alkaloids.[19] The roxburghines apparently derive from two tryptamine and one monoterpenoid C_{10} units. **4b** would contain a C_{10} unit which does not correspond to any of the three so far recognized as ubiquitous in the Corynanthe, Iboga and Aspidosperma type alkaloids, whereas **4a** contains a loganin-derived C_{10} unit of the Corynanthe type. The following is a rough possible biogenetic scheme:[20]



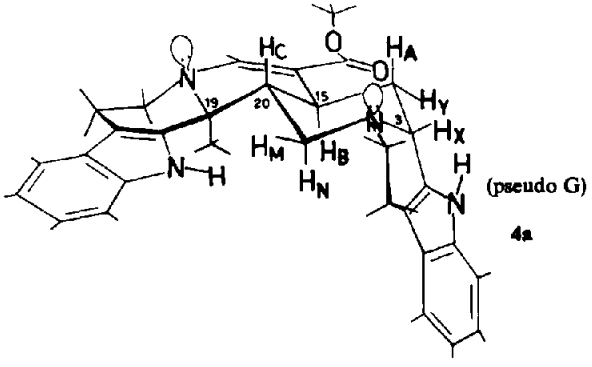
The presence of tetrahydroalstonine* (**14**) and one isomer of it (**15**) in the stems of the plant indirectly supports this hypothesis.

Results of NMR analysis of roxburghine D can also be used to elucidate its stereochemistry. It must be noted that the skeleton of ABCDE rings (**4**) in the roxburghines is the same as that of heteroyohimbines. The four asymmetric centers in both alkaloid types are equivalent (C-3, C-15, C-19, C-20). The piperidine ring D is assumed, as usual, to be more stable in the chair conformation, and the coupling constant value between H_{15} and H_{20} (J_{BC} = 11·0 Hz) requires a diaxial interaction, hence D/E ring junction must be *trans*. As H_X (H at C-3) has already been shown to be equatorial, C/D ring junction must be *cis*. By comparing NMR data with those of conformations

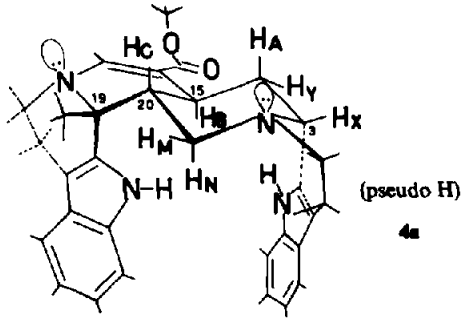
* K. C. Chan, *Phytochemistry* 8, 219 (1969) has very recently reported the isolation of tetrahydroalstonine from an unknown *Uncaria* species.

corresponding to each of the possible configurations,* the latter are reduced to the four *pseudo* ones (*pseudo G*, 3β, 15α, 20β, 19α, and *pseudo H*, 3β, 15α, 20β, 19β, and their antipodes).[21] Nothing can be said about the configuration at C-19 in respect to the other centers.†

The *pseudo G* and *pseudo H* (or their antipodes) proposed as the most probable for roxburghine D (**4**) are in agreement with the chemical shift values of the protons of ring D. It is now possible to explain the strong difference ($\Delta\delta = 2{\cdot}0$) between the two geminal protons H_A and H_Y. The anomalous low field shift of H_Y (on a carbon linked to others all saturated) is due to the strong deshielding effect of both the indole AB



4a



4a

rings and the conjugated carbonyl group. H_Y is almost coplanar with the carbonyl and the indole ring, and it lies very near (according to Dreiding models, ca. 2·5 Å) to the $\rangle N_{(a)}$-H group. The allylic proton H_B may be slightly shielded (2·15δ) by the perpendicular conjugated carbonyl. H_M and H_N are shown by decoupling experiments to have similar chemical shifts (ca. 3·0δ), whereas a difference of about 1 ppm should

* With the assumption, supported by examination of Dreiding molecular models, that the steric hindrance due to the presence of the two tetrahydro-β-carboline rings ABC and A′B′C′ is not very different for all the conformations of each configuration.

† The *pseudo G* may exist in two conformations (E/C′ ring junction *trans* and *cis*) of comparable energy, whereas *pseudo H* (E/C′ *cis*) seems slightly favoured in respect to *pseudo H* (E/C′ *trans*), where ring E becomes a boat.

be expected[22] between the equatorial and the axial proton, due to the N lone pair effect.* In *pseudo H*, but not in *pseudo G*, this difference could be compensated by the deshielding effect of the indole (A'B') rings on the axial proton (H_N), which is parallel to the aromatic plane, and very near to the $\rangle N_{(a)}$-H group.

If sufficient material is available, it is hoped to support these working hypotheses by NMR data of other roxburghines, by epimerization and correlation experiments. Furthermore, the preceding biogenetical scheme indicates also a possible synthetic pathway[23] which could not only confirm the structure, but also the stereochemistry of roxburghines. Synthetic experiments along this line are in progress.

Analysis of the NMR spectrum of roxburghine D

The spectrum of roxburghine D. The pattern of protons in ring E, and their interacting neighbours ($H_{X,A,Y,C,B,M,N,Z,Q,P}$) was determined by decoupling experiments. Proton H_X (4·31δ) is the most clearly visible. Upon irradiation at 2·28 δ it sharpens to give a well resolved double doublet of J = 2·0 and 5·0 Hz (Fig. 4m). The small allylic-type interaction with the proton at 2·80 δ, already found in vallesiachotamine,[10] is probably due to one of the hydrogens on C-6 (H_Q) and is estimated to be $J_{X,Q} \leqslant 0{\cdot}2$ Hz. The irradiation of H_X changes only the upper part of the spectrum, or more precisely the 8-line pattern centered at 1·46 collapses to a double doublet of 13·0 and 15·5 Hz, the separation of 5·0 Hz (J_{AX}) having disappeared (Fig. 4f). The reverse experiment (irradiation of H_A at 1·46 δ) converts H_X into a poorly resolved signal (Fig. 4o) resulting from J_{XQ} and the remaining coupling of 2·0 Hz. Since the last coupling of H_X vanishes upon irradiation at 3·45 δ (Fig. 4n), a proton (H_Y) hidden by other complex absorptions must be responsible for this interaction (J_{XY} = 2·0 Hz). Triple resonance experiment proved this point: on simultaneous irradiation of H_A at 1·46 and H_Y at 3·45 δ H_X collapses into a singlet† (Fig. 4p). H_A (Fig. 4e) is also decoupled on irradiation at 3·45 δ (H_Y). It becomes a double doublet of 11·5 and 5·0 Hz. Simultaneous irradiation of H_X simplifies it further (Fig. 4g) to a doublet (11·5 Hz). The H_A pattern is complete as follows: J_{AY} = 13·0 Hz, and J_{AB} = 11·5 Hz, which is the third coupling constant of H_A, with the proton H_B at 2·15 δ. H_B consists of a triplet of doublets with splittings of 11·5 and 2·5 Hz. This sharpens upon irradiation of the vinylic proton (H_Z) at 7·25 δ (Fig. 4a), and further simplifies to a triplet by decoupling H_Y (Fig. 4b) and to a doublet structure of 11 Hz‡, upon irradiation of H_A (Fig. 4c). The relationship between H_A and H_B can be proved by triple resonance. Irradiation of H_B leads to the poorly resolved (Fig. 4h) double doublet pattern of H_A. However on simultaneous irradiation of H_B and H_Y the 8-line signal of H_A collapses into a doublet of 5·0 Hz (Fig. 4i, residual coupling J_{AX}).

The fifth proton of the sequence lies at 1·76 δ (H_C) and appears as a triplet of doublets with splittings of 11·5 and 3·0 Hz. Upon strong irradiation at 3·00 δ, it collapses to a sharp doublet of 11 Hz, which is not removed by double irradiation on

* The averaging of δ_M and δ_N by flipping of $N_{(b)}$ is made unlikely by the formation of a boat D ring, which requires dihedral angles not consistent with NMR coupling constants values.

† J_{XQ} is too small to be detected here.

‡ This doublet is indeed broad, as J_{BY} = 2·5 Hz is still present. The splitting of 2·5 is not well resolved, because of the strong irradiation necessary to decouple the adjacent proton H_A, and the presence of another interacting nucleus at 1·76 δ (H_C, see below).

sweeping the whole field. Thus H_C appears to interact with two protons (H_M and H_N) of about the same chemical shift ($\sim 3\ \delta$)* and is also probably coupled to the near proton H_B, with $J_{BC} = 11{\cdot}5$ Hz.

Irradiation of H_B perturbs, as was expected, the signal of H_C (Fig. 4h). However, the interaction between H_C and H_B is proved by triple resonance. Upon irradiation of H_C at 1·76 and H_A at 1·46 δ, J_{AB} and J_{BC} vanish and H_B collapses into a narrow signal, resulting from $J_{BY} = 2{\cdot}5$ Hz (Fig. 4d). Some information on J_{CM} and J_{CN} is needed to complete the analysis of H_C. H_M and H_N are hidden by other absorptions. As they probably have similar chemical shifts, the value of J_{CM} and J_{CN} cannot be deducted from H_C pattern alone. Only their sum $|J_{CM} + J_{CN}| = 14{\cdot}5$ Hz is obtained. The last decoupling experiment proves the interactions between the vinylic proton H_Z, H_B and H_P: H_Z is a doublet of 0·6 Hz (Fig. 4q). This sharpens upon irradiation at 3·60 δ (Fig. 4r) (H_P, $J_{ZP} \leqslant 0{\cdot}2$ Hz) and further collapses to a singlet (Fig. 4s) by decoupling of H_B at 2·15 δ. From the chemical shift value, H_P is assigned to one proton at C-5 near to nitrogen.

Analysis of the mass spectrum of roxburghine D (**4**)

The mass spectrum of the main alkaloid roxburghine D (**4**) is shown in Fig. 5. In Table 2 the elemental compositions of the important fragment ions together with the corresponding peaks in the spectra of d_2-roxburghine D (d_2-**4**), decarbomethoxy-roxburghine D (**8**), dihydroroxburghine D (**6**) and the dihydrocarbinol **10** are given.

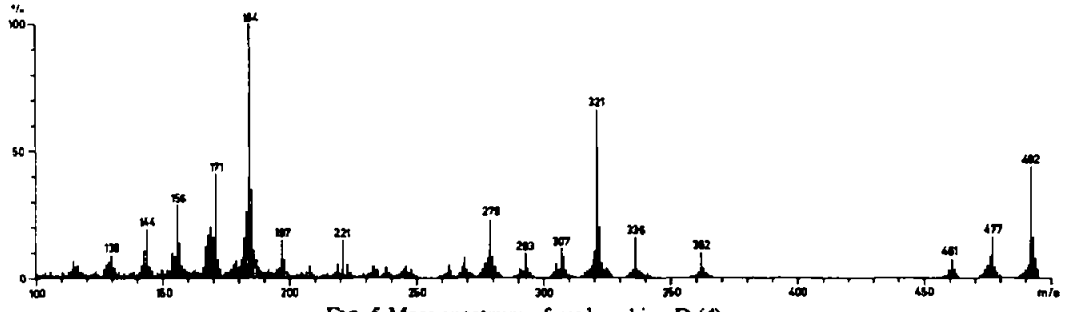
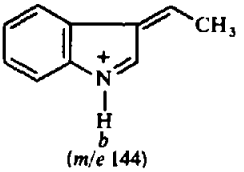


FIG. 5 Mass spectrum of roxburghine D (**4**).



b
(*m/e* 144)

Peaks at *m/e* 130 (*a*), 144 (*b*) and 156 (*c*), typical for indole alkaloids[24] unsubstituted in the aromatic part, are present in the mass spectrum of **4**. In the molecular ion region, besides the M^+ at *m/e* 492 there are signals for M^+-H (*m/e* 491), M^+-CH_3O (*m/e* 461) and an intensive one for M^+-CH_3 (*m/e* 477). Interestingly in the mass spectrum of **4** eight pairs of peaks exist which by addition of their elemental composition give the elemental composition of the molecular ion. These pairs are: *m/e*

* $\Delta\nu_{MN} \leq 20$–30 Hz.

TABLE 2. IMPORTANT PEAKS IN THE MASS SPECTRA OF d_2-ROXBURGHINE D (d_2—4), DECARBOMETHOXY ROXBURGHINE D (8), DIHYDRO ROXBURGHINE D (6) AND THE DIHYDROCARBINOL 10[b]

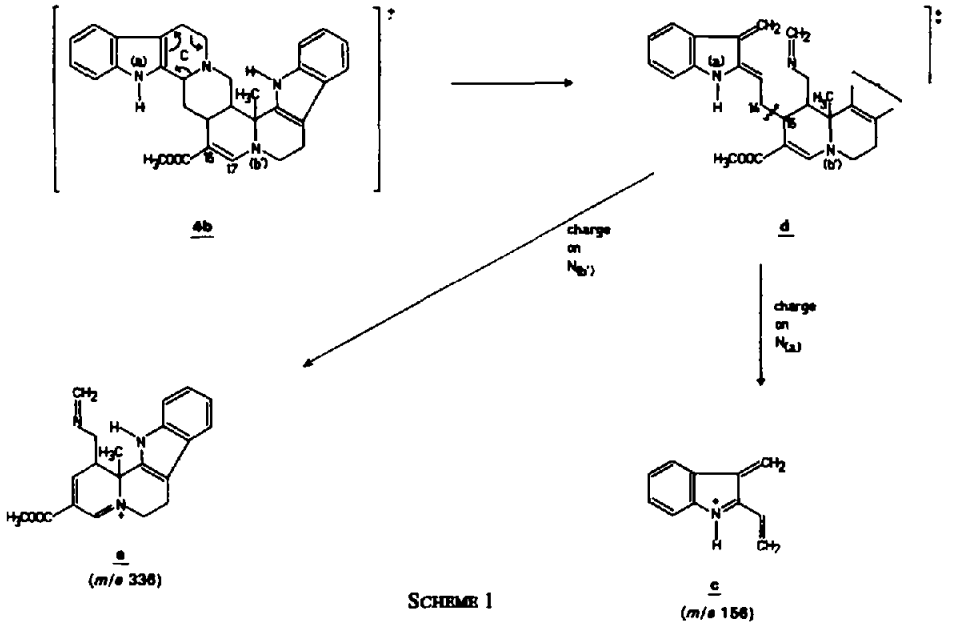
		4		d_2-4	8[c]		6		10[a]	
m/e	%	Formula	Elemental Composition*	*m/e*	*m/e*	%	*m/e*	%	*m/e*	%
492	43	M^+	$C_{31}H_{32}N_4O_2$	494	434	43	494	74	466	57
491	14	M^+-1		493	433	12	493	5	465	14
477	16	M^+-CH_3	$C_{30}H_{29}N_4O_2$	479	419	16	479	38	451	50
461	7	M^+-OCH_3	$C_{30}H_{29}N_4O$	463	—	—	463	5	—	—
362	10	g	$C_{22}H_{24}N_3O_2$	363	304	3	364	16	336	17
336	16	e	$C_{20}H_{22}N_3O_2$	337	278	6	—	—	—	—
321	66	k	$C_{20}H_{21}N_2O_2$	322	263	100	323	5	—	—
307	12	o	$C_{19}H_{19}N_2O_2$	308	[d]	—	[d]	—	—	—
294	3			294	236	15	294	27	268	21
293	10	q	$C_{18}H_{17}N_2O_2$	294	235	20	295	28	267	20
279	23	r	$C_{17}H_{15}N_2O_2$	280	221	34	281	43	253	14
246	5	M^{++}		247	217	10	247	22	—	—
221	15	l	$C_{15}H_{13}N_2$	222	221	34	223 221	81 71	223 221	86 51
197 198	15 8	p	$C_{13}H_{13}N_2$	198 199	198	28	198	40	198	91
184	100	n	$C_{12}H_{12}N_2$	185	184	42	184	100	184	90
171	41	i	$C_{11}H_{11}N_2$	172	171	28	[d]	—	171	38
156	29	c	$C_{11}H_{10}N$	157	156	22	156	20	156	47
144	19	b	$C_{10}H_{10}N$	145	144	14	144	17	144	42
130	9	a	C_9H_8N	131	130	13	130	15	130	60

* Established for all the fragments by high resolution measures.
[a] Base peak in mass spectrum of **10** is *m/e* 183.
[b] Relative percentage.
[c] Hydrochloride measurements given for **8**.
[d] Correct mass shift not detected.

362/130, 336/156, 321/171, 307/184.* Similar pairs are also registered in the spectra of d_2-**4** and **8**. The genesis of the corresponding ions from the molecular ion must be similar for each ion pair.†

1. m/e 336/156 (*Scheme* 1). Retro-Diels Alder reaction in ring C gives an intermediate *d* in which the charge could be localized either on $N_{(a)}$ or $N_{(b')}$. In both cases the diallylic activated bond C-14–C-15 has to be split giving *c* (*m/e* 156, charge on $N_{(a)}$) or *e* (*m/e* 336, charge on $N_{(b')}$). For charge stabilization in ion *d* the C-16–C-17

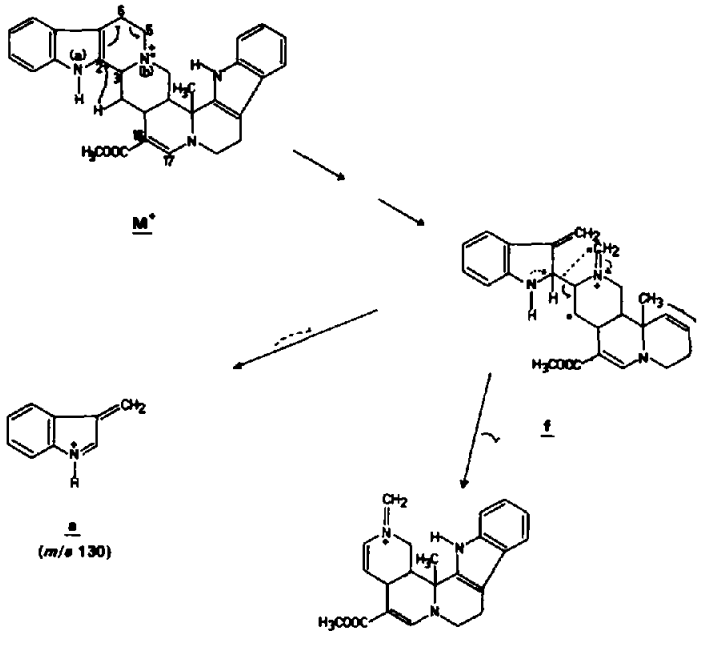
* The addition of these two peaks produces the ion *m/e* 491.
† Mass spectrometrical fragmentation is discussed on the basis of structure **4a**.



SCHEME 1

double bond is necessary. When this double bond is absent, signals in the spectra of the dihydroderivatives **6** and **10** corresponding to *e* disappear. Ion *c* however (*m/e* 156) is present. This is in agreement with the postulated intermediate *d*.

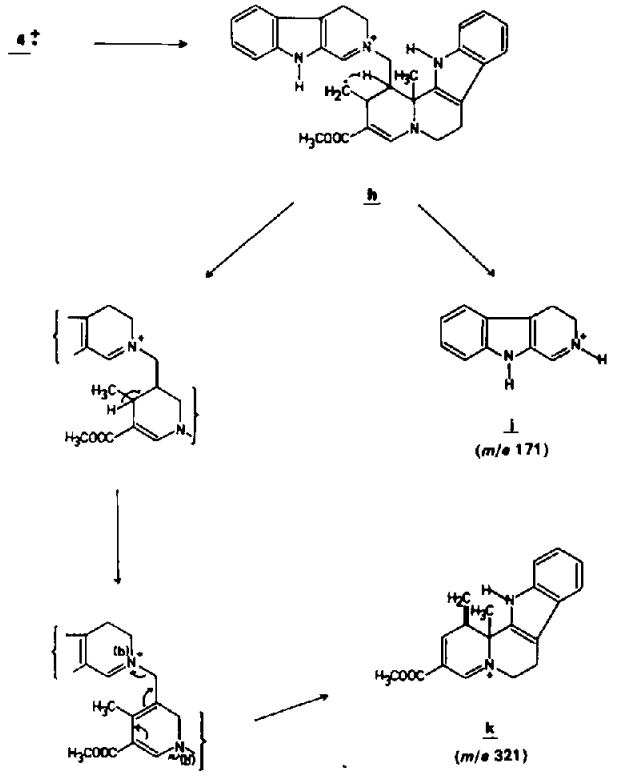
2. m/e 362/130 (*Scheme* 2). In contrast to the ion pair *m/e* 336/156 the appearance of this ion pair is not dependent on the C-16–C-17 double bond (see Table 2). Ion 362



SCHEME 2

contains the "vallesiachotamine" part [10] and a third nitrogen. On the basis of the d_2-4 spectrum and the elemental composition the third N could be but the $N_{(b')}$ atom.* Therefore $N_{(b')}$ must be connected in some way to the vallesiachotamine part. Fragmentation starts in ring C by rupture of the C-5–C-6 bond, charge on $N_{(b')}$, followed by a hydrogen abstraction (C-2/H-14; *f*). Stabilization of ion *f* could take place in two ways: rupture of C-2–C-3 bond giving *g* (*m/e* 362); charge transfer reaction $N_{(b)} \rightarrow N_{(a)}$ shown in Scheme 2 leading to the production of ion *m/e* 130 (*a*).

3. m/e 321/171 (*Scheme* 3). Formation of ion 321 depends—as in scheme 3—on the C-16–C-17 double bond. In the mass spectra of dihydrocompounds **6** and **10** the corresponding signals are of very low intensities compared with that of **4**. The genesis

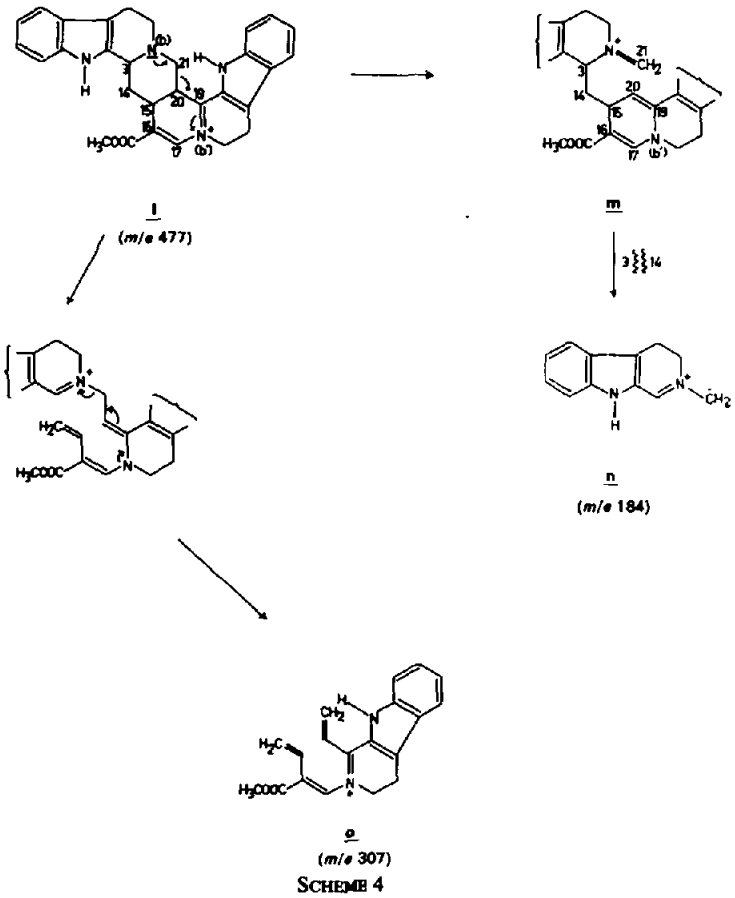


SCHEME 3

of both ions is given in scheme 3. α-cleavage to $N_{(b)}$ in the molecular ion gives *h*. This could behave like an imonium species[25] to form *i* (*m/e* 171) or following shift and loss of one hydrogen produce *j* (M^+-1). A charge transfer reaction in *j* ($N_{(b)} \rightarrow N_{(b')}$) yields *k* (*m/e* 321).

4. *m/e* 307/184 (*Scheme* 4). In contrast to the three discussed mechanisms it seems that the reaction leading to *m/e* 307 and 184 starts with the fragmentation of M^+-CH_3 ion (*l*). A charge transfer from $N_{(b')}$ to $N_{(b)}$ including the C atoms 21, 20 and 19 gives

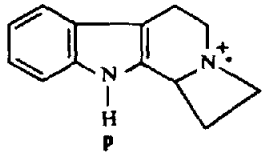
* The possibility that the ions 362, 336, 156 and 130 are formed by fragmentation of ring C' could not be excluded.



SCHEME 4

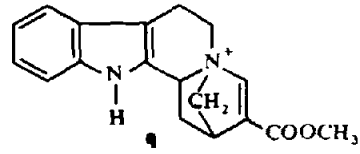
the intermediate *m*. Splitting of C-3–C-14 bond in **m** yields the most intensive ion (*m/e* 184). As for ions **a**, **c** and **i**, ion **n** could be built up in a different way. If the charge transfer in **1** includes the atoms 3, 14, 15, 20 and 19 a reverse charge transfer reaction directly yields ion *o* (*m/e* 307).

Ion **m** seems to be the precursor of some other fragment ions. By splitting the bond C-14–C-15 *p* (*m/e* 198) is formed. By a direct attack of $N_{(b)}$ at C-14 under splitting of the C-3–C-14 bond and formation of a new bond (C-3–C-21) ion *q* (*m/e* 293)[26] is made. Finally the peaks at *m/e* 279 and 221 have to be considered.* *M/e* 221 is formed



p

(*m/e* 198)

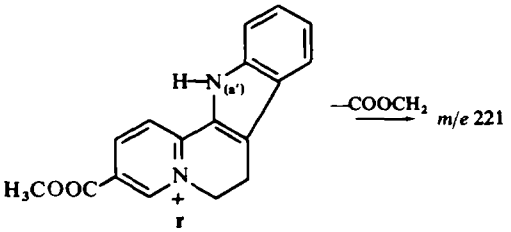


q

(*m/e* 293)

* In the mass spectra of the dihydroderivatives **6** and **10** peak 221 is also registered. It should be formed by dehydrogenation of *m/e* 223.

by the loss of $C_2H_2O_2$ from *m/e* 279 (m*); the latter (*r*) could be formulated as the product of a charge transfer reaction in *l* ($N_{(b)} \rightarrow N_{(b')}$) including the atoms 19, 20, 15, 21. Generally, the genesis of ions in the mass spectrum of **4** is dependent on the C-16–C-17 double bond (e.g. *m/e* 307). The intensity of the corresponding ions in the mass



r
(*m/e* 279)

spectra of the dihydroderivatives **6** and **10** is much lower than in **4**; otherwise they are not influenced.

Interpretation of the mass spectrum of roxburghine D in relation to the structure **4a** seems to be plausible. However, in the absence of deuterated derivatives, it cannot be proved. A similar interpretation can be drawn when considering the structure **4b**.

EXPERIMENTAL

NMR spectra were measured with A-60 and HA-100 Varian spectrometers. Spin-decoupling experiments were performed by the "frequency sweep" method. The integrals were measured with a 405/CR Hewlett–Packard digital voltmeter. Chemical shifts are in ppm (δ) from TMS, used as the internal standard, *J* are in Hz. In the text, s = singlet, d = doublet, t = triplet, q = quartet. IR spectra of KBr disks were recorded with a Perkin–Elmer Infracord (values in μ). UV spectra of solutions in 95% EtOH (λ_{max} in nm) were taken with a Beckman DK-2 apparatus. M.ps (uncorrected) were measured with a Kofler apparatus. Silica gel Merck 0·05–0·20 mm was used for column chromatography, and Merck HF_{254} for TLC. Mass spectra were measured on CEC instrument type 21–110B (70 eV, direct inlet system). High resolution values were obtained by using the peak matching method. The values are given in *m/e* (rel %).

Extraction and separation. Leaves from young plants grown near Singapore were usually collected in June. 1 kg was powdered and extracted with ether for 3 days in a Soxhlet, then with MeOH. The MeOH extract was concentrated to a syrup, made alkaline with conc NH_4OH and extracted with ether. Evaporation of ether gave a crude extract (5 g), which was adsorbed onto silica gel and charged on a chromatographic column (50 × 3·5 cm) of the same silica gel. Elution with hexane-ether mixtures, followed by ether-AcOEt, gave in the following order: *roxburghine A* (**1**), *B* (**2**), *C* (**3**), *D* (**4**), and *E* (**5**), see Table 1. Mixtures of **2** and **3** could be separated by chromatography through Al_2O_3 Woelm act. 2, eluting **2** with hexane-ether 8/1 and **3** with hexane-ether 1/1. From 1 kg of stems, extracted as described above, the following compounds were obtained: (i) *dihydrocorynantheine*, crystd. from aqueous MeOH, $[\alpha]_D^{20} = +33·2°$ (*c* = 0·087 in MeOH)[5], and identified by UV, IR, mass spectra and TLC comparison with an authentic sample; (ii) *tetrahydroalstonine* (**14**), m.p. 227–30° (hexane-ether), $[\alpha]_D^{20} = -60°$ (*c* = 0·092 in MeOH), −81° (*c* = 0·11 in Py), UV: 227, 250sh, 284, 291 (ε 43500, 11400, 7800, 6750), identified by IR, mass and NMR spectra[27]; (iii) a *heteroyohimbine alkaloid* (**15**), $C_{21}H_{24}N_2O_3$, m.p. 97–100° (hexane-ether), $[\alpha]_D^{20} = -69·5°$ (*c* = 0·095 in MeOH) and −25° (*c* = 0·11 in Py), UV: 227, 250sh, 284, 292 (ε 29000, 9400, 6150, 5300), IR: 3·0 (NH), 5·85 and 6·1 (unsatd. ester) and 3·5–3·6 (Bohlmann bands), ORD (*c* = 0·037 in MeOH): $\Phi_{295} = -4260°$, $\Phi_{255} = +6690°$, $\Phi_{237} = -11490°$, NMR (acetone-d_6): CH_3 (1·25, d, *J* = 6·5), 11 H (1·5–3·6), OMe (3·68), CH_3—C*H*— (4·38, 8 lines, *J* = 5 and 6·5), 4 indole H (6·8–7·4), =CHO— (7·46), NH (9·84). Mass: 352 (M^+, 100, Found: 352·1787 ± 0·0017; Calc. for $C_{21}H_{24}N_2O_3$: 352·1787), 351 (73), 337 (70), 293 (13), 251 (32), 223 (47), 209 (14), 197 (19), 184 (20), 169 (28), 156 (66).

Roxburghine D (**4**)

(Table 1) UV: 226, 287sh, 292 (ε 62000, 40300, 42000) (Fig. 1). ORD: (*c* = 0·027 in MeOH): $\Phi_{305} = +54000°$, $\Phi_{282} = -86500°$, $\Phi_{255} = +1800°$, $\Phi_{235} = +43500°$, $\Phi_{224} = -258000°$.

Dihydroroxburghine D (**6**)

(a) 150 mg of **4** were hydrogenated with 50 mg PtO_2 in 10 ml AcOH. After 2 days the soln was evaporated, taken up with dil NH_4OH and extracted with ether. Chromatography through silica gel with hexane-AcOEt gave **6** ($C_{31}H_{34}N_4O_2$) (40%), m.p. 225–230°, UV: 227, 276sh, 284, 291 (ε 80000, 17100, 18000, 15800), IR: 2·95 (NH), 5·80 (CO), 6·18; NMR (60 MHz, acetone-d_6): Me—C (1·32), OMe (3·73), N—C*H*—CH_2— (m, 4·32, *J* ca. 3 and 6), 8 indole H (6·8–7·6), 2 NH (9·67 and 9·85) and other abs. between 1·8 and 3·7. Mass: see Table 2.

(b) 20 mg of **4** in 3 ml AcOH were treated with 150 mg $NaBH_4$. Evaporation, taking up with dil NH_4OH and extraction with ether gave **6**.

Decarbomethoxyroxburghine D (**8**)

Compound **4** (150 mg) in 5 ml HCl and MeOH (5 ml) to complete soln were heated on a steam bath for 20 min. Evaporation taking up with dil NH_4OH and extraction with ether gave **8**, as an unstable solid, the soln of which rapidly becomes violet; UV: 227, 284, 291 (ε 64800, 15300, 13200). IR: 3·0 and 3·1 (NH), 6·1 (C=C); NMR (60 MHz, acetone-d_6): Me—C (1·30), N—CH_α=CH_β— (4·2), N—C*H*—CH_2— (m, 4·37), N—CH_α=CH_β— (6·01, d of 7 Hz again splitted of 1 Hz), 8 indole H (6·8–7·5), 2 NH (9·7). Elemental composition of important peaks in mass spectrum (Table 2):

Found	Calcd.	Composition
434·2463 ± 0·0022	434·2470	$C_{29}H_{30}N_4$
419·2238 ± 0·0020	419·2236	$C_{28}H_{27}N_4$
263·1550 ± 0·0013	263·1548	$C_{18}H_{19}N_2$
198·1140 ± 0·0020	198·1157	$C_{13}H_{14}N_2$
184·1000 ± 0·0009	184·1000	$C_{12}H_{12}N_2$

D-*exchange of roxburghine D* (d_2-**4**)

Roxburghine D (**4**, 10 mg) was dissolved in 1 ml D_2O/abs dioxan (1/1) and evaporated to dryness. The procedure was repeated 3 times. Mass: 494 (49), 493 (44), 479 (17), 478 (12), 463 (7), 363 (10), 337 (20), 322 (71), 321 (30), 308 (12), 294 (10), 293 (7), 280 (21), 270 (13), 222 (11), 198 (13), 186 (32), 185 (100), 184 (53), 172 (35), 157 (25).

Roxburghine D monomethiodide (**9**)

Compound **4** (30 mg) in 5 ml MeOH were treated with 3 ml MeI and 20 mg dry Na_2CO_3 and refluxed 15 hr. Filtration, evaporation, taking up with MeOH and pptn. with ether gave **9** (Found: I, 22·81. $C_{32}H_{35}N_4O_2I$ requires: I 20·0%), UV: 221, 284, 291 (ε 75000, 36500, 36000). NMR (60 MHz, Py-d_5): MeC— (1·18), N^+-Me (3·62), OMe (3·76), 9 H (7·0–8·0), 2 NH (11·8 and 12·3). Mass: mixed mass spectrum of **4** (M^+ = 492) and MeI (M^+ = 142).

Dihydrocarbinol **10**

Compound **4** (80 mg) in 5 ml of liq. NH_3 were treated with Na until the blue colour was stable, then left 10 min. Evaporation, treatment with NH_4Cl, water and ether and evaporation of ether gave a mixture from which **10** was isolated with preparative TLC on silica gel (ether-MeOH 7/1): colorless cryst., m.p. 217–220°, UV: 227, 275sh, 284, 291 (ε 47000, 11600, 12150, 10600). Mass: see Table 2.

Dehydrogenation of roxburghine D

(a) 200 mg of **4** and 200 mg Se were heated at 300° for 5 min. The crude product was extracted with MeOH and subjected to prep TLC on silica gel. A first elution with hexane-AcOEt 4/1 gave 3-*ethylindole* identified from UV, NMR spectrum and comparison with a pure sample. Elution with AcOEt gave *harman*, identified from UV, mass spectrum and direct comparison.

(b) A similar reaction with Pd black gave a complex mixture, from which a red product was isolated by TLC, with UV: 285, 292, 310sh, 460, 478, mass: 428 (M^+, 89), 413 (100), 245 (43), 207 (52), 182 (63).

(c) 60 mg of **4** in 8 ml MeOH were treated with 200 mg AcONa and 80 mg I_2 and refluxed 30 min. To the warm soln Na_2SO_3 was added, the soln was filtered, evaporated, taken up with $CHCl_3$ and pptd with ether to give **13**, yellow solid, dec > 300°, $[\alpha]_D^{20} = -520°$ (*c* = 0·072 in MeOH), UV: 222, 255, 273, 279, 290sh, 316, 430 (ε 87000, 17800, 29700, 32700, 23800, 18800, 27800), mass: 486 (M^+, 9), 485 (8), 471 (17), 442 (7), 428 (34), 427 (34), 413 (100), 214^{++} (5), 206^{++} (34), 142, 127. NMR: see Fig. 3.

Roxburghine A (1)

The small amount of material available prevented other measurements being made besides those reported in Table 1. Mass: 492 (M^+, 45), 491 (10), 477 (33), 461 (5), 433 (1), 362 (1), 336 (1), 321 (9), 307 (6), 305 (4), 294 (3), 293 (13), 279 (15), 246 (M^{++}, 8), 239 (15), 221 (9), 197 (9), 184 (100), 171 (20), 156 (19), 144 (11), 130 (6).

Roxburghine B (Table 1)

UV: 226, 283, 291 (ε 80000, 37800, 36200). ORD (c = 0·027 in MeOH): $\Phi_{302} = -127800°$, $\Phi_{275} = +171000°$, $\Phi_{234} = +124000°$; NMR (acetone-d_6, 60 MHz): Me-C (1·67), 15 H (1·2-3·8), OMe (3·57), 8 indole H (6·8–7·5), =CHN— (7·38), 2 NH (9·25, 9·90). Mass: 492 (M^+, 56), 491 (14), 477 (8), 461 (5), 433 (12), 362 (2), 336 (2), 321 (4), 307 (6), 305 (5), 294 (35), 293 (31), 279 (15), 246 (M^{++}, 7), 239 (4), 221 (100), 197 (39), 184 (20), 171 (5), 156 (12), 144 (11), 130 (6).

Reduction with $NaBH_4$ in AcOH as described for **6** gave *dihydroroxburghine B*, mass: 494 (M^+, 74), 479 (37), 463 (5), 364 (15), 310 (18), 295 (27), 294 (26), 281 (42), 270 (40), 269 (57), 247 (M^{++}, 22), 239 (25), 223 (81), 221 (70), 211 (28), 198 (41), 185 (71), 184 (100), 183 (49), 156 (20), 144 (18).

Dehydrogenation with I_2 and AcONa as described for **13** afforded *dehydroroxburghine B*, with UV, mass and NMR spectra identical with those of dehydroroxburghine D (**13**), and $[\alpha]_D^{20} = +580°$ (*c* = 0·114 in MeOH).

Roxburghine C (Table 1)

UV: 226, 286sh, 292 (ε 64000, 39600, 40500). ORD (c = 0·253 in MeOH): $\Phi_{298} = -4320°$, $\Phi_{294} = -13600°$, $\Phi_{290} = -23300°$, $\Phi_{234} = -135000°$, $\Phi_{222} = +197000°$. NMR (60 MHz, acetone-d_6): Me-C (1·68), 15 H (1·2-3·8), OMe (3·73), 8 indole H (6·8–7·5), =CHN— (7·55), 2 NH (8·15, 8·28). Mass: 492 (M^+, 57), 491 (19), 477 (9), 461 (7), 434 (10), 362 (4), 336 (10), 321 (52), 307 (13), 305 (6), 294 (6), 293 (13), 279 (20), 263 (14), 221 (19), 184 (100), 171 (32), 156 (33), 144 (32), 130 (24).

Dehydrogenation with I_2 and AcONa as described for **13** gave *dehydroroxburghine C*, with UV and mass spectra identical with those of **13**, and $[\alpha]_{589}^{20} = -670°$, $[\alpha]_{578}^{20} = -735°$, $[\alpha]_{546}^{20} = -960°$ (*c* = 0·095 in MeOH).

Roxburghine E (Table 1)

UV: 224, 284, 291 (ε 52600, 27400, 24600); ORD (*c* = 0·031 in MeOH): $\Phi_{302} = -62900°$, $\Phi_{290} = -21900°$, $\Phi_{273} = +117500°$, $\Phi_{234} = +153900°$, $\Phi_{225} = -157000°$; NMR (100 MHz, acetone-d_6): Me-C (1·70), 14 H (1·2–3·7), OMe (3·59), C_3-H (4·47), 8 indole H (6·8–7·4), =CHN (7·41), 2 NH (9·18, 9·87); Mass: 492 (M^+, 12), 491 (3), 477 (21); 461 (4), 433 (1), 362 (1), 336 (1), 321 (10), 307 (4), 305 (5), 294 (3), 293 (14), 279 (10), 246 (M^{++}, 2), 239 (2), 221 (5), 197 (6), 184 (100), 171 (18), 156 (22), 144 (10), 130 (5). Elemental composition of important peaks:

Found	Calcd.	Composition
492·2503 ± 0·0025	492·2525	$C_{31}H_{32}N_4O_2$
477·2277 ± 0·0023	477·2290	$C_{30}H_{29}N_4O_2$
461·2355 ± 0·0023	461·2341	$C_{30}H_{29}N_4O$
321·1594 ± 0·0016	321·1603	$C_{20}H_{21}N_2O_2$
279·1124 ± 0·0014	279·1133	$C_{17}H_{15}N_2O_2$
184·1000 ± 0·0009	184·1000	$C_{12}H_{12}N_2$

Dehydrogenation with I_2 and AcONa as described for **13** gave *dehydroroxburghine E*, identical in TLC behaviour, UV and mass spectra with *dehydroroxburghine D* (**13**), $[\alpha]_D^{20} = +430°$, $[\alpha]_{578}^{20} = +510°$, $[\alpha]_{546}^{20} = +580°$ (*c* = 0·094 in MeOH). Epimerization: 20 mg of roxburghine E (**5**) and 30 mg Zn in 8 ml AcOH were heated under N_2 for 15 hrs. Evapn., taking up with NH_3, extn. with ether, and prep. TLC on silica gel with ether afforded roxburghine B (**2**), $[\alpha]_D^{20} = -233°$ (*c* = 0·044, MeOH), identified by comparison on TLC (Al_2O_3, $CHCl_3$ or silica gel, ether or $CHCl_3/Et_3N$ 9/1).

Reaction with phenyl chloroformate

Compound **5** (40 mg) in 10 ml CH_2Cl_2 were treated with 2 drops of PhOCOCl and left for 3 hr. Evaporation and prep TLC of the residue on silica gel with ether gave **11**, $C_{40}H_{42}N_4O_5$, UV: 224, 285, 291 sh (ε 60500, 34400, 33400); IR: 2·98 (NH), 5·82 (PhCOO), 5·90 ($COOCH_3$); mass: 658 (M^+, 14; Found: 658·3163 ± 0·0033; Calc. for $C_{40}H_{42}N_4O_5$: 658·3155), 643 (36), 626 (15), 611 (32), 597 (83), 580 (40), 565 (43), 305 (30), 293 (75), 291 (78), 279 (91), 156 (100), 144 (48), 130 (25), 94 (100). When the reaction was conducted by using CH_2Cl_2 previously treated with H_2SO_4, water, dried on KOH and distd., **12** was

obtained, $C_{38}H_{38}N_4O_5$, mass: 630 (M^+, 1), 615 (2), 597 (7), 580 (2), 565 (3), 503 (9), 293 (18), 291 (30), 279 (19), 183 (10), 167 (18), 156 (38), 143 (15), 129 (16), 115 (18), 94 (100).

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