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THE STRUCTURE ELUCIDATION OF THREE TETRAHYDROQUINOLYLIMIDAZOLE ALKALOIDS FROM *MACRORUNGIA LONGISTROBUS* C.B.Cl. (ACANTHACEAE)

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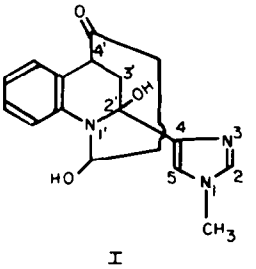
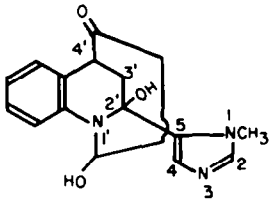
Abstract—Evidence is presented which confirms the structures I–III for three new tetrahydroquinolylimidazole alkaloids.

THE ISOLATION and structure elucidation of four alkaloids from *Macrorungia longistrobus* have been described previously.[1,2] From the ethanol extract of the dried aerial portion of the plant, another three alkaloids, longistrobine ($C_{17}H_{19}N_3O_3$), isolongistrobine ($C_{17}H_{19}N_3O_3$) and dehydroisolongistrobine ($C_{17}H_{17}N_3O_3$) for which we propose structures I–III, have been isolated.



I



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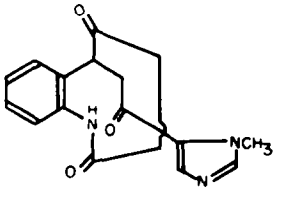

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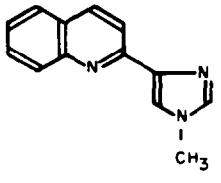
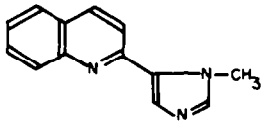


IV



V

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The spectrometric and chemical properties exhibited by longistrobine and isolongistrobine were very similar and, sometimes, as in the case of their mass spectra, even identical. Zinc-dust distillation of longistrobine and isolongistrobine yielded the known alkaloids, macrorine IV, and isomacrorine V, respectively. This experiment, and the similarity in spectrometric properties, suggested that the two alkaloids were substituted quinolylimidazoles differing only in the position of the methyl substituent on the imidazole ring.

The interrelationship of isolongistrobine and dehydroisolongistrobine was established by Jones' oxidation of the former to give the dehydro-alkaloid in quantitative yield. A dehydro product was also obtained on Jones' oxidation of longistrobine, but unlike dehydroisolongistrobine, dehydrolongistrobine was not present in the crude alkaloid mixture.

Longistrobine I had molecular formula $C_{17}H_{19}N_3O_3$ and was optically inactive, λ_{max}^{EtOH} 257 mμ (ε 11,300), $\lambda_{max}^{EtOH/HCl}$ 235 mμ (ε 14,900). There was a strong resemblance between this spectrum and those of tetrahydroquinoline λ_{max}^{EtOH} 250 mμ (ε 8600) and 1-methyl-5-acetyl-imidazole[3], λ_{max}^{EtOH} 255 mμ (ε 14,950), $\lambda_{max}^{EtOH/HCl}$ 235 mμ (ε 12,250). The UV spectrum was not affected by alkali. ν_{max} 3160 cm^{-1} (broad) (NH and/or OH), 1680 cm^{-1} (strong) (C=O). Aromatic bands were present at 1500 cm^{-1}, 1455 cm^{-1} and 1415 cm^{-1}.

The NMR spectrum of longistrobine showed two one-proton singlets at τ 2·48 and τ 2·62, typical of the C-2 and C-4 protons of an imidazole ring. Four other aromatic protons were present in the region τ 2·65 to τ 3·0. A signal at τ 4·46 was ascribed to a proton on a carbon atom, bearing an oxygen function, while a three-proton singlet at τ 6·32 was typical for a N–Me group of an imidazole. Eight protons resonated in the methylene-methine region τ 6·5–8·1. After equilibration with deuterium oxide, this region integrated for only seven protons, and a broad one-proton signal at τ 3·3 also disappeared.

Of the 19 protons accounted for in the above analysis, two were therefore present as the active hydrogens of hydroxyl or amine groups.

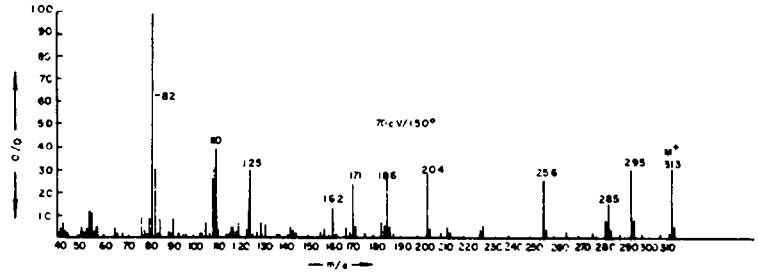
Isolongistrobine II was optically inactive and also analysed for $C_{17}H_{19}N_3O_3$. λ_{max}^{EtOH} 253 mμ (ε 16,750), $\lambda_{max}^{EtOH/HCl}$ 233 mμ (ε 14,400), ν_{max} 3350 cm^{-1} (broad) (NH and/or OH), 1660–1680 cm^{-1} (strong) (C=O). Aromatic bands appeared at 1540 cm^{-1}, 1495 cm^{-1} and 1415 cm^{-1}.

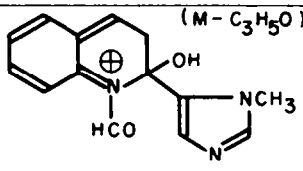
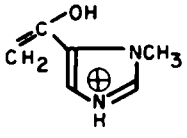
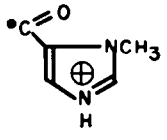
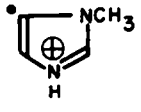
The NMR spectrum of isolongistrobine displayed the same resonance pattern as longistrobine with six aromatic protons between τ 2·48 and 2·95 and a one-proton signal at τ 4·60 (proton on carbon bearing an oxygen atom). The three-proton signal of a N–Me group was present at τ 6·25. One of eight protons resonating between τ 6·8 and 8·0 as well as a one-proton signal at τ 4·08 disappeared on equilibration with D_2O.

The mass spectra of longistrobine and isolongistrobine were identical. The mass spectral data for isolongistrobine are given in Table 1.

The peaks at *m/e* 125 and *m/e* 110 were significant, their elemental composition indicating that isolongistrobine had an oxygen atom on the carbon atom attached to C–5 of the imidazole ring. Loss of a C_3H_5O fragment from the molecular ion gave rise to the peak at *m/e* 256. That the oxygen atom in this fragment was present as a carbonyl function in isolongistrobine, was apparent from the mass spectrum of the mono-oxime of the alkaloid (M^+ 328). Expulsion of a C_3H_6NO fragment again resulted in formation of the *m/e* 256 peak. In the mass spectrum of the mono-acetate of

TABLE 1. MASS SPECTROMETRIC DATA: ISLONGISTROBINE

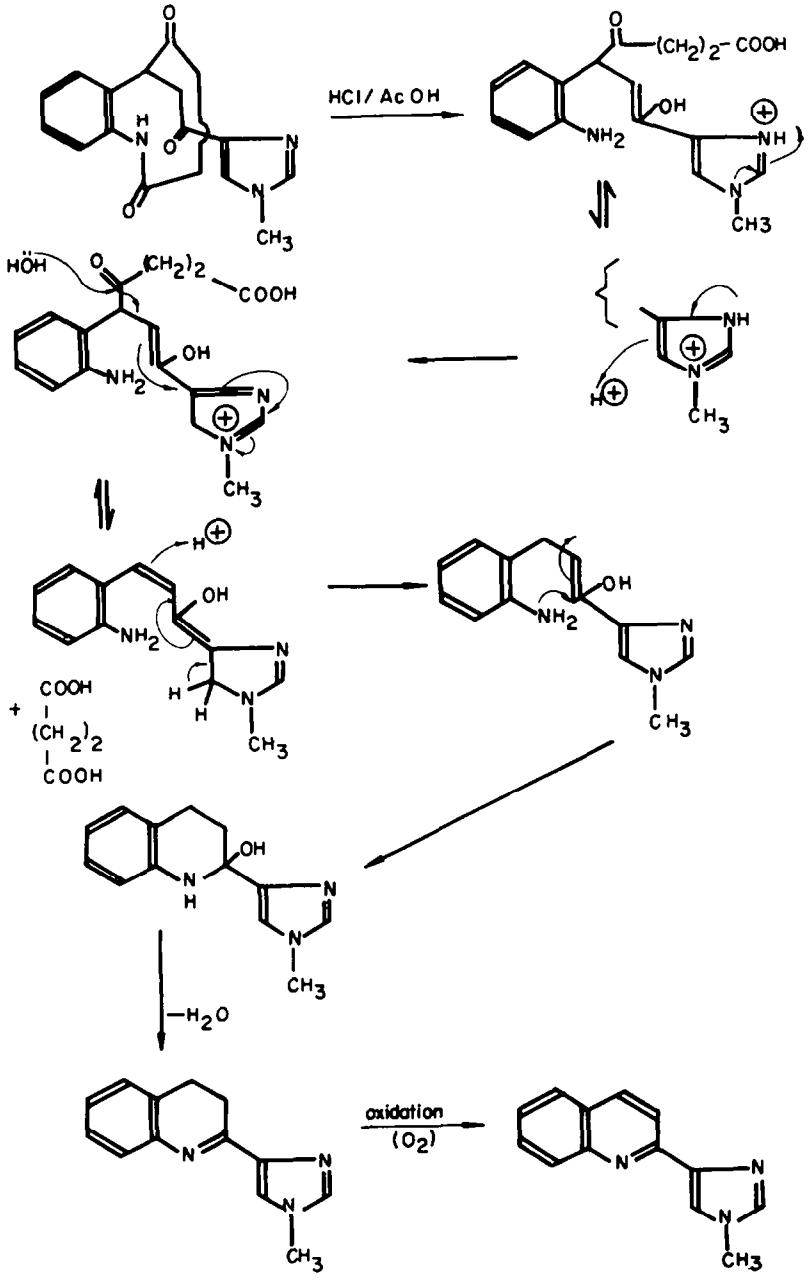


m/e	ELEMENTAL COMPOSITION	PROPOSED STRUCTURE	D_2O / MeOD	NaOMe / MeOD
313	$C_{17}H_{19}N_3O_3$	M^+	315	319
295	$C_{17}H_{17}N_3O_2$	(M - 18)	296	301
256·1088	$C_{14}H_{14}N_3O_2$* requires 256·1086	 (M - C_3H_5O)	258	259
125·0690	$C_6H_9N_2O$* requires 125·0715		126	128
110·0493	$C_5H_6N_2O$* requires 110·0480		110	111
109	$C_5H_5N_2O$		109	110
82	$C_4H_6N_2$		82	83

* Based on high-resolution accurate mass determination.

isolongistrobine (M^+ 355), prominent peaks were observed at *m/e* 125 and *m/e* 110. It thus seemed probable that a hydroxyl group on a carbon atom not directly attached to the imidazole ring had been acetylated. The prominent elimination of acetic acid from the molecular ion mitigated against the presence of an N–acetyl group.

SCHEME 1.

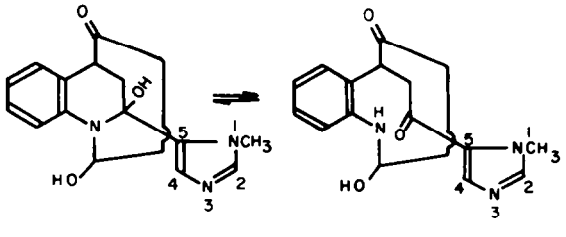


Two protons could be exchanged for deuterium, when longistrobine or isolongistrobine was equilibrated with methanol-d_1–deuterium oxide in the inlet of the mass spectrometer, confirming the results previously obtained by NMR spectrometry. The fact that the *m/e* 125 peak was shifted to *m/e* 126, while the *m/e* 82 peak remained unchanged was regarded as an indication that the oxygen on C-2, of the tetrahydroquinolyl moiety formed part of a carbinolamine group.

Working on the assumption that the alkaloids were substituted tetrahydroderivatives of macrorine IV and isomacrorine V, it remained to establish the point(s) of attachment of the $C_4H_6O_2$ moiety, as well as the relative positions of the oxygen atoms in the moiety. Acid hydrolysis of dehydroisolongistrobine and dehydrolongistrobine through refluxing in a mixture of glacial acetic and concentrated hydrochloric acids, resulted in the formation of isomacrorine and macrorine, respectively. In addition, in both experiments the acid fraction obtained after acid-base separation, was shown to contain succinic acid. The relative positions of the oxygen atoms in the four-carbon moiety had thus been established.

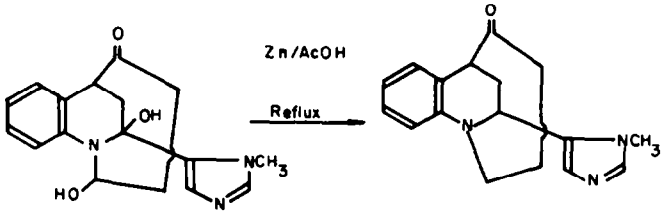
It was not immediately clear by what mechanism the hydrolysis of dehydrolongistrobine would lead to the elimination of succinic acid. This reaction may, however, be rationalized on the basis of a *retro*-Michael reaction as outlined in Scheme 1.

It was suspected that one end of the four-carbon moiety was linked to the tetrahydroquinolyl nitrogen atom, since the acetyl group in longistrobine mono-acetate was an O-acetyl group. Dehydration experiments described below confirmed this suspicion. In order to gain information about the surroundings of the carbonyl function in the four-carbon moiety, isolongistrobine was treated with sodium methoxide in methanol-d_1. A total of six protons was exchanged for deuterium, as indicated in Table 1. That one of these was situated on the imidazole ring (probably on C-2)[3] was evident from the peaks at *m/e* 82 + 1 and *m/e* 110 + 1. The presence of five enolizable hydrogens can only be rationalized on the basis of a linkage of the four-carbon moiety to the C-4, atom of the tetrahydroquinolyl moiety. Furthermore, isolongistrobine either had a diketone structure as in VII, or the species VII nad been present in an equilibrium during the exchange reaction, as indicated by the shift of the *m/e* 125 peak to *m/e* 128, after exchange. That longistrobine and isolongistrobine existed in the carbinolamine (see for example VI) and not the keto-amine form in chloroform solution, was deduced from the NMR spectra of two model compounds, 1-methyl-5-acetyl-imidazole[4] and 1-methyl-5-hydroxyethyl-imidazole. It appeared that the carbonyl function had a marked deshielding effect on the two low-field protons. Two one-proton singlets were observed at τ 2·27 and τ 2·41 in the NMR spectrum of 1-methyl-5-acetyl-imidazole as compared to two one-proton singlets at τ 2·68 and τ 3·16 in the spectrum of 1-methyl-5-hydroxyethyl imidazole. As no proton resonated below τ 2·5 in the NMR spectra of longistrobine and isolongistrobine the structures I and II were preferred to structures as in VII.



VI VII

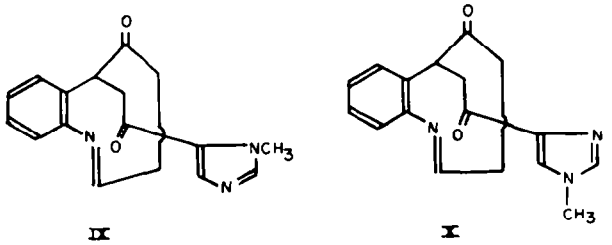
Refluxing longistrobine with zinc and acetic acid led to the reduction of the two carbinolamine groups. The mass spectrum of the product mixture showed a peak at *m/e* 281 ($C_{17}H_{19}N_3O$), i.e. replacement of two hydroxyl groups by hydrogen to give VIII. Prominent peaks were also present at *m/e* 283 and *m/e* 96 indicating that the alkaloid had also been reduced in the diketone form VII. Reduction of this diketone led to loss of the hydroxyl group from the carbinolamine and Clemmensen reduction of the carbonyl α to C-4 of the imidazole ring. As the Clemmensen reduction usually requires the presence of a mineral acid, the reduction of a model imidazole ketone in acetic acid was also investigated. Treatment of 1-methyl-5-acetyl-imidazole with zinc and acetic acid again illustrated that the ketone group of an imidazole-ketone could be reduced under acidic conditions weaker than those generally required for Clemmensen reduction. Two products, 1-methyl-5-ethyl- and 1-methyl-5-hydroxyethylimidazole were formed. The latter was acetylated under the reaction conditions.



II VIII

Additional information in support of the dicarbinolamine structure for isolongistrobine was obtained, when the alkaloid was treated with methanolic hydrogen chloride at room temperature. Mono- and dimethoxy derivatives (M^+ 327 and M^+ 341, respectively), were isolated from the mixture.

When the above reaction mixture was refluxed, a product of dehydration (M^+ 295) resulted, in addition to traces of the methyl ethers and isomacrorine. This anhydroisolongistrobine was obtained quantitatively, when isolongistrobine was refluxed in a 1:2 mixture of concentrated hydrochloric and acetic acids. Anhydroisolongistrobine analysed for $C_{17}H_{17}N_3O_2$, λ_{max}^{EtOH} 257 mμ (ε 27,200), $\lambda_{max}^{EtOH/HCl}$ 244 mμ (ε 23,200), ν_{max} 1690 cm^{-1} and 1660 cm^{-1} (C=O). The NMR spectrum showed a doublet representing one proton centred at a very low-field position τ 1·25 (J 8·0 c/s), typical of an aromatic proton deshielded by a carbonyl in a peri position. In this case the deshielding was due, not to a carbonyl on the ortho carbon atom, but to the influence of an imine double bond (see IX). Two further one-proton singlets



IX X

were present at τ 2·13 and τ 2·40. These two positions were again typical of the C-2 and C-4 protons on an imidazole ring deshielded by a carbonyl α to the C-5 position. In addition, three protons resonated in the normal aromatic region τ 2·7–3·0, giving a total of six aromatic protons as in isolongistrobine. The signals at τ 4·60 and τ 4·08 assigned to the protons H—C—OH in the spectrum of isolongistrobine were absent. On adding deuterium oxide no change in the spectrum was noted. Apart from the molecular ion M^+ 295, the mass spectrum of the anhydro product also showed prominent fragments at *m/e* 110 and *m/e* 82. Accurate mass determinations established that, just as in the mass spectrum of longistrobine, these fragments have the elemental compositions $C_5H_6N_2O$ and $C_4H_6N_2$ respectively.

No exchangeable hydrogens could be demonstrated mass spectrometrically after treatment with methanol-d_1–deuterium oxide in the inlet.

As had been found for isolongistrobine, treatment of longistrobine with absolute methanol and hydrogen chloride, also led to a product of dehydration which could be prepared in quantitative yield by refluxing the alkaloid with glacial acetic acid–concentrated hydrochloric acid 2:1. Unlike the reaction of isolongistrobine, the intermediate mono- and dimethyl ethers could not be detected after treating longistrobine with methanol-hydrogen chloride. After prolonged refluxing, the presence of traces of macrorine IV in the reaction mixture was demonstrated by paper chromatography. Anhydrolongistrobine had spectrometric properties closely resembling those of the analogous anhydroisolongistrobine. The mass spectrum of anhydrolongistrobine X was indistinguishable from that of anhydroisolongistrobine IX.

An attempt was made to reduce the imine double bond in anhydrolongistrobine with sodium borohydride. One very polar product, M^+ 297, was formed. The peak at *m/e* 110 in the spectrum of anhydrolongistrobine was replaced by a peak at *m/e* 112 showing that only the carbonyl group α to C–4 of the imidazole ring had been reduced. Lithium aluminium hydride also failed to reduce the carbon–nitrogen double bond.

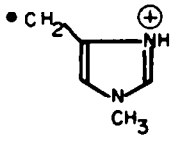
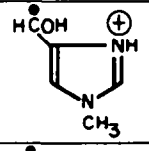
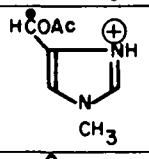
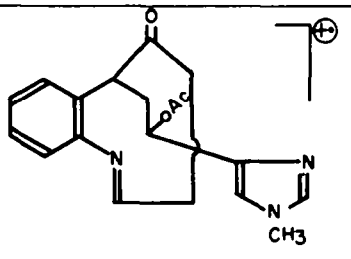
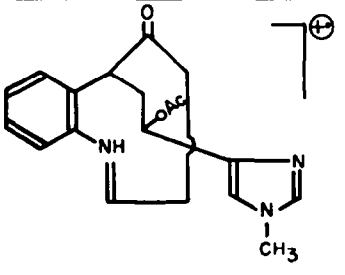
Anhydrolongistrobine was reduced with zinc dust in refluxing acetic acid to give a mixture of products. The mass spectrum of the major fraction obtained by thin-layer chromatography of the mixture showed the fragments which are listed in Table 2.

A molecular ion at *m/e* 339 corresponded to the acetate of a reduction product, in which the carbonyl α to C–4 of the imidazole ring had been reduced to an alcohol. The location of the reduced carbonyl was fixed by a prominent peak at *m/e* 154, which had replaced the peak at *m/e* 110 in the unreduced material. Peaks of low intensity were also observed at *m/e* 111 and *m/e* 112.

A peak at *m/e* 96 showed that a reduced species in which Clemmensen reduction of the carbonyl α to C–4 had taken place, was also present in the mixture. That further reduction of either the second carbonyl group, or the carbon–nitrogen double bond had also taken place, was indicated by a peak at *m/e* 341. The IR spectrum of the reduced material showed that the second carbonyl group had not been attacked, ν_{max} 3375 cm^{-1} (broad) (NH and/or OH), 1740 cm^{-1} (OAc) and 1690 cm^{-1} (C=O), making it clear that the normal reduction of a Schiff base by zinc and acetic acid had taken place.

Heating isolongistrobine in dimethyl sulphoxide also led to dehydration of the alkaloid. The temperature (95°) at which the reaction took place was lower than that usually required for the dehydration of alcohols to olefins.[5] The anhydro product from DMSO had the same molecular formula, but spectroscopic properties differed from those of the methanol–hydrogen chloride product. It was more polar on TLC and the

TABLE 2. MASS SPECTROMETRIC DATA OF REDUCTION MIXTURE OBTAINED FROM Zn/AcOH REDUCTION OF ANHYDROLONGISTROBINE IX.

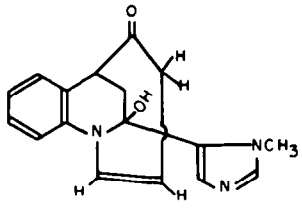
m/e	ELEMENTAL COMPOSITION	PROPOSED STRUCTURE
96	$C_5H_8N_2$	
(111), 112	$C_5H_8N_2O$	
154	$C_7H_{10}N_2O_2$	
339	$C_{19}H_{21}N_2O_2$	
341	$C_{19}H_{23}N_3O_3$	

mass spectra, and in particular the NMR spectra of the two products were different. The NMR spectrum of anhydroisolongistrobine (ex DMSO) showed a triplet (2 protons) at τ 5·58 (J=2 c/s) and a doublet of triplets (1 proton) at τ 3·73 (J = 2 c/s and 6 c/s) indicative of the system $\underset{\tau<3}{HC}=\underset{\tau3\cdot73}{CH}-\underset{\tau5\cdot58}{CH_2}-C-$

In spin decoupling experiments, irradiation at τ 3·73 resulted in collapse of the signal at τ 5·58 to a doublet, while irradiation at τ 5·58 gave a doublet at τ 3·73. In both cases some modification of the aromatic region was observed. One proton was present as a singlet at τ 2·46. Six other protons resonated in the aromatic region τ 2·5–2·9, while the signal of the N-methyl protons appeared at τ 6·31. The region τ 6·5–7·2

contained the resonance signals of a further three or four protons. The absence of two one-proton singlets below τ 2·5 showed that the compound could not have a carbonyl α to C–5 of the imidazole rong. A "closed" ring system as in XI was therefore indicated. Anhydroisolongistrobine (ex DMSO) which was very unstable and could not be crystallized, showed UV-absorption at λ_{max}^{EtOH} 256 mμ (ε 9200) shifting to λ_{max} 228 mμ (shoulder) (ε 11,350) on the addition of acid. Equilibration of the compound with methanol-d_1–deuterium oxide in the inlet of the mass spectrometer showed that one active hydrogen was present in the molecule.

The above data established the structure XI for the anhydroisolongistrobine obtained from DMSO.



XI

Dehydroisolongistrobine had molecular formula $C_{17}H_{17}N_3O_2$, λ_{max}^{EtOH} 258 mμ (ε 17,350), $\lambda_{max}^{EtOH/HCl}$ 235 mμ (ε 14,350). The spectrum was unchanged in alkali. The IR spectrum showed no absorption maxima in the region 3100–4000 cm^{-1} (OH and NH), but carbonyl absorption was present at ν_{max} 1710 cm^{-1}. The NMR spectrum of dehydroisolongistrobine showed a four-proton multiplet between τ 2·7 and τ 3·0, a three-proton peak at τ 6·14 (N—CH_3) and a multiplet of ca. 8 protons around τ 7·1. Two one-proton singlets were present at τ 2·28 and τ 2·49 which compares well with the two low-field resonances at τ 2·27 and τ 2·41 found for 1-methyl-5-acetylimidazole. On oxidation of isolongistrobine one of the carbinolamine groups was therefore oxidized to a lactam. This, together with the opening of the remaining carbinolamine led to III.

The mass spectrum of dehydroisolongistrobine had base peak at *m/e* 81, ascribed to the imidazole moiety. The only other prominent peaks were at *m/e* 310 (M–1) and *m/e* 109, 110. The last two peaks had the same elemental composition as in the spectrum of isolongistrobine (Table 1).

Refluxing dehydroisolongistrobine with aqueous potassium hydroxide gave a polar product from which the alkaloid could be recovered after treating with acid and neutralizing with sodium carbonate. This was in accordance with the structure III proposed for dehydroisolongistrobine, where the relative positions of the carbonyl groups precluded the occurrence of *retro*-Aldol or *retro*-Michael reactions under alkaline conditions.

EXPERIMENTAL

Analytical samples were dried *in vacuo* at 60°. The IR spectra were measured in chloroform solution on a Perkin–Elmer Infracord 237 spectrometer and UV spectra in ethanol on a Unicam Model S.P. 800 Spectrometer. NMR spectra were determined with a Varian A–60 or HA–100 instrument in $CDCl_3$ with TMS as internal standard (τ 10·0). Mass spectra were determined with an A.E.I. MS–9 spectrometer, using the direct insertion technique and an ionizing voltage of 70 Ev. Melting points were determined on a Kofler block. Dragendorff's spray reagent was used for the development of spots on all thin-layer plates.

Isolation of the alkaloids

The separation of macrorine, isomacrorine, normacrorine and macrorungine from Macrorungia longistrobus C.B.Cl. has been described previously.[1,2] The basic residue (3·1 g) remaining after the above-mentioned alkaloids had been separated from the alkaloidal extracts of 100 kg dried plant material was chromatographed on alumina. Elution with 100:1 and 50:2 methylene chloride–methanol separated dehydroisolongistrobine (30 mg) from isolongistrobine (750 mg) and longistrobine (1·1 g).

Longistrobine (I)

Longistrobine crystallized from acetone–hexane as colourless needles, m.p. 145–148°. λ_{max} 257 mμ (ε 11,300). λ_{max} (in 1% HCl–EtOH) 235 mμ (ε 14,900) ν_{max} 3160 cm^{-1} (broad) (OH and/or NH). 1680 cm^{-1} (strong) (C=O). Aromatic bands were present at 1500 cm^{-1}, 1455 cm^{-1} and 1415 cm^{-1}. (Found: M$^+$ 313·1428. $C_{17}H_{19}N_3O_3$ requires: 313·1426).

Isolongistrobine (II)

The alkaloid crystallized from acetone–hexane as yellowish needles, m.p. 132–136°. λ_{max} 253 mμ (ε 16,750). λ_{max} (in 1% HCl–EtOH) 235 mμ (ε 14,400) ν_{max} 3350 cm^{-1} (broad) (NH and/or OH) 1660–1680 cm^{-1} (strong) (C=O). Aromatic bands appeared at 1540 cm^{-1}, 1495 cm^{-1} and 1415 cm^{-1}. (Found: C, 65·0; H, 6·0; N, 13·3. $C_{17}H_{19}N_3O_3$ requires C, 65·2; H, 6·1; N, 13·4%).

Isolongistrobine-d_6

Longistrobine (5 mg) was dissolved in methanol-d_1 (2 ml) to which sodium metal (5 mg) had been added. The mixture was kept at 0° for 24 hr, the methanol removed *in vacuo* and the residue extracted with methylene chloride. The methylene chloride was dried over sodium sulphate and removed under vacuum to give an oil (5 mg). The above treatment was repeated twice on the oil. The mass spectrum of the product was determined (Table 1).

Isolongistrobine monoxime

Isolongistrobine (10 mg) was refluxed in the presence of hydroxylamine hydrochloride (20 mg) and potassium acetate (50 mg) in methanol (25 ml) for 1 hr. Sodium bicarbonate (20 mg) was added to the solution, the methanol removed *in vacuo* and the residue chromatographed on a silica plate with methylene chloride–methanol 19:1 as mobile phase to give the monoxime as a yellowish oil. M$^+$ 328 (molecular weight $C_{17}H_{20}N_4O_3$ = 328). ν_{max} 1660 cm^{-1}. The band at 1680 cm^{-1} present in the spectrum of isolongistrobine was absent.

Acetylation of isolongistrobine

On treatment of isolongistrobine (4 mg) with acetic anhydride–pyridine (1:1) (5 ml) at 25° for 12 hr, a monoacetate was obtained as an oil. ν_{max} 1730 cm^{-1} (OAc). M$^+$ 355. Other prominent fragments in the mass spectrum were present at *m/e* 296, 295, 256, 218, 186, 171, 125, 109, 82.

Dehydroisolongistrobine (III)

Dehydroisolongistrobine crystallized from acetone–hexane as colourless needles, m.p. 131°. λ_{max} 258 mμ (ε 17350). λ_{max} (in 1% HCl–EtOH) 235 mμ (ε 14,350). ν_{max} 1710 cm^{-1} (C=O). (Found: M$^+$ 311·1270. $C_{17}H_{17}N_3O_3$ requires 311·1269).

Zinc dust distillation of longistrobine

Longistrobine (5 mg) was intimately mixed with zinc powder (3 g) and the mixture heated for 3 hr at 300° under a flow of nitrogen in a glass tube. The oil which condensed in the cooler part of the tube, crystallized, and was shown to be identical in mass spectrum and chromatographic behaviour to macrorine (IV).

Zinc dust distillation of isolongistrobine

Using the same method as for longistrobine, zinc dust distillation of isolongistrobine (4 mg) gave crystals identical in mass spectrum and chromatographic behaviour to isomacrorine (V).

Chromium trioxide oxidation of longistrobine

To longistrobine (50 mg) in acetone (15 ml) at 0° was added 0·8N CrO_3 in 9N H_2SO_4 (0·5 ml) and the

mixture left at 0° for 10 min. The acetone was removed *in vacuo* and sodium carbonate (1 g) and water added. The solution was extracted with methylene chloride (3 × 25 ml), the methylene chloride solution dried over sodium sulphate, filtered, and the solvent removed under reduced pressure to give an oil (45 mg), which was purified by preparative chromatography on a silica plate with methylene chloride–methanol 19:1 as mobile phase. Unchanged material (10 mg) and dehydrolongistrobine were obtained. Dehydrolongistrobine crystallized from acetone–hexane as colourless needles, m.p. 177–178°. λ_{max} 255 mμ (ε 12,600), λ_{max} (in 1% HCl–EtOH 236 mμ (ε 15,200). The IR spectrum showed no absorption in the region 3100–4000 cm^{-1} (OH and NH) but strong absorption was present at ν 1715 cm^{-1} (C=O). The NMR spectrum showed a one-proton singlet at τ 4·45 and the absorption of five other aromatic protons in the region τ 2·5–3·0. A three-proton singlet was present at τ 6·31, while eight protons resonated in the low methylene-methine region between τ 7·4 and τ 8·3. After deuterium exchange the region τ 7·4 to τ 8·3 integrated for seven protons only. (Found: M^+ 311·1268. $C_{17}H_{17}N_3O_3$ requires 311·1270).

Chromium trioxide oxidation of isolongistrobine

Isolongistrobine (320 mg) was oxidized in acetone (30 ml) with 0·8N CrO_3 in 9N H_2SO_4 (5 ml) and the reaction mixture treated as described for the oxidation of longistrobine to yield colourless crystals, m.p. 131°, identical in m.p., mixed m.p. and IR to the alkaloid dehydroisolongistrobine.

Acid hydrolysis of dehydrolongistrobine

Dehydrolongistrobine (5 mg) in glacial acetic acid–concentrated hydrochloric acid 2:1 (5 ml) was refluxed for 36 hr. During the whole experimental procedure a blank was also run with the same reagents. The solvent was removed *in vacuo*, sodium carbonate (4 mg) and water (2 ml) added and the solution extracted with chloroform (3 × 5 ml). The chloroform was dried over sodium sulphate, filtered and removed under reduced pressure to give a semi-crystalline residue (2 mg), identical in mass spectrum and chromatographic behaviour to authentic macrorine (IV). The aqueous solution was evaporated to dryness *in vacuo*, concentrated hydrochloric acid (0·2 ml) added to the residue and all solvent again removed *in vacuo*. The crystalline residue, consisting mainly of sodium chloride, when introduced into a mass spectrometer, gave a spectrum identical to that of succinic acid (*m/e* 100, 75, 74, 55, 45). Due to loss of the elements of water, succinic acid shows no molecular peak at *m/e* 118. The crystalline residue was then suspended in methanol (1 ml) and an ethereal solution of diazomethane added. After standing for 24 hr at 0°, the solvent was removed *in vacuo*, methanol (0·5 ml) added, the solution filtered and concentrated to 0·2 ml. By gas chromatography on Chromosorb 35/80 with 30% PEG 20M as stationary phase (column length 2 m, temperature 175°, argon pressure 30 lb, ionization detector), the presence of dimethyl succinate in the solution could be established. No succinic acid or dimethyl succinate was found in the blank.

Acid hydrolysis of dehydroisolongistrobine

Dehydroisolongistrobine (5 mg) was hydrolysed as for dehydrolongistrobine. In the same manner as has been described for dehydroisolongistrobine, it could be shown that hydrolysis leads to the formation of isomacrorine (V) and succinic acid.

1-Methyl-5-hydroxyethyl-imidazole

To 1-methyl-5-acetyl-imidazole[4] (50 mg) in methanol (25 mg) was added sodium borohydride (30 mg) and the mixture refluxed for 1 hr. The methanol was removed *in vacuo* and 0·5N hydrochloric acid (40 ml) added to the residue. The solution was basified with solid sodium carbonate and extracted with methylene chloride (3 × 50 ml). The methylene chloride was dried over sodium sulphate, filtered and removed under vacuum to give an oil (24 mg) which crystallized from acetone-hexane as colourless prisms, m.p. 128–130°. In the NMR spectrum of the compound the two aromatic protons resonated as two singlets at τ 2·71 and τ 3·12. The spectrum also contained a one-proton quartet at τ 5·13 (HO—CH—CH_3) (J = 7 c/s), a three-proton singlet at τ 6·28 (NCH_3), and a three-proton doublet at τ 8·37 (HCOH—CH_3) (J = 7 c/s). A one proton signal at τ 7·32 disappeared on equilibration with deuterium oxide. (Found: C, 56·8; H, 7·8; N, 21·6. $C_6H_{10}N_2O$ requires C, 57·1, H, 8·0; N, 22·2%).

Reduction of longistrobine with zinc and acetic acid

Longistrobine (6 mg) was refluxed with glacial acetic acid (7 ml) and zinc powder (500 mg) for 7 hr. The

mixture was filtered, the zinc residue washed with acetic acid (3 × 2 ml) and acetic acid removed from the combined filtrates *in vacuo*. The residue was dissolved in water (10 ml) basified with sodium carbonate and extracted with methylene chloride (3 × 10 ml). The methylene chloride was dried over sodium sulphate and removed under vacuum to give an oil (3 mg). TLC of the oil on silica with methylene chloride-methanol 9:1 as mobile phase showed only one polar spot. The mass spectrum of the oil showed prominent peaks at *m/e* 283, 281 and 96. The oil was therefore not homogeneous.

Reduction of 1-methyl-5-acetyl-imidazole with zinc and acetic acid

1-Methyl-5-acetyl-imidazole (60 mg) and zinc powder (700 mg) was refluxed in glacial acetic acid (7 ml) for 7 hr and the reaction mixture treated as has been described for the reduction of longistrobine to give a mixture of products as an oil (50 mg). The mass spectrum of this oil indicated that different degrees of reduction of the carbonyl group had taken place. Clemmensen reduction of the carbonyl led to fragments at *m/e* 95 and 110 while partial reduction to a hydroxyl, followed by acetylation, could be inferred from the ion at *m/e* 168 (MW starting material 124).

Dehydration of isolongistrobine in methanol–hydrogen chloride

Isolongistrobine (20 mg) was dissolved in a saturated solution of hydrogen chloride in dry methanol (25 ml) and the mixture left at 25° for 18 hr. The methanol was removed *in vacuo*, the residue taken up in water (10 ml), the solution basified with sodium carbonate and extracted with methylene chloride (4 × 10 ml). Drying over sodium sulphate and removal of the methylene chloride under vacuum led to a product (16 mg) which could be separated into unchanged starting material and three other components by preparative TLC on silica with methylene chloride–methanol 20:1 as mobile phase. The two fastest moving components were the major products, and were shown by mass spectrometry to be the mono- and dimethyl ethers of isolongistrobine (M^+ 327 and M^+ 341, respectively). The third product, a product of dehydration, could be obtained in higher yield (30 mg) by refluxing isolongistrobine (90 mg) in methanol–hydrogen chloride for 1 hr. The presence of traces of isomacrorine (V) in the reaction product could also be demonstrated by TLC and mass spectrometry. The anhydroisolongistrobine (IX) crystallized from acetone–hexane as colourless needles, m.p. 206–207°. λ_{max} 257 mμ (ε 27,200), λ_{max} (in 1% HCl–EtOH) 244 mμ (ε 23,200). (Found: M^+ 295·1323. $C_{17}H_{17}N_3O_2$ requires 295·1320).

Dehydration of isolongistrobine in hydrochloric acid–acetic acid

Isolongistrobine (20 mg) in concentrated hydrochloric acid–acetic acid 1:2 was refluxed for 3 hr, the acetic acid removed *in vacuo*, the residue dissolved in water (10 ml), the solution basified with sodium carbonate, extracted with methylene chloride (4 × 10 ml) and the methylene chloride removed under vacuum to give colourless crystals (18 mg), identical in m.p., mixed m.p. and spectrometric behaviour to the anhydro product obtained after refluxing isolongistrobine with methanolic hydrogen chloride.

Anhydrolongistrobine (X)

Refluxing longistrobine (20 mg) in methanolic hydrogen chloride using the same conditions as for the dehydration of isolongistrobine, also led to the isolation of a product of dehydration (5 mg). The presence of trace amounts of macrorine could be demonstrated in the mixture of reaction products by TLC and mass spectrometry. Anhydrolongistrobine was formed in quantitative yield when longistrobine was refluxed in concentrated hydrochloric acid–acetic acid 1:2 for 4 hr. Anhydrolongistrobine crystallized from acetone–hexane as colourless needles, m.p. 218–222°. λ_{max} 255 mμ (ε 21,600), λ_{max} (in 1% HCl–EtOH) 245 mμ (ε 22,800), ν_{max} 1680 cm^{-1} (C=O).

The NMR spectrum showed a doublet representing one proton centred at a very low-field position τ 1·25 (J = 8 c/s), typical of an aromatic proton deshielded by a carbonyl on the ortho carbon atom. Two other protons appeared as singlets at τ 2·36 and τ 2·57, these respective signals being attributed to the protons on C–4 and C–2 of the imidazole ring. An additional three protons resonated in the aromatic region τ 2·7–3·0, giving a total of six aromatic protons as in the spectrum of longistrobine. The signals at τ 3·30 and τ 4·46 assigned to H—C—OH in the spectrum of longistrobine were absent. The mass spectrum of anhydrolongistrobine was indistinguishable from that of the analogous anhydroisolongistrobine. No hydrogen could be exchanged in the inlet of the mass spectrometer by equilibration with deuterium oxide–methanol-d_1. (Found: M^+ = 295·1323. $C_{17}H_{17}N_3O_2$ requires: 295·1320).

Reduction of anhydrolongistrobine with sodium borohydride

Anhydrolongistrobine (4 mg) in methanol (5 ml) was refluxed in the presence of an excess of sodium borohydride for 1 hr and the solvent then removed *in vacuo*. Concentrated hydrochloric acid (0·25 ml) was added to destroy the excess reagent, the solution basified with solid sodium carbonate and potassium fluoride (1 g) and dioxane (5 ml) added. The mixture was refluxed for 7 hr, cooled and extracted with chloroform (4 × 10 ml). The chloroform extract was dried over sodium sulphate, filtered and the solvent removed under reduced pressure to give an oil (2 mg). TLC of the oil on silica indicated the presence of only one very polar product: ν_{max} 1680 cm^{-1} (C=O) and 3670 cm^{-1}, 3400 cm^{-1} (broad) (OH). The mass spectrum showed M^+ 297. The peak at *m/e* 110, present in the spectrum of anhydrolongistrobine, was absent. A peak at *m/e* 112 showed that only the carbonyl group α to C–4 of the imidazole ring had been reduced.

Reduction of anhydrolongistrobine with lithium aluminium hydride

Anhydrolongistrobine (4 mg) in dry THF (4 ml) was treated with an excess of LAH and the mixture kept at room temperature for 48 hr. The excess reagent was destroyed by adding a few drops of aqueous acetic acid, the solution basified with sodium carbonate and extracted with methylene chloride (3 × 10 ml). The combined extracts were dried over sodium sulphate and the solvent removed *in vacuo* to give an oil (2 mg) which was shown to be a mixture of at least three components on TLC. The mass spectrum of the mixture showed a very strong peak at *m/e* 112 (reduction of the imidazole-ketone) and an unknown peak at *m/e* 282. No further information could be obtained from the mass spectrum.

Reduction of anhydrolongistrobine with zinc and acetic acid

Anhydrolongistrobine (10 mg) was refluxed with zinc powder (600 mg) in glacial acetic acid (7 ml) for 13 hr, the solution filtered, the acetic acid removed *in vacuo*, the residue dissolved in water (10 ml) and the solution basified with sodium carbonate and extracted with methylene chloride (3 × 10 ml). The methylene chloride was dried over sodium sulphate, filtered and removed under vacuum to leave an oil (7 mg). Preparative TLC on silica with methylene chloride–methanol 9:1 as mobile phase separated the oil into one major and three minor components. The mass spectrum of the major reduction product, ν_{max} 3375 cm^{-1} (OH/NH), 1740 cm^{-1} (OAc) and 1690 cm^{-1} C=O), showed peaks at *m/e* 341, 339, and 154. Peaks of low intensity were also present at *m/e* 112, 111 and 96.

Dehydration of isolongistrobine in dimethyl sulphoxide

Isolongistrobine (100 mg) was heated in freshly distilled dry dimethyl sulphoxide (1 ml) for 1 hr at 95°, the reaction mixture poured into water (50 ml) and extracted with methylene chloride (4 × 25 ml). The methylene chloride extract was dried over sodium sulphate, filtered and removed *in vacuo* to leave an oil (95 mg) which was chromatographed on a preparative alumina plate with methylene chloride–methanol 20:1 as mobile phase to give mainly unchanged starting material (45 mg) and an anhydro-product (20 mg) as a colourless unstable glass λ_{max} 256mμ (ε 9200), λ_{max} (in 1% HCl–EtOH) 228 mμ (ε 11,350), ν_{max} 1690 cm^{-1} (C=O), M^+ 295. Prominent peaks were observed at *m/e* 186, 168, 109 and 82 in the mass spectrum.

Attempted alkaline hydrolysis of dehydroisolongistrobine

Dehydroisolongistrobine (16 mg) was refluxed with 5% aqueous potassium hydroxide in methanol for 1 hr. The methanol was removed under vacuum to leave a product which did not move on a silica TLC plate with methylene chloride–methanol 10:1. The product was dissolved in 3N hydrochloric acid, the solution basified with sodium carbonate and extracted with methylene chloride (4 × 25 ml). After drying of the extract over sodium sulphate, filtration and removal of the methylene chloride *in vacuo*, unchanged dehydroisolongistrobine (14 mg) was recovered.

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