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THE ISOLATION AND STRUCTURE OF CYCLOPIAZONIC ACID, A TOXIC METABOLITE OF *PENICILLIUM CYCLOPIUM* WESTLING

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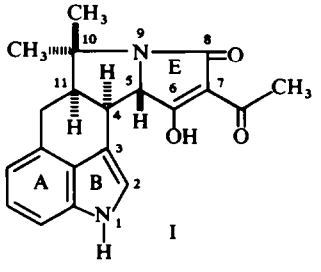
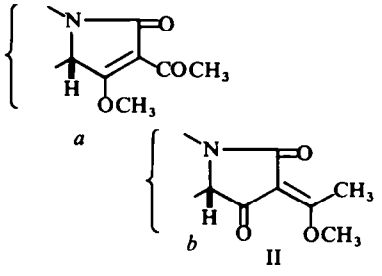
Abstract—Cyclopiazonic acid is the main toxic principle of a strain of *Penicillium cyclopium* Westling. On the basis of chemical and spectrochemical evidence it has been deduced that cyclopiazonic acid has the structure and relative stereochemistry shown in I.

Penicillium cyclopium Westling has a world-wide distribution and is frequently isolated from stored grain and cereal products. Several strains of this species, recovered from samples of domestic cereal products, were found to cause acute toxicoses in ducklings and rats. Maize-meal was used for the large-scale cultivation of the most toxic strain and the toxic principles were extracted quantitatively with chloroform-methanol. From the fraction soluble in sodium hydrogen carbonate solution, cyclopiazonic acid (I) was isolated by chromatography on formamide-impregnated cellulose and ion-exchange (Dowex I) columns. This compound was found to be the main cause of the toxicity of the fungus.



I



II

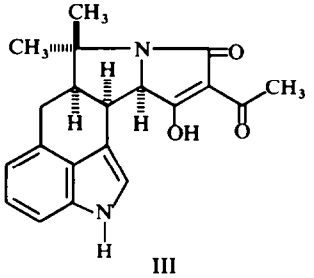
Cyclopiazonic acid (I) is an optically active colourless crystalline compound which analysed for $C_{20}H_{20}N_2O_3$. It does not contain an OMe or a N-Me group and is inert towards hydrogenation in the presence of 30% Pd-C (in acetic acid) or Adams catalyst (in methanol). The compound titrated as a monobasic acid and gave a monohydrazone $C_{20}H_{22}N_4O_2$. The IR spectrum of cyclopiazonic acid in chloroform showed an intense band at 1618 cm^{-1} which shifted to 1600 cm^{-1} on the addition of morpholine. Absorption of this type is generally considered to be characteristic of intramolecularly hydrogen bonded enolized β-diketones.[1] The presence of a system of this type in cyclopiazonic acid accounts for its intense orange-red ferric colour reaction and the formation of a copper chelate on treatment of its sodium salt with copper acetate. Furthermore, reaction with acetic anhydride and pyridine gave a monoacetate which showed IR bands at 1770 and 1180 cm^{-1}, typical of an

enol-acetate. The PMR spectrum* of cyclopiazonic acid shows absorption corresponding to one proton at τ −2·4 which disappears on shaking with deuterium oxide. The position of this signal is characteristic of strongly hydrogen bonded protons.[2]

Treatment of cyclopiazonic acid with diazomethane gave an amorphous O-Me derivative $C_{21}H_{22}N_2O_3$ (II)† as one of the products. Its PMR spectrum showed, *inter alia*, a singlet at τ 5·83 due to the OMe group, 3-proton singlets at τ 8·42 and 8·50 ascribed to two quaternary-attached C-Me groups and a 3-proton signal at τ 7·57 indicative of a Ac group. The reaction of cyclopiazonic acid with alkaline iodine to give iodoform is consistent with the presence of a methyl ketone moiety.

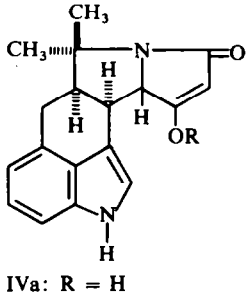
Cyclopiazonic acid gave a purple-blue Erlich colour reaction which suggested the presence of an indole system unsubstituted at either the α or β positions.[3] The indole and β-diketone systems account for one nitrogen and two of the oxygen functions of the toxin. The remaining oxygen and nitrogen functions were assigned to an amide group in order to account for the essentially non-basic character of the compound.

Vigorous treatment of cyclopiazonic acid with aqueous alkali caused partial conversion into an isomer (III), while hydrolysis with 0·05N mineral acid gave acetic

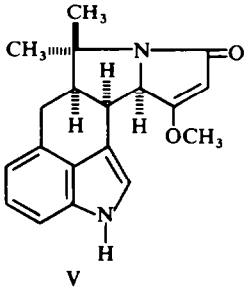


III

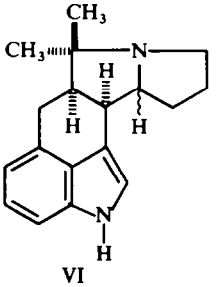
acid in good yield together with a non-basic residue from which crystalline deacetylcyclopiazonic acid (IVa) was obtained. Treatment of the mother liquors with diazomethane yielded the O-Me derivatives of deacetylcyclopiazonic acid (IVb) and



IVa: R = H
IVb: R = Me



V



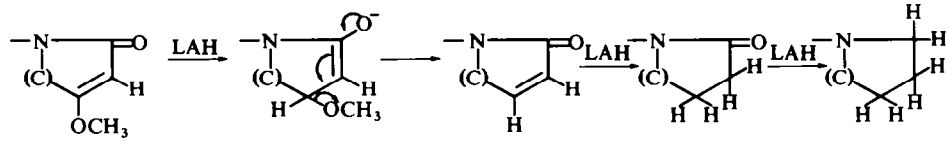
VI

its isomer (V) which were separated by chromatography. Compound IVb was reacted with LAH in tetrahydrofuran. A colourless crystalline amine $C_{18}H_{22}N_2$ (VI) was isolated from the basic fraction of the reaction product. Its UV spectrum (λ_{max} 223, 274(sh), 280 and 292 mμ; log ε 4·52, 3·78, 3·79 and 3·67, respectively) is character-

* PMR spectra were recorded at 100 Mc/s for solutions in $CDCl_3$ (except where otherwise stated) using TMS as internal reference.

† From the available evidence, no distinction could be made between the possibilities IIa and IIb.

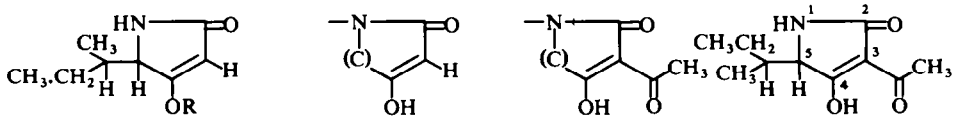
istic of a 3,4-disubstituted indole. Formation of this amine may be rationalized on the basis of partial structure VII, modified as depicted in Scheme 1. The PMR spectrum of the O-Me derivative of deacetylcyclopiazonic acid (IVb) showed a 1-proton singlet at τ 5·02 consistent with the partial structure VII. The proton on the double bond of the O-Me derivative (VIIIb) of the known deacetyltenuazonic



VII VIIa

SCHEME 1

acid (VIIIa)[4] absorbs at τ 4·94. Deacetylcylopiazonic acid (IVa), like deacetyltenuazonic acid (VIIIa),[4] titrates as a monobasic acid. On the basis of these data, it is concluded that deacetylcyclopiazonic acid has the partial structure IX. Cyclopiazonic acid must therefore have the partial structure X, if the presence of a chelated β-diketone system is taken into account. The UV spectrum of the toxin in methanol (λ_{max} 225, 253, 275(sh), 284 and 292 mμ; log ε 4·60 4·22, 4·28, 4·31 and 4·24, respectively) can now be interpreted as the combination of an isolated indole system and a cross-conjugated chromophore similar to that of tenuazonic acid (XI)[4] (λ_{max} 241 and 280;



VIIIa: R = H
VIIIb: R = Me

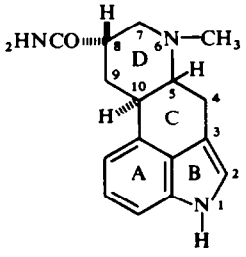
IX X XI

log ε 4·08 and 4·13, respectively). It is of interest to note that deacetylation of cyclopiazonic acid occurred under conditions similar to those described[4] for the conversion of tenuazonic acid (XI) into its deacetyl derivative (VIIIa).

The PMR spectrum of cyclopiazonic acid showed broad absorption corresponding to one proton at τ 1·82 which disappeared on shaking with deuterium oxide. This signal is assigned to an indole-NH group which also gives rise to a sharp IR peak at 3478 cm^{-1}. The PMR spectrum further showed the presence of four aromatic protons absorbing in the region τ 2·85–3·2 in deuteriochloroform. A doublet at τ 2·95 (J 2 c/s) reduced to a singlet when the indole-NH was exchanged with deuterium oxide, indicating that the indole system contains a proton at position 2 and a substituent at position 3.[5] In indole systems it is possible for a 3-proton to couple to a 1-proton, but the signal of the 3-proton generally occurs at much higher field than τ 2·95.[5] Furthermore, from the change in the multiplets in the region τ 2·5–3·2 on 1-deuteration, it was quite definitely ascertained that the proton coupled to the indole-NH absorbs at τ 2·71 in the PMR spectrum of cyclopiazonic acid in DMSO-d_6. This solvent dependence of the signal position of the proton in question is consistent with its location at position 2.[5b] The 2-proton of dihydroisolysergamide-I (XII)[6] was found to show similar absorption at τ 3·20 in deuteriochloroform. The three remaining aromatic protons of both cyclopiazonic acid and dihydroisolysergamide-I

gave rise to practically identical PMR multiplets (2-proton absorption around τ 2·91; 1-proton absorption around τ 3·20). These data support the UV evidence for the presence of a 3,4-disubstituted indole system in cyclopiazonic acid.

Structure elucidation of cyclopiazonic acid was now reduced to establishing the



XII

manner in which the partial structure X and the indole system are linked through the remaining carbon atoms. The required information was obtained mainly from a study of the mass spectrum of the toxin (I; Fig. 1). This spectrum showed a strong molecular ion (*m/e* 336) and prominent fragment ions at *m/e* 154, 155, 181, 182 and 196. Accurate mass measurements showed that the peaks at mass 154 and 155 correspond to $C_{11}H_8N$ and $C_{11}H_9N$, respectively. A metastable peak at *m/e* 153 lent support to the assumption that at least part of the fragment ion of mass 154

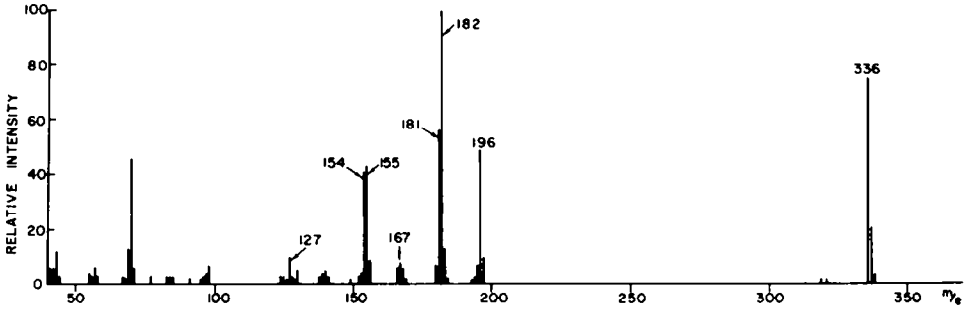
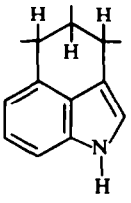


FIG. 1. Mass spectrum of cyclopiazonic acid (I).

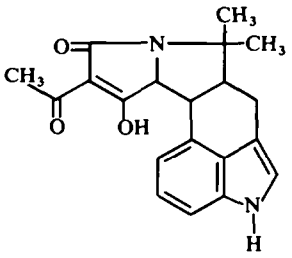
results from the species of mass 155 by ejection of a hydrogen atom. These fragment ions must contain the indole system and three additional carbon atoms. Taking into account what has been reported on the substitution of the indole system of cyclopiazonic acid, the ions of mass 154 and 155 can be represented by XIII and (XIII + H). Fragment ions of identical composition and relationship are prominent in the mass spectrum of dihydroisolysergamide-I (XII) and in this case the species of mass 155 must arise by cleavage of the C_5—N_6 and C_9—C_{10} bonds of ring D.

The fragment ion of mass 196 (Fig. 1) has the composition $C_{14}H_{14}N$ and corresponds to the species of mass 154 (or 155) with an additional three carbon atoms. The species of mass 181 corresponds to $C_9H_{11}NO_3$ and since it contains all the oxygen functions of the molecule it must contain the moiety X with three additional carbon atoms. From these facts it follows that the partial structures X and XIII must

be joined by a C_3H_6 moiety. Taking into account the presence of two quaternary-attached C-Me groups, the alternative structures I and XIV were considered for the toxin. Support for the presence of a *gem*-dimethyl group was obtained from the IR spectrum of deacetylcyclopiazonic acid (IVa) which showed, in the region corresponding to symmetrical CH_3 deformation, two bands of roughly equal intensity at

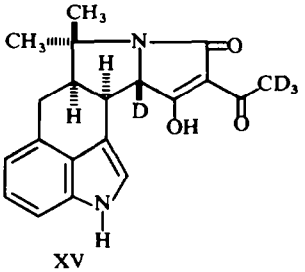


XIII

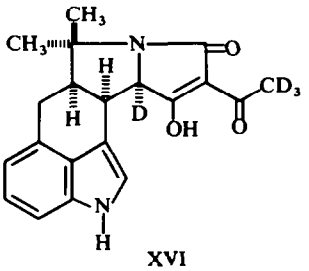


XIV

1371 and 1385 cm^{-1}.[7] The presence of four protons on carbons adjacent to CO groups was demonstrated as follows. Cyclopiazonic acid was treated under vigorous conditions with deuterium oxide containing NaOD and then briefly (to exchange labile deuterium atoms) with methanol. Separation of the products yielded tetradeuteriocyclopiazonic acid (XV) and tetradeuterioisocyclopiazonic acid (XVI).



XV



XVI

A distinction between the structures I and XIV considered for cyclopiazonic acid was made on the basis of the following results. The PMR spectra of cyclopiazonic acid and its derivatives showed that the chemical shift of the indole α-proton was very sensitive to changes affecting the orientation and diamagnetic anisotropy of ring E (Table 1). The chemical shifts of the remaining aromatic protons were essentially insensitive to these changes. This suggests that, of the aromatic protons, the indole α-proton is situated closest to ring E. Inspection of Dreiding models showed that this situation exists in structures of type I but not in XIV.

The PMR spectrum of the O-Me derivative of cyclopiazonic acid (II) confirmed the presence of the grouping (—CH.CH.CH.CH_2—). This grouping absorbed as an XMA_2B system with coupling limited to vicinal protons (Fig. 2). Proton X gives rise to a sharp doublet at τ 5·95 due to coupling with the M proton (J_{MX} 11 c/s). The M proton gave rise to a sharp quartet around τ 6·34 due to further coupling with the B proton (J_{BM} 6 c/s). Two magnetically equivalent *gem*-protons form the A_2-part of the system, giving rise to a sharp doublet around τ 7·02 due to coupling with the B proton (J_{AB} 8 c/s). The B proton, being coupled to both the A and M protons gives

rise to a sextet of lines, centred around τ 7·44, only partly separated from the Ac signal at τ 7·57. In the PMR spectrum of cyclopiazonic acid, the Ac group gives rise to two signals at τ 7·61 and 7·45 with integrated intensities in the ratio 4:1. The PMR signals due to C_5-H appear as two doublets (J 6 c/s) centred at τ 6·03 and 5·87 with integrated intensities in the ratio 4:1. These data indicate that cyclopiazonic acid exists in two tautomeric enol forms between which there is no rapid interchange in

TABLE 1. CHEMICAL SHIFTS OF INDOLE α-PROTON IN DERIVATIVES OF CYCLOPIAZONIC ACID

Compound	τ*
I	2·95
II	2·93
III	3·20
IVb	3·00
V	3·37
XIX	3·02

* PMR spectra were determined at a fixed concentration (0·15M) since the chemical shift of the 2-proton of certain indoles is concentration dependent.[5c]

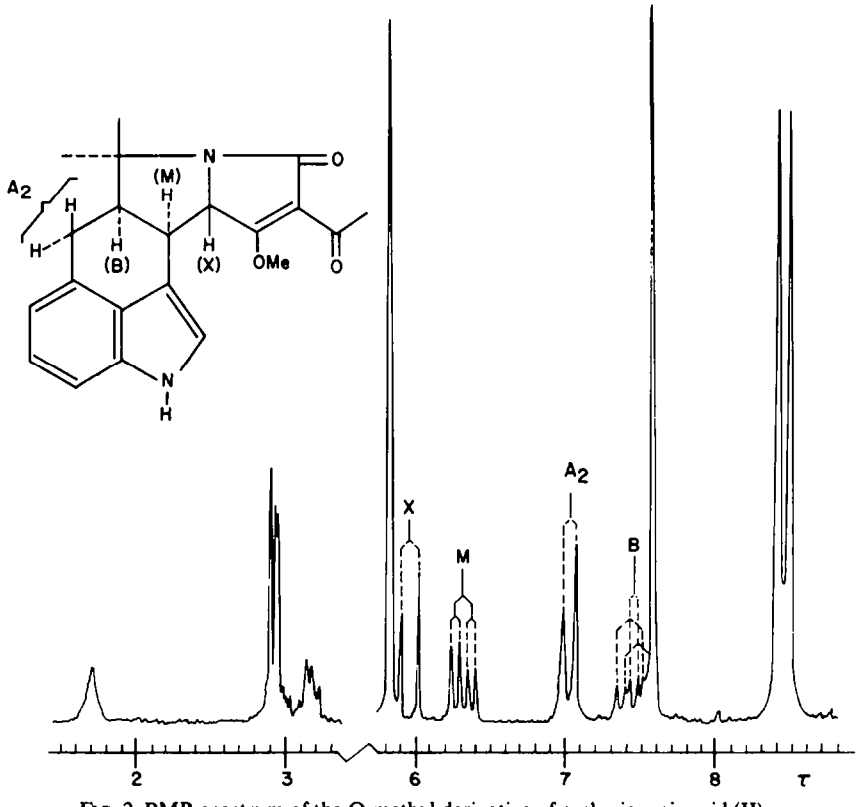


FIG. 2. PMR spectrum of the O-methyl derivative of cyclopiazonic acid (II).

neutral solution at room temperature. The structure I assigned to cyclopiazonic acid therefore represents but one of its possible tautomeric forms. The PMR spectrum of tenuazonic acid (XI) indicated a similar equilibrium of tautomeric forms. This is reminiscent of the behaviour of the β-triketones flavesone,[3c] tasmonone[3a] and grandiflorone.[8]

The PMR spectrum of isocyclopiazonic acid (III) showed that the compound was epimeric with cyclopiazonic acid (I) at position 5. First order analysis of the PMR spectrum of the O-Me derivative of deacetylisocyclopiazonic acid (V) showed that the grouping (—CH.CH.CH.CH_2) absorbed essentially as an XMABC system with coupling within the system limited to vicinal protons (Fig. 3). The methylene group was responsible for the AB part of the system. Proton A absorbed as a quartet centred at τ 6·7 (J_{AB} 18 c/s, J_{AC} 6 c/s), but the signals due to proton B were poorly separated from those of proton C. Proton X gave rise to a doublet at τ 5·30 due to coupling with proton M (J_{XM} 6 c/s). Proton M was further coupled to proton C (J_{MC} 6 c/s) and to the α-proton of the indole system (J 2 c/s). The latter coupling was proved by a double resonance experiment. The indole α-proton absorbed as a triplet (J 2 c/s) at τ 3·37 which collapsed to a doublet (J 2 c/s) on deuteration of the indole-NH. By irradiation at the frequency of this doublet, the 6-line pattern due to proton M changed to a sharp triplet. This allylic coupling between the protons in question is only observed in the "iso" series of compounds.

An interesting product from the reaction of compound IVb with LAH was an amide, $C_{18}H_{18}N_2O$. It gave an immediate purple Erlich colour reaction but its UV

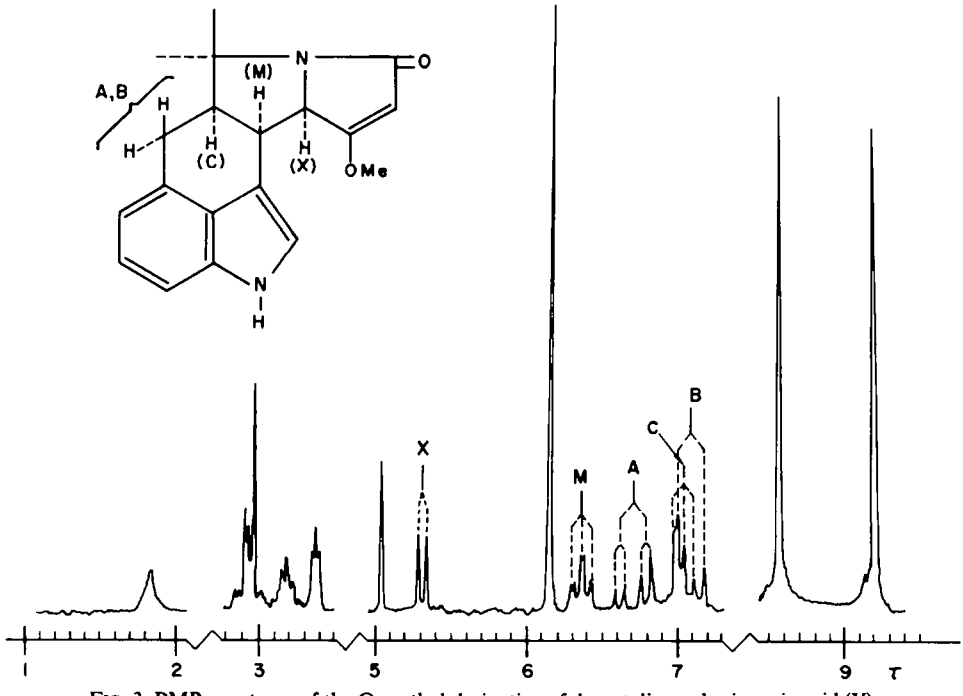
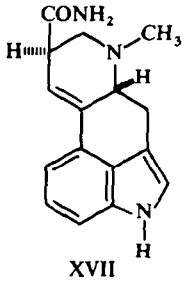


FIG. 3. PMR spectrum of the O-methyl derivative of deacetylisocyclopiazonic acid (V).

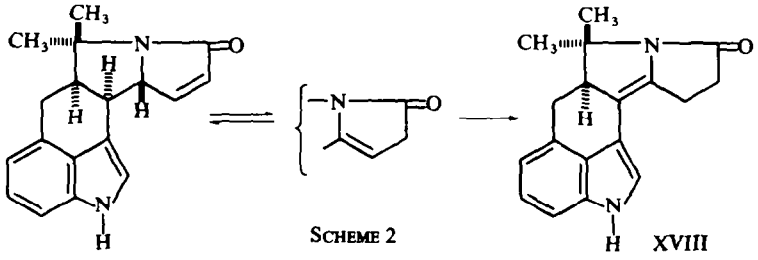
absorption (λ_{max} 232, 284 and 313 mμ, log ε 4·34, 4·09 and 4·03, respectively) was not that of an unconjugated indole. Comparison of its UV spectrum with those of lysergamide (XVII),[6b,9] uleine[10] and diacetyl-allo-cinchonamine[11] led to the conclusion that the compound contained a double bond in conjugation with an indole system as in XVIII. This compound may conceivably arise from the postulated



XVII

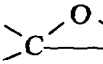
intermediate VIIa (*vide supra*) by base-catalysed (LAH or LiOMe) migration of the C_6—C_7 double bond into conjugation with the indole system as depicted in Scheme 2.

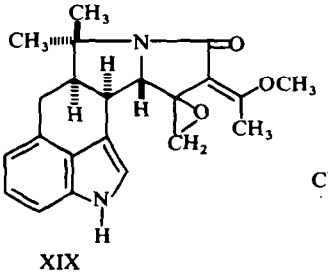
The reaction of diazomethane with cyclopiazonic acid (I) yielded, in addition to a simple O-Me derivative (*vide supra*), a compound $C_{22}H_{24}N_2O_3$ corresponding to the addition of two CH_2 groups to the starting material. Its PMR spectrum showed,



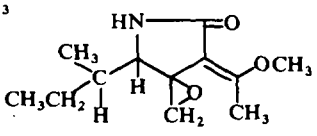
SCHEME 2

XVIII

inter alia, signals due to an OMe group (τ 6·17) and a Me group (τ 7·80) on a sp^2 hybridized carbon atom. A two-proton singlet at τ 6·58 is assigned to a  group.[12] The compound can best be represented by structure XIX. It is known that certain activated CO groups react readily with diazomethane to give epoxides.[13] It is of interest to note that the reaction of diazomethane with tenuazonic acid (XI) yielded mainly a compound $C_{12}H_{19}NO_3$, formulated as XX on the basis of its IR, UV and PMR spectra.

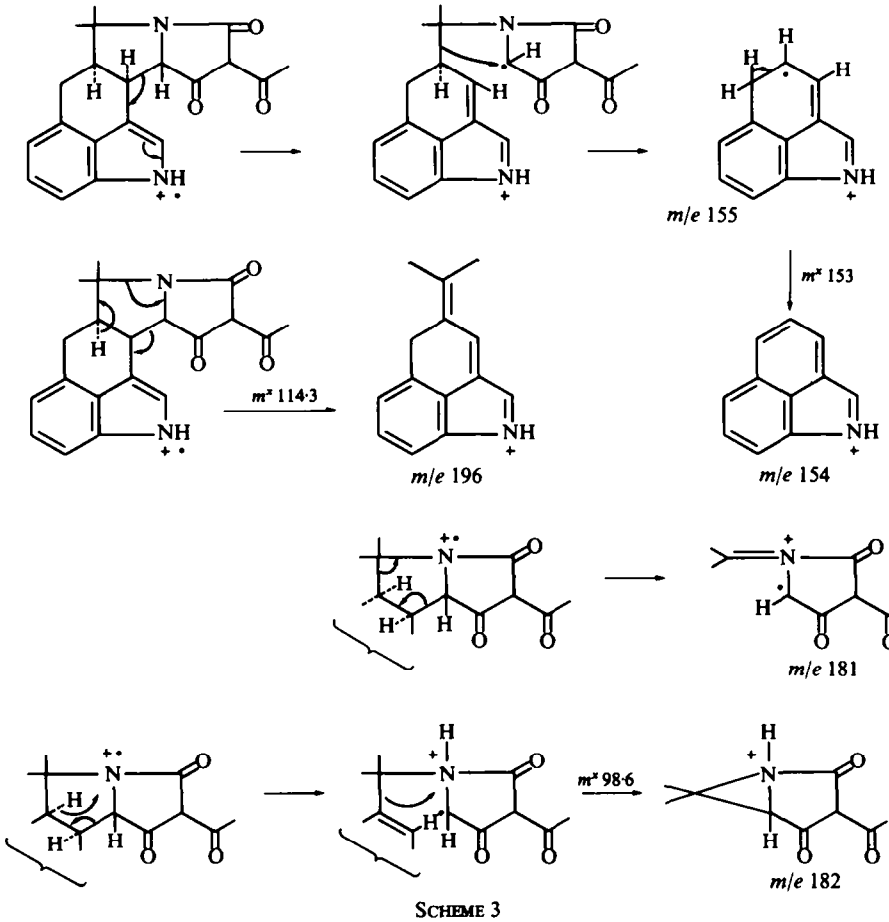


XIX



XX

On the basis of structure I, the main fragmentation of cyclopiazonic acid under electron impact can be interpreted as shown in Scheme 3. This rationalization of the fragmentation mechanism is based on the assumption that it is a non-bonding electron of one of the nitrogen atoms which is first removed and that the charged centres so produced are responsible for the course and direction of the subsequent

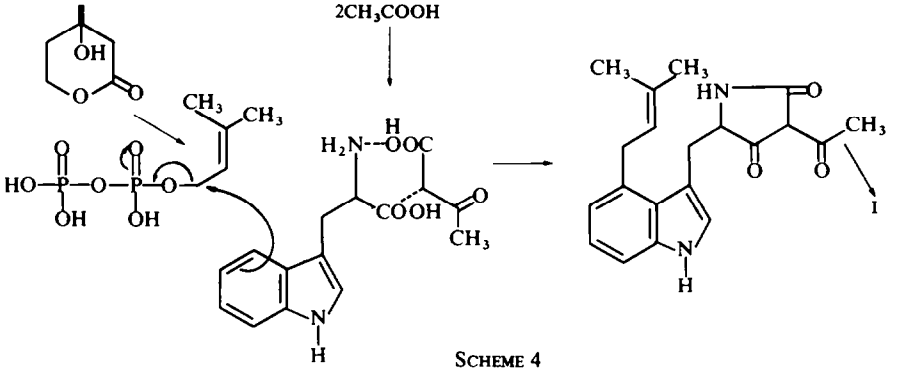


SCHEME 3

bond ruptures. Several of the less prominent fragment ions (Fig. 1) may conceivably be due to further decomposition of the main fragment ions. Thus the ion of mass 167 may be formed from the ion of mass 182 by ejection of a Me group while the ion of mass 127 may arise from the species of mass 154 by expulsion of hydrogen cyanide.[14] The mass spectrum of dideuteriocyclopiazonic acid, in which the enolic proton and the indole-NH are replaced by deuterium (treatment with deuteriomethanol), showed major peaks at *m/e* 338, 197, 182, 183, 156 and 155. Not unexpectedly, the mass spectra of the deacetyl compounds IV a and IVb as well as that of the amine VI all show prominent peaks at 154 and 196 together with prominent peaks at *m/e* 140, 153 and 111, respectively, corresponding to ring E of these compounds together with the CMe_2 moiety. However, the mass spectrum of the conjugated indole XVIII

was entirely different. It showed an intense peak for the molecular ion but, apart from the simple loss of one Me, showed very little further fragmentation. This greatly increased stability of the compound may be due to the presence of a double bond in ring D.

Structure I assigned to cyclopiazonic acid is plausible from a biogenetic point of view. Structure analysis of its molecular skeleton shows that the compound is probably derived from tryptophan, a C_5-unit derived from mevalonic acid (the precursor of active isoprene, e.g. γ,γ-dimethylallyl pyrophosphate) and two molecules of acetic acid as outlined in Scheme 4. The biological introduction of a



SCHEME 4

mevalonate-derived C_5-unit into the 4-position of an indole system is known to occur, e.g. in the biosynthesis of the lysergic acid portion of ergot alkaloids in *Claviceps* species.[15] The condensation of tryptophan and two molecules of acetic acid (or acetoacetate) to form a substituted tetramic acid would be analogous to the formation of tenuazonic acid (XI) from L-isoleucine and two molecules of acetic acid in *Alternaria tenuis* Auct.[16] So far as is known, cyclopiazonic acid, tenuazonic acid and erythroskyrene[17] are the only examples of substituted tetramic acids occurring as natural products.

The relative stereochemistry of cyclopiazonic acid

Cyclopiazonic acid has three asymmetric centres (C-4, C-5 and C-11); this gives rise to the possibility of four configurations, each of which can exist as an enantiomorphic pair. Inspection of Dreiding models showed that the dihedral angle between C_4-H and C_{11}-H is close to 180° in structures with a *trans* C/D ring juncture (ring C assumes a "five-point coplanar" conformation). in 6-membered ring systems,[18] including those with a "five-point coplanar" conformation,[19] such an orientation is known to give rise to large coupling constants (8–14 c/s). The coupling constant (6 c/s*) between C_4-H and C_{11}-H in both cyclopiazonic acid (I) and isocyclopiazonic acid (III) falls outside this range and is in better agreement with a structure in which rings C and D are *cis* fused. The coupling constants for vicinal protons with an approximate axial-equatorial or equatorial–equatorial orientation in 6-membered rings fall

* Splittings in the patterns due to protons at C-4, C-5 and C-11 were identical in spectra at both 60 Mc/s and 100 Mc/s and are therefore good approximations to the coupling constants.[20]

within the range 1–7 c/s.[18] Support for a *cis* C/D ring juncture in cyclopiazonic acid and its derivatives is derived from the fact that allylic coupling between C_2-H and C_4-H is only observed in the "iso" series of compounds (*vide supra*). This must be due to a considerable difference in the angle (θ) between the 2,3-double bond and the adjacent C_4-H bond. [21] PMR data (*vide supra*) showed that isomerization of cyclopiazonic acid involved only C-5. The change in the angle θ must therefore be associated with a rotation about the C_3—C_4 bond on passing from the "normal" to the "iso" series of compounds. This is only possible in a structure with a *cis* C/D ring juncture.

Structures with a *cis* C/D ring juncture may have C_4-H and C_5-H either in a *syn* or *anti* relationship. Dreiding models of these configurational possibilities show that there are no important non-bonded interactions and that in each case there exists a definite preferred conformation corresponding to minimum bond and angle strain. Figure 4 depicts the preferred conformations of two C-5 diastereoisomers with *cis* C/D ring junctures. The PMR data of the "normal" and "iso" series of compounds

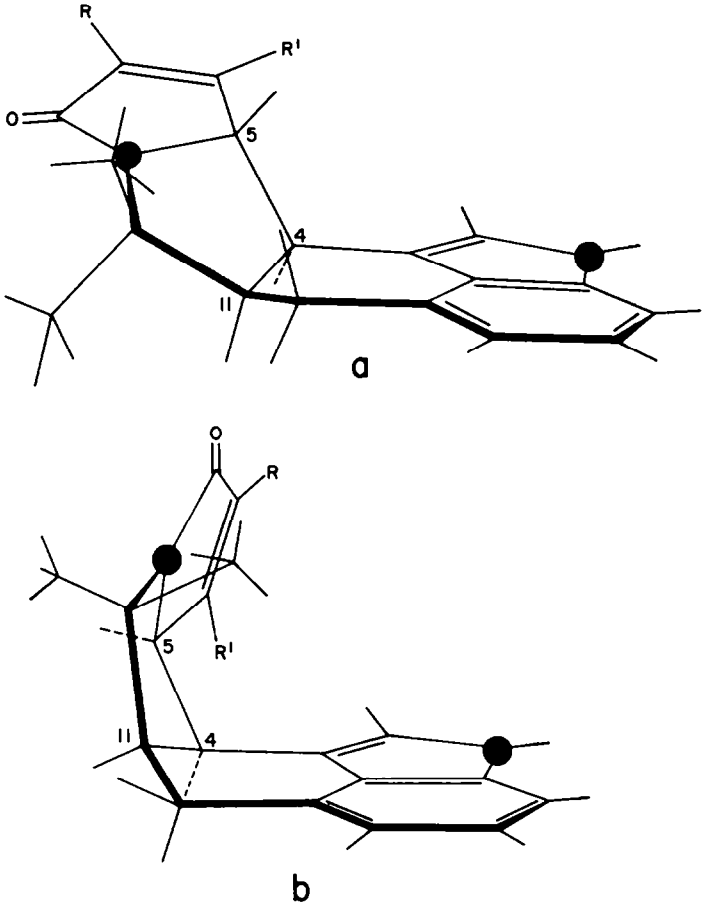


FIG. 4. Preferred conformations of two C-5 diastereoisomers with cis A/B ring junctures.
a = C_4-H, C_5-H *anti* + "normal" b = C_4-H, C_5-H *syn* + "iso"
Full circles: nitrogen; R = $COCH_3$ or H,R' = OH or OCH_3.

can be interpreted in terms of the conformations and relative stereochemistry depicted in Figs 4a and 4b, respectively. Thus, the large coupling constant (11 c/s*) between C_4-H and C_5-H in cyclopiazonic acid is consistent with a dihedral angle close to 180°, while the smaller coupling constant (6 c/s*) between C_4-H and C_5-H in isocyclopiazonic acid is consistent with a dihedral angle close to 35°.[22] In cyclopiazonic acid, the proton on C-11 lies outside the *gem*-protons at C-12 and this is compatible with the relatively large average coupling constant (8 c/s) associated with the interaction of these protons.[23] In the "iso" series of compounds, the proton on C-11 lies inside the *gem*-protons at C-12 and the dihedral angles between C_4-H and C_5-H, between C_5-H and C_{11}-H and between C_{11}-H and one of the C_{12}-H protons are all close to 35°. This is consistent[22] with the fact that the coupling constants between these pairs of protons are practically equal (6 c/s) in the iso-compound V. The proton on C-4 assumes a quasi-equatorial orientation in the "normal" series but a quasi-axial orientation in the "iso" series. This accounts for the fact that allylic coupling between C_2-H and C_4-H is observed only in the "iso" series, since in the latter case the angle between the 2,3-double bond and the C_4-H bond is much nearer the value (90°) for maximum allylic coupling.[21]

The frequencies of the Me groups attached to C-10 in cyclopiazonic acid (I) and the deacetyl compound IVb are very close (τ 8·35 and 8·42 in I, τ 8·40 and 8·42 in IVb). On the other hand, those of the Me groups attached to C-10 in both isocyclopiazonic acid (III) and the deacetyl compound V are very different (τ 8·54 and 9·16 in III, τ 8·56 and 9·18 in V). It follows that one of the Me groups on C-10 becomes considerably more shielded on passing from the "normal" to the "iso" series of compounds. Figure 4b shows that in the "iso" series one of the C_{10}-Me groups is situated close to the hexagonal axis of ring A. In this position the magnetic field due to the ring current associated with the indole system may be expected to have a shielding effect.[24] A difference in deshielding of the methyl groups by the amide CO group in the two series should, however, also be considered.

The CD spectra (Fig. 5) of the O-Me derivative of deacetylcyclopiazonic acid (IVb) and the corresponding 5-epi compound V show Cotton effects at ca. 223 mμ and also in the region 270 to 300 mμ which can be ascribed to the indole system. In addition, both compounds show Cotton effects at ca. 210 and ca. 250 mμ. These are ascribed to the β-methoxy α,β-unsaturated amide chromophore, since UV spectra of α,β-unsaturated lactams[25] show maxima at ca. 200 and 240 mμ. The effect of a β-OMe group would be to shift these maxima to higher wavelengths. Furthermore, the CD spectrum (Fig. 5D) of the O-Me derivative of deacetyltenuazonic acid (VIIIb)† shows Cotton effects at 210 and 241 mμ. It may be noted that optically active compounds with an indole system as the only chromophore absorbing above 220 mμ (e.g. pseudoyohimbine[26] and dihydroisolysergamide-I) show no Cotton effects (cf. Fig. 5C) in the 250 mμ region.

The sign of the Cotton effects due to the ring-E chromophores in compounds IVb and V appears to be clearly related to the stereochemistry at C-5. The stereochemistry at this centre also appears to determine the sign of the strong Cotton effect at ca.

* See footnote on p. 2110.

† Compound VIIb was prepared by methylation (diazomethane) of compound VIIa obtained by deacetylation of tenuazonic acid (Xa) under conditions which did not effect complete racemization at C-5.

223 mμ due to the indole chromophore. This is somewhat surprising since the signs of the Cotton effects due to an aromatic chromophore are generally determined by asymmetric centres in rings fused to the aromatic system.[27] The difference in the sign of the Cotton effects at ca. 223 mμ in IVb and V may, however, be due to the different orientation of C-11 with respect to the indole chromophore in these compounds (see Fig. 4).

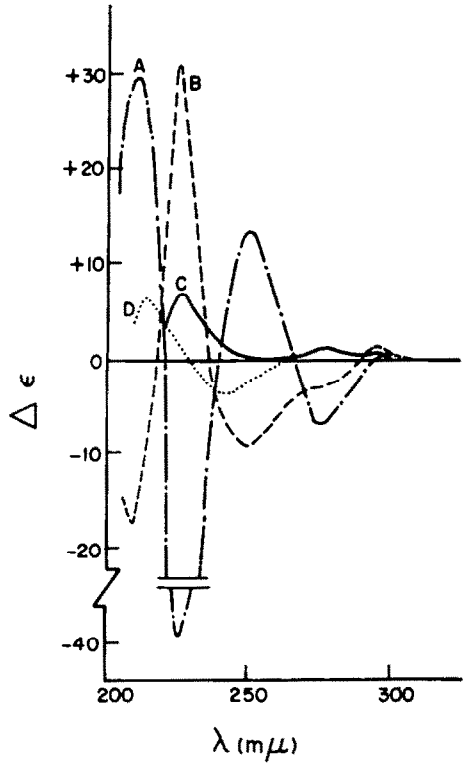


FIG. 5. CD curves (in MeOH) for:
A —.—.— O-methyl compound IVb
B — — — — O-methyl compound V
C ———— dihydroisolysergamide — I
D ..... O-methyl compound VIIIb.

The intensities of the Cotton effects of isocyclopiazonic acid (III) (Fig. 6B) in the region 270 to 300 mμ are an order of magnitude greater than those of the deacetyl compound V. This increase in intensity is probably not due to the contribution of the ring-E chromophore since tenuazonic acid (XI) shows a relatively weak Cotton effect at 280 mμ (Fig. 6C). In fact, the high intensity of the Cotton effects of III may be taken as *prima facie* evidence for the presence of an inherently dissymmetric chromophore.[28] Fig. 4b shows that the two chromophores of isocyclopiazonic acid are closely situated and very much skewed with respect to one another. Electronic interaction between these chromophores should give rise to large Cotton effects.[29] The small energy separation between the transitions increases the likelihood of

electronic interaction.[30] The much smaller Cotton effects of cyclopiazonic acid (I) (Fig. 6A) in the region 270–300 mμ are consistent with the fact that the two chromophores are not very closely situated and are only slightly skewed with respect to one another (see Fig. 4a).

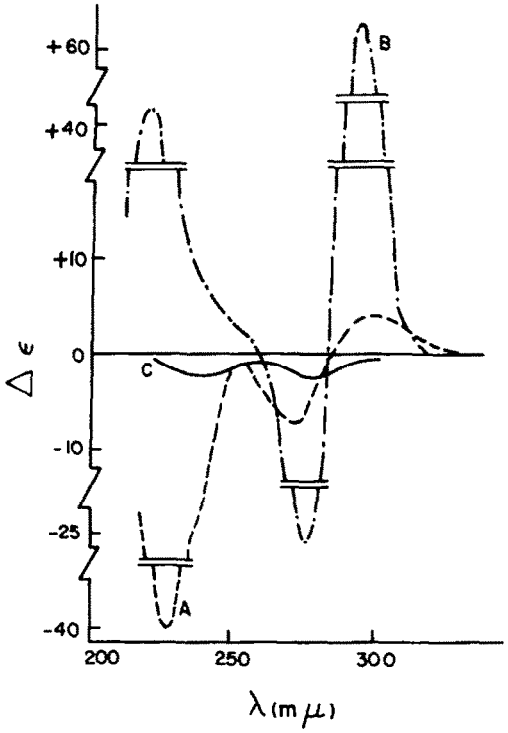


FIG. 6. CD curves (in MeOH) for:
A – – – – – – – – cyclopiazonic acid (I)
B — . — . — . isocyclopiazonic acid (III)
C ———— tenuazonic acid (XI).

EXPERIMENTAL

UV absorption refers to MeOH and IR absorption to $CHCl_3$ solns. UV spectra (Unicam Model S.P. 800 Spectrometer) and IR spectra (Perkin–Elmer Model 237 Spectrometer). Mass spectra were taken on an MS-9 double focusing mass spectrometer. The CD curves were measured at 20° with a Jasco O.R.D./U.V.-5 instrument with CD attachment. Concentrations of solns used for UV* and CD measurements are given in mg/100 ml throughout. For describing PMR signals, the following conventions are used: s (singlet), d (doublet), t (triplet); coupling constants are given in c/s. For preparative TLC, chromatoplates were coated with Merck's Silica Gel G containing a fluorescent indicator (thickness of silica gel layer ca. 2 mm).

Isolation of cyclopiazonic acid

Penicillium cyclopium Westling (strain 1082) was grown in bulk on sterilized wet maize meal as previously described.[31] The dried mouldy meal (20 kg) was extracted with $CHCl_3$—MeOH and the solvent removed *in vacuo*. The toxic extract (2·2 kg) was distributed between $CHCl_3$ (5 l.) and water (8 l.). The $CHCl_3$ was evaporated and the residue (920 g) distributed between 95% MeOH (3 l.) and hexane (4 l.).

* The degree of enolization of the β-diketones described herein is concentration dependent.

The MeOH was evaporated and the residue (255 g) chromatographed on cellulose powder (3 kg) impregnated with 50:3 $HCONH_2$—$(COOH)_2$. Lipids were eluted with hexane (6 l.). The column was then developed with hexane—C_6H_6 mixtures (20:1 → 3:1) and 70 × 150 ml fractions collected. Fractions 36 to 48 accounted for most of the toxicity. This toxic concentrate (12·5 g) in ether (500 ml) was extracted with 0·5M aqueous $NaHCO_3$ (4 × 200 ml), the acidified aqueous phase extracted with $CHCl_3$ and the isolated acids (2·5 g) separated on "Dowex 1 × 8" (50 g, 200–400 mesh, formate form) in 1:1 aqueous MeOH. The column was developed by gradient elution with 3·5M formic acid in 1:1 aqueous MeOH and 50 × 25 ml fractions collected. $CHCl_3$ extraction of the combined fractions 28 to 38 and crystallization from MeOH gave pure *cyclopiazonic acid* (I; 395 mg), m.p. 245–246°, λ_{max} (*c*, 0·63) 225, 253, 275(sh), 284 and 292(sh) mμ (log ε 4·60, 4·22, 4·28, 4·31 and 4·24, respectively), ν_{max} 3478, 3200–2600, 1708(m) and 1618(s) cm^{-1}. CD (*c*, 1·5): Δε (320 mμ) +1·06, (298) +4·24, (286) 0, (273) −9·53, (254) −1·06, (228) −40·27, (220) −22·28. The high resolution mass spectrum showed: *m/e* 336·1463 (M^+, $C_{20}H_{20}N_2O_3$ requires: 336·1474), 196·1123 ($C_{14}H_{14}N$ requires: 196·1126), 182·0802 ($C_9H_{12}NO_3$ requires: 182·0817), 181·0735 ($C_9H_{11}NO_3$ requires: 181·0739), 155·0727 ($C_{11}H_9N$ requires: 155·0735) and 154·0651 ($C_{11}H_8N$ requires: 154·0657). [Found: C, 71·29; H, 6·04; N, 8·60%; equiv. by titration (phenolphthalein), 328. $C_{20}H_{20}N_2O_3$ requires: C, 71·41; H, 5·99; N, 8·33%; equiv. weight (monobasic acid) 336.] The compound gave a blue-violet Erlich colour reaction and an orange-red $FeCl_3$ colour reaction, but the Dragendorff colour reaction was negative. Dropwise addition of a 10% soln of I_2 in aqueous 20% KI to a soln of I in 5N NaOH gave an immediate yellow ppt and the characteristic smell of iodoform.

Zeisel and Herzig-Meyer analyses showed that cyclopiazonic acid contained no OMe or N-Me groups. Treatment of an aqueous soln of the Na salt of I with a chemically equiv volume of 0·5M $Cu(OAc)_2$ gave the *copper salt* as a green ppt. Crystallized from $CHCl_3$ it had m.p. > 300°. [Found: C, 65·15; H, 5·03; N, 7·35. $Cu(C_{20}H_{19}N_2O_3)_2$ requires: C, 65·44; H, 5·22; N, 7·63%.] The free acid is readily recovered from the $CHCl_3$ layer after shaking the copper salt with $CHCl_3$ and 3N HCl until all the solid has disappeared.

Acetylation with Ac_2O and pyridine and chromatography of the product on $HCONH_2$-impregnated cellulose in 4:1 hexane—C_6H_6 gave an acetate which would not crystallize. TLC indicated that the compound was substantially pure; ν_{max} 3478, 1770, 1650 and 1180 cm^{-1}. The mass spectrum showed: M^+, 378 ($C_{22}H_{22}N_2O_4$ requires: M, 378).

Cyclopiazonic acid (15 mg) in 1:1 $CHCl_3$—MeOH (2 ml) was treated with 85% $NH_2.NH_2$ 0·05 ml) at room temp for 2½ hr. Dilution with water and extraction with $CHCl_3$ yielded *cyclopiazonic acid hydrazone*, m.p. 189–190° (from $CHCl_3$), λ_{max} (*c*, 1·0) 223, 250, 285(sh), 292 and 310 mμ (log ε 4·56, 3·96, 4·08, 4·15 and 4·15, respectively), ν_{max} 3478, 3400–3200, 1665(m) and 1623(s) cm^{-1}. The mass spectrum showed: *m/e* 350 (M^+, 24), 332 (31), 196 (100), 177 (74) and 154 (39). (Found: C, 68·25; H, 6·10; N, 15·35. $C_{20}H_{22}N_4O_2$ requires: C, 68·55; H, 6·33; N, 15·99%.) The compound gave a green $FeCl_3$ colour reaction.

Reaction of cyclopiazonic acid with diazomethane

Cyclopiazonic acid (50 mg) in MeOH (4 ml) was treated with ethereal diazomethane (20 ml) at room temp for 30 min. The excess of diazomethane was decomposed with HOAc and the reaction product separated on formamide-impregnated Whatman No. 3MM filter paper with 1:1 C_6H_6—hexane as mobile phase. The two main absorbing bands were eluted with 1:1 $CHCl_3$—MeOH. Band 1 (R_f 0·45) yielded the colourless amorphous O-*methyl compound* II (16·5 mg) moving as one spot when examined by TLC and taken as substantially pure. It showed λ_{max} 224, 265(sh), 281 and 293(sh) (log ε 4·53, 4·26, 4·28 and 4·18, respectively), ν_{max} 3480, 1680 and 1635 cm^{-1}. The mass spectrum showed: *m/e* 350 (M^+, 100), 196 (90), 155 (25) and 154 (56). [Found: M, 350·1622. $C_{21}H_{22}N_2O_3$ requires: M, 350·1630].

Band 2 (R_f 0·33) yielded the *epoxide* XIX (11·5 mg), m.p. 193–195° (from MeOH), λ_{max} 223, 271, 280(sh) and 291 mμ (log ε 4·55, 3·82, 3·78 and 3·66, respectively), ν_{max} 3480 and 1660 cm^{-1}. The mass spectrum showed: *m/e* 364 (M^+, 17), 209 (23), 196 (100), 166 (82), 154 (18) and 127 (6). The compound showed *inter alia* the following PMR signals: τ 1·70, broad, N1-H; τ 5·92, d J = 11, C5-H; τ 6·17, s, OCH_3; τ 6·58, s, ); τ 7·02, d J = 8, C12-H_2; τ 7·80, s, ($CH_3.C(OMe) = C$—) and τ 8·46, s, (*gem* CH_3). (Found: M, 364·1783. $C_{22}H_{24}N_2O_3$ requires: M, 364·1787.) The compound gave negative $FeCl_3$ and Dragendorff colour reactions.

Isomerization of cyclopiazonic acid (I)

A soln of I (60 mg) in 0·3N NaOH (8 ml) was heated under reflux in an atmosphere of N_2 for 10 hr. The

cooled mixture was acidified (HOAc), extracted with $CHCl_3$ and the organic solvent evaporated. The residue (56 mg) was separated by chromatography on Whatman No. 3MM filter paper impregnated with 8:1 $HCONH_2$—$(COOH)_2$. The chromatogram was developed with 3:1 hexane—C_6H_6 and the two absorbing bands eluted with 1:1 $CHCl_3$—MeOH. Band 1 (R_f 0·48) yielded *isocyclopiazonic acid* (III; 19 mg), m.p. 238–239° (from Et_2O), λ_{max} (c, 0·62) 225, 253, 275(sh), 284, and 292(sh) mμ (log ε 4·62, 4·23, 4·26, 4·32 and 4·23, respectively), CD (c, 0·5): Δε (310 mμ) +3·53, (304) +28·25, (296) +63·53, (284) 0, (275) −26·49, (260) 0, (222) +42·36, (210) 0. The mass spectrum showed: *m/e* 336 (M^+, 69), 196 (67), 182 (100), 181 (54), 155 (62), 154 (43) and 127 (11). The compound showed *inter alia* the following PMR signals: τ 2·0, broad N1-H; τ, 5·58, d J = 6, C5-H; τ 6·25, t of d J = 2 and 6, C4-H; and τ 7·62, s, CH_3CO. (Found: C, 71·05; H, 6·15; N, 8·30. $C_{20}H_{20}N_2O_3$ requires: C, 71·41; H, 5·99; N, 8·33%.)

Band 2 (R_f 0·43) yielded cyclopiazonic acid (I; 28 mg).

Acid hydrolysis of cyclopiazonic acid (I)

(i) A soln of cyclopiazonic acid (17 mg) in 2N H_2SO_4 (5 ml) and MeOH (3 ml) was heated under reflux in an atmosphere of N_2 for 6 hr. The mixture was then steam-distilled for volatile-acid determination. The distillate gave a titre of 0·91 ml of 0·05N NaOH. The titrated distillate was acidified with 2N H_2SO_4 and distilled. The volatile-acid was identified as HOAc (R_f 0·14)[32] by paper chromatography of its ammonium salt.

(ii) A soln of I (100 mg) in 0·1N HCl (120 ml) and MeOH (120 ml) was heated under reflux in an atmosphere of N_2 for 22 hr. The mixture was cooled and exhaustively extracted with $CHCl_3$. Evaporation of the organic solvent gave a residue (79 mg) which crystallized partly on trituration with a large volume of ether. Recrystallization from $CHCl_3$ gave *deacetylcyclopiazonic acid* (IVa; 11 mg) m.p. 218–220° decomp, λ_{max} (c, 2·0) 223, 273, 280(sh) and 293 mμ (log ε 4·59, 3·91, 3·87 and 3·67, respectively), ν_{max} 3480 and 1682 cm^{-1}. The mass spectrum showed: *m/e* 294 (M^+, 81), 196 (19), 155 (38), 154 (45), 140 (100) and 127 (9). (Found: C, 73·15; H, 6·05; N, 9·20. $C_{18}H_{18}N_2O_2$ requires: C, 73·45; H, 6·16; N, 9·52%.) The compound gave a blue-violet Erlich colour reaction but negative $FeCl_3$ and Dragendorff colour reactions.

The residue (65 mg) from the combined mother liquors of the above crystallizations was treated with diazomethane in 9:1 Et_2O—MeOH for 1 hr. The excess of diazomethane was decomposed with HOAc and the solvent evaporated. The reaction product was separated on a preparative chromatoplate with 9:1 $CHCl_3$—MeOH as mobile phase. The two main absorbing bands were eluted with MeOH. Band 1 (R_f 0·68) yielded the *O-methyl derivative of deacetylcyclopiazonic acid* (IV b; 35 mg), m.p. 253–254° (from $CHCl_3$—Et_2O), λ_{max} 223, 270, 280(sh) and 292 mμ (log ε 4·58, 3·81, 3·78 and 3·67, respectively), ν_{max} 3480, 1668 and 1615 cm^{-1}), CD (c 1·0): Δε (300 mμ) +1·4, (295) 0, (275) −6·93, (266) 0, (250) +13·3, 240 (0), (226) −45·08, (220) 0, (210) +34·83. It showed *inter alia* the following PMR signals: τ 1·77, broad, N1-H; τ 5·02, s, C7-H; τ 5·73, d J = 11, C5-H; τ 6·50, d of d J = 6 and 11, C4-H and τ 6·23, s, OCH_3. The mass spectrum showed: *m/e* 308 (M^+, 28), 196 (90), 155 (20), 154 (53), 153 (100), 138 (18) and 127 (11). [Found: M, 308·1530. $C_{19}H_{20}N_2O_2$ requires: M, 308·1525]. Compound IVb was also prepared directly by treatment of IVa with diazomethane in Et_2O—MeOH.

Band 2 (R_f 0·58) yielded the *O-methyl derivative of deacetylisocyclopiazonic acid* (V; 16 mg), m.p. 236–238° (from C_6H_6), λ_{max} 223, 270, 280(sh) and 292 mμ (log ε 4·56, 3·80, 3·77 and 3·66, respectively), ν_{max} 3475, 1666 and 1618 cm^{-1}, CD (c, 0·5): Δε (295 mμ) +1·77, (290) 0, (270) −3·10, (250) −8·8, (235) 0, (225) +30·97, (219) 0, (210) −17·70. (Found: M, 308·1521. $C_{19}H_{20}N_2O_2$ requires: M, 308·1525.)

Reaction of compound IVb *with LAH*

Compound IVb (30 mg) and LAH (30 mg) in THF (5 ml) were heated under reflux in an atmosphere of N_2 for 6 hr. The cooled mixture was carefully poured into crushed ice and extracted with CH_2Cl_2. The CH_2Cl_2 was washed with 3N HCl and the organic solvent evaporated. The residue (18 mg) was separated on a preparative chromatoplate with 20:1 $CHCl_3$—MeOH as mobile phase. The main absorbing band (R_f 0·65) was eluted with 1:1 $CHCl_3$—MeOH. Evaporation of the solvent gave the colourless *conjugated indole* XVIII (10 mg), m.p. 244–246° decomp (from CH_2Cl_2), λ_{max} 232, 245(sh), 284, 295(sh) and 313 mμ (log ε 4·34, 4·16, 4·09, 4·05 and 4·03, respectively), ν_{max} 3370 and 1665 cm^{-1}. The mass spectrum showed: *m/e* 278 (M^+, 100), 263 (30), 208 (11) and 154 (5). (Found: M, 278·1409. $C_{18}H_{18}N_2O$ requires: M, 278·1419.) The compound gave a positive Erlich colour reaction.

Neutralization of the above 3N HCl washings and extraction with $CHCl_3$ gave material (8 mg) which was separated on a preparative chromatoplate (Woelm neutral alumina containing fluorescent indicator) with 25:1 $CHCl_3$—MeOH as mobile phase. The main absorbing band (R_f 0·75) was eluted with 1:1 $CHCl_3$—MeOH as mobile phase. The main absorbing band (R_f 0·75) was eluted with 1:1 $CHCl_3$—MeOH.

Evaporation of the solvent gave the *amine* VI (5 mg), m.p. 196–197° (from Et_2O), λ_{max} 223, 274(sh), 280 and 292 mμ (log ε 4·52, 3·78, 3·79 and 3·67, respectively), ν_{max} 3480 and 1603 cm^{-1}, CD (*c*, 5·1): Δε (275 mμ) +1·29. The mass spectrum showed: *m/e* 266 (M^+, 18), 251 (7), 196 (3), 154 (14) and 111 (100). (Found: M, 266·1768. $C_{18}H_{22}N_2$ requires: M, 266·1783.) The compound gave positive Erlich and Dragendorff colour reactions.

Preparation of the tetradeuterio compounds XV *and* XVI

Na (20 mg) and then cyclopiazonic acid (40 mg) were added to D_2O (4 ml) and the mixture heated under reflux in an atmosphere of N_2 for 6 hr. The cooled mixture was acidified (DCl) and extracted with $CHCl_3$. The $CHCl_3$ was evaporated and the residue treated twice with NaOD—D_2O as before. The reaction product was treated briefly with MeOH and then chromatographed on formamide-impregnated cellulose powder (10 g). Two compounds were eluted with 5:1 hexane—C_6H_6. After purification by rechromatography and crystallization from Et_2O, the first compound was identified as *tetradeuterioisocyclopiazonic acid* (XVI; 11 mg), m.p. 235–237°, ν_{max} 3480, 3200–2600, 1708(m) and 1620(s) cm^{-1}. The mass spectrum showed: *m/e* 340 (M^+, $C_{20}H_{16}D_4N_2O_3$ requires: M^+, 340; relative intensity 62), 196 (69), 186 (100), 185 (53), 155 (63), 154 (45) and 127 (10). The compound showed *inter alia* the following PMR signals: τ 1·97, broad, N1-H and τ 6·23, d of d J = 2 and 6, C4-H.

The second compound was purified by rechromatography and was identified, after recrystallization from MeOH, as *tetradeuteriocyclopiazonic acid* (XV; 14 mg), m.p. 243–245°, ν_{max} 3478, 3200–2600, 1708(m) and 1618 cm^{-1}. It showed *inter alia* the following PMR signals: τ −2·35, broad s, enolic OH; τ 1·77, broad, N1-H and τ 6·43, d J = 6, C4-H. The mass spectrum showed: *m/e* 340 (M^+, $C_{20}H_{16}D_4N_2O_3$ requires: M^+, 340; relative intensity 80), 196 (47), 186 (100), 185 (58), 155 (48), 154 (46) and 127 (9).

Model compounds

(i) Lysergamide (XVII) [m.p. 242° (lit.,[9] m.p. 242°); λ_{max} 240 and 310 mμ (log ε 4·27 and 3·91, respectively)] and isolysergamide, m.p. 133–135° (lit.,[9] m.p. 132–134°), were obtained by hydrolysis of ergotamine tartrate with NaOH in aqueous MeOH and separation of the $CHCl_3$ extract on silica chromatoplates. Hydrogenation of isolysergamide over 30% Pd-C in HOAc and separation of the reaction product on silica chromatoplates yielded dihydroisolysergamide-II, m.p. 211–212° (lit.,[33] m.p. 211–212°), and dihydroisolysergamide-I (XII), m.p. 273° (lit.,[6b] m.p. 275°). The high resolution mass spectrum of XII showed: *m/e* 269·1524 (M^+, $C_{16}H_{19}N_3O$ requires: M, 269·1528), 155·0728 ($C_{11}H_9N$ requires 155·0735) and 154·0658 ($C_{11}H_8N$ requires 154·0657). Compound XII showed CD (*c*, 1·19): Δε (300 mμ) +0·27, (295) +0·96, (290) +0·40, (275) +1·33, (255) +0·27, (225) +6·67, (220) +1·33.

(ii) Tenuazonic acid (XI) was isolated from culture filtrates of *Alternaria tenuis* Auct. as previously described.[34] It showed λ_{max} (*c* 0·8) 241 and 280 mμ (log ε 4·08 and 4·13, respectively), ν_{max} 3440, 3200–2700, 1704(m), 1655(s) and 1620(s) cm^{-1}; CD (*c*, 2·0): Δε (300 mμ) −0·47, (280) −2·8, (260) −1·17, (240) −2·34, (220) −0·47. (Found: M, 197·1044. Calc. for $C_{10}H_{15}NO_3$: M, 197·1052.) The copper salt had $[\alpha]^{20}_{5461}$ −114° in MeOH (lit.,[4] $[\alpha]^{20}_{5461}$ −117° in MeOH).

(iii) Deacetyltenuazonic acid (VIIIa) was prepared by hydrolysis of XI with 0·1N H_2SO_4 as previously described.[4] After crystallization from AcOEt, it had m.p. 116–118° (lit.,[4] m.p. 117·5–119°), λ_{max} (in 0·1N methanolic NaOH) 264 mμ (log ε 4·07), ν_{max} 3425 and 1702 cm^{-1}. [Found: C, 61·6; H, 8·3; N, 8·7. Calc. for $C_8H_{13}NO_2$: C, 61·9; H, 8·4; N, 9·0%]. Treatment with diazomethane in 9:1 Et_2O—MeOH gave the corresponding O-*methyl derivative* VIIIb, m.p. 150–151° (from Et_2O), λ_{max} 211 mμ (log ε 4·12), ν_{max} 3455, 1680 and 1600 cm^{-1}. [Found: C, 63·80; H, 8·85; N, 8·05. $C_9H_{15}NO_2$ requires: C, 63·88; H, 8·94; N, 8·28%]. Compounds VIIIa and VIIIb prepared as described above are mixtures of the C-5 epimers in approximately equal amounts[4] and show no Cotton effects in the region above 210 mμ.

In another experiment, tenuazonic acid (XI) was boiled with 0·05N HCl in 50% aqueous MeOH for 3 hr. The $CHCl_3$ extract contained some deacetyltenuazonic acid (10%) which was separated from unchanged XI by chromatography on silica (elution with $CHCl_3$). This preparation of VIIIa showed a strong Cotton effect at ca. 300 mμ. The corresponding O-methyl derivative showed CD (*c*, 1·8): Δε (260 mμ) −0·47, (242) −3·76, (230) O, (214) +6·42 and (210) +3·28.

(iv) Tenuazonic acid (200 mg) was treated with diazomethane in 9:1 Et_2O—MeOH for 30 min and the reaction product separated on formamide-impregnated cellulose powder (20 g). Elution with 5:1 hexane—C_6H_6 first gave mixed fractions and then the crude *epoxide* XX (86 mg). Chromatography on a preparative chromatoplate with 20:1 $CHCl_3$—MeOH as mobile phase and elution of the absorbing band (R_f 0·44) with 1:1 $CHCl_3$—MeOH yielded the pure epoxide as a colourless oil. It had λ_{max} 208 mμ (log ε 3·83)

and ν_{max} 3450 and 1685 cm^{-1}. It showed *inter alia* the following PMR signals: τ 3·42, broad signal, NH; τ 6·04, s, OCH_3, superimposed on C5-H multiplet; τ 6·50, s, $(H_2C\!-\!C\!<^{O}_{}\!>)$; and τ 7·80, s, $(CH_3.C(OMe)\!=\!C\!-)$. [Found: M, 225·1354. $C_{12}H_{19}NO_3$ requires: M, 225·1365].

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