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THE ISOLATION AND STRUCTURE OF TWO NEW INDOLE DERIVATIVES FROM *PENICILLIUM CYCLOPIUM* WESTLING

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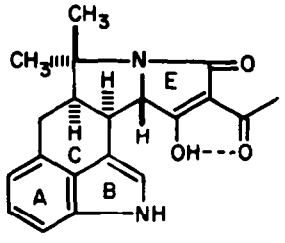
Abstract—Two new indole derivatives, cyclopiazonic acid imine (II) and bissecodehydrocyclopiazonic acid (V), have been isolated from a strain of *Penicillium cyclopium* Westling grown on liquid culture. The structures of these compounds have been deduced on the basis of chemical and spectrochemical evidence.

PENICILLIUM CYCLOPIUM Westling is frequently encountered on stored grain and cereal products. Strain 1082 of this fungus, isolated from ground nuts, produces the toxic substance cyclopiazonic acid (I)[1] on maize,[1] semisynthetic media[2] and synthetic



I

media.[2] The best yield of cyclopiazonic acid was obtained by growing strain 1082 in shake culture on a (basically) Czapek medium. A new indole derivative, designated cyclopiazonic acid imine ($C_{20}H_{21}N_3O_2$) accumulated during the later stages of the fermentation. Another indole derivative, designated bissecodehydrocyclopiazonic acid ($C_{20}H_{22}N_2O_3$) could be detected during the early stages of the fermentation. Initially its concentration increased rapidly and then decreased rapidly as soon as cyclopiazonic acid production accelerated. However, this indole continued to accumulate when the fungus was grown in media having a low ferrous—or zinc–ion concentration.[2] The structures of the two new indole derivatives were deduced on the basis of the following evidence.

Cyclopiazonic acid imine

This compound was insoluble in aqueous sodium bicarbonate. Its molecular

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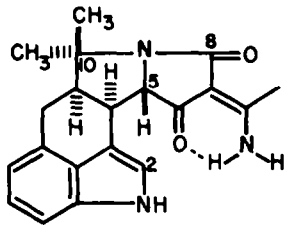
formula ($C_{20}H_{21}N_3O_2$) and spectroscopic properties showed that it was closely related to cyclopiazonic acid. Thus its mass spectrum showed intense fragments ions with *m/e* 154 ($C_{11}H_8N$) and 196 ($C_{14}H_{14}N$) identical with those of cyclopiazonic acid and a peak at *m/e* 180 corresponding to an ion with the composition $C_9H_{12}N_2O_2$. These facts could be rationalised on the basis of formula II. In agreement with this



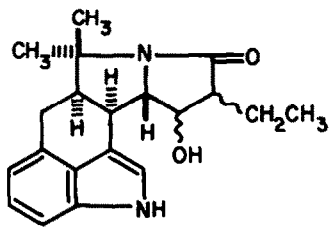
II

deduction the compound could be prepared by treatment of a solution of cyclopiazonic acid with an equal volume of 25% aqueous ammonia. The reaction was carried out at room temperature. Under these conditions the reaction was slow and did not go to completion. At the attainment of equilibrium (6 hr) approximately 72% of the cyclopiazonic acid was converted into the imine. Cyclopiazonic acid could also be partly converted into the imine simply by treatment with aqueous ammonia. The amount of the imine present in the equilibrium mixture decreased rapidly with decreasing ammonia concentration. However, even with the ammonia concentration as low as 0·1 N, detectable amounts of the imine were formed. The possibility must, therefore, be considered that the naturally occurring imine (II) is not the product of an enzymic reaction, but results from a direct reaction of cyclopiazonic acid and ammonia of the ammonium-pool of the fungus.

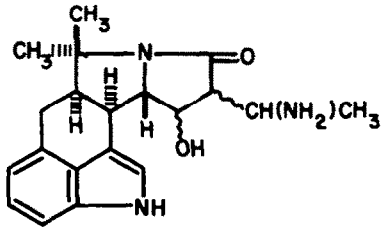
The above evidence does not establish the position of the imine-group. The NMR spectrum of cyclopiazonic acid imine did not provide unequivocal evidence for the position of this group. However, treatment of the imine with an excess of sodium borohydride yielded, in addition to two neutral diastereomers ($C_{20}H_{24}N_2O_2$, formulated as III), a mixture of basic diastereomers $C_{20}H_{25}N_3O_2$, formulated as IV. The presence of the grouping $-CH(NH_2)CH_3$ in these compounds was confirmed by their mass spectra which showed an intense fragment ion (*m/e* 44) with the composition C_2H_6N (formulated as $CH_3CH{=}\overset{+}{N}H_2$). The mass spectra also showed a strong fragment ion at *m/e* 296 ($C_{18}H_{20}N_2O_2$) corresponding to the loss of $CH_3CH{=}NH$ from the parent ion.

Dudek and Holm[3] showed that the 1:1 condensation products of simple β-diketones with monoamines are capable of existing in any of three tautomeric forms (the Schiff-base, the ketamine- and enimine-forms) but that the ketamine-form predominates in most solvents. The NMR spectrum of cyclopiazonic acid imine (II) in deuteriodimethylsulphoxide indicated that the compound exists in two tautomeric forms (probably the ketamine and enimine) present in the ratio 3:2. Thus the indole—NH absorbed* as two broad signals at δ 8·75 and 8·85 with integrated intensities in the ratio 3:2. Another exchangeable hydrogen, probably the non H-bonded hydrogen of

* The indole—NH of cyclopiazonic acid in deuteriodimethylsulphoxide absorbed around δ 9·0.



III

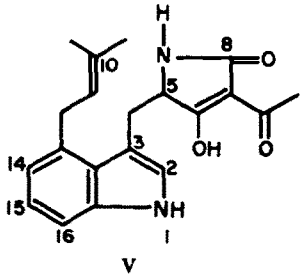


IV

the ketamine and enimine systems, gave rise to signals at δ 9·55 and 9·75 with integrated intensities in the ratio 3:2. The H-bonded proton of the ketamine and enimine forms absorbed as a broad signal at δ 10·75. The proton at position 5 gave rise to two overlapping doublets (J = 11 Hz) around δ 4·4. However, after the addition of sodium deuteroxide to the solution, the proton at position 5 gave rise to a sharp doublet (J = 11 Hz) at δ 4·6. In this solution the proton at position 4 absorbed as a quartet (J = 11 and 5·5 Hz) at δ 4·36 while the *gem*-protons at position 12 absorbed as a doublet (J = 8 Hz) around δ 3·88. This simplification of the NMR spectrum is probably due to the fact that both the ketamine and enimine forms were converted into the same anion on the addition of sodium deuteroxide.

Bissecodehydrocyclopiazonic acid

Bissecodehydrocyclopiazonic acid is a bicarbonate-soluble compound $C_{20}H_{22}N_2O_3$. Its UV spectrum (λ_{max} 225, 276 and 296 (sh) mμ; log ε 4·55, 4·28 and 4·07, respectively) showed a close similarity with that of cyclopiazonic acid and could be interpreted as due to indole and enolised β-diketone chromophores. The compound also showed absorption bands (1708, 1640 and 1618 cm^{-1}) in the carbonyl region of the IR identical with those of cyclopiazonic acid. However, in the N—H stretching region it showed, in addition to an indole—NH band, an absorption band at 3475 cm^{-1} which was tentatively assigned to a secondary amide group. These facts, when considered together with the molecular formula of the new indole suggested that it may be formally derived from cyclopiazonic acid by opening of rings C and D. In agreement with this deduction, the NMR spectrum of the indole-derivative in deuteriodimethylsulphoxide could be interpreted completely on the basis of structure

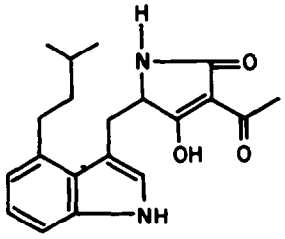


V

V. The proton at C-2 absorbed as a broad signal at δ 7·12. Each of the protons at C-14 and C-16 gave rise to a doublet of doublets (J = 7·5 and 1·5 Hz) at δ 6·71 and 7·18 due to mutual (meta) coupling (J = 1·5 Hz) and coupling with the proton at position 15 which absorbed as a sharp doublet (J = 7·5 Hz) at δ 6·94. The grouping

($ArCH_2$—CH=) gave rise to a two-proton doublet (J = 6·5 Hz) at δ 3·68 and a one-proton triplet (J = 6·5 Hz) at δ 5·28. The three protons in the grouping (Ar—CH_2—CH—CO—) absorbed as an ABX system (δ_A 3·38, δ_B 2·94, δ_X 4·16, J_{AB} = 15, J_{AX} = 9 and J_{BX} = 4 Hz). A sharp three-proton signal at δ 2·38 was assigned to the acyl group. The Me groups of the isopropylidene group absorbed at δ 1·72.

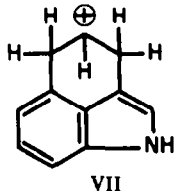
The presence of an isopropylidene group was confirmed by ozonolysis which yielded acetone, identified as its 2,4-dinitrophenylhydrazone-derivative. On hydrogenation in the presence of palladised carbon, bissecodehydrocyclopiazonic acid (V) absorbed 1 mole of hydrogen. The NMR spectrum of the product (VI) confirmed the



VI

presence of an isopropyl-group (6-proton doublet with J = 6·5 Hz at δ 1·02). The mass spectrum of VI showed a prominent peak at m/e 57, corresponding to the ion $(CH_3)_2CH—\overset{+}{C}H_2$, and a peak at m/e 283 ($C_{16}H_{15}N_2O_3$) corresponding to the loss of $(CH_3)_2CH—CH_2$ from the parent ion. The presence of a metastable ion of m/e 103·7 indicated that the base peak at m/e 144 ($C_{10}H_{10}N$) arose from the ion of mass 200 ($C_{14}H_{18}N$) by the loss of the fragment $(CH_3)_2C = CH_2$. The fragment of mass 200 arose from the parent ion by the loss of the grouping ($C_6H_6NO_3$).

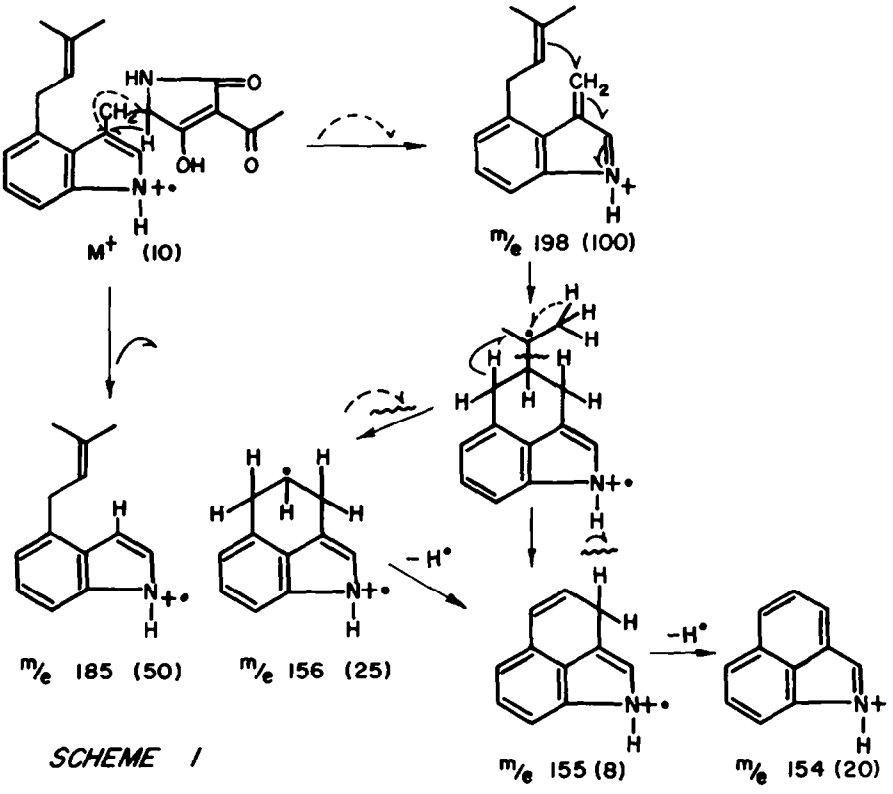
The evidence discussed above can, however, also be interpreted on the basis of the isomeric structure in which the γ,γ-dimethylallyl-group is located at position 16 instead of position 13. This structure is, however, untenable on the basis of the mass spectrum of bissecodehydrocyclopiazonic acid which showed prominent fragment ions with m/e 156, 155 and 154. Accurate mass determination showed that these fragment ions had the composition $C_{11}H_{10}N$, $C_{11}H_9N$ and $C_{11}H_8N$, respectively. The



VII

ions with m/e 155 and 154 have the same composition as the tricyclic ions (cf. formula VII) with m/e 155 and 154 observed in the mass spectrum of cyclopiazonic acid. The occurrence of these ions in the mass spectrum of bissecodehydrocyclopiazonic acid, therefore, indicates that the initial fragmentation is followed by a cyclisation process involving the two side-chains of the indole nucleus. Cyclisation of this kind can only be envisaged if the side-chains are located in *peri*-positions of the indole nucleus as in (V). Schabort[4] has recently described the isolation of five iso-enzymes from *Penicillium cyclopium* Westling strain 1082 which are capable of quantitatively con-

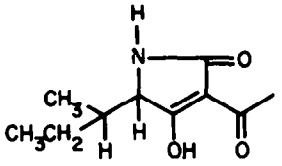
verting bissecodehydrocyclopiazonic acid into cyclopiazonic acid in the presence of air. This result constitutes independent evidence for the structure V of bissecodehydrocyclopiazonic acid. On the basis of structure V, the main fragmentation of cyclopiazonic acid under electron impact can be interpreted as shown in Scheme 1.



SCHEME 1

Information on the absolute stereochemistry of bissecodehydrocyclopiazonic acid was obtained from a study of its circular dichroism spectrum. The determination of the stereochemistry of complex molecules by comparison of their circular dichroism (or optical rotatory dispersion) spectra with those of simpler molecules is based on the fact that the sign of a Cotton effect associated with a given chromophore is generally determined by the chirality of the nearest asymmetric centre. Houghton and Saxton,[5] for example, deduced the absolute stereochemistry of echinulin by comparison of its amide Cotton effects with those of simple model compounds, e.g., L-alanyl-L-seryl-dioxopiperazine and L-alanyl-L-alanyl-dioxopiperazine. It should, therefore, be possible to deduce the absolute stereochemistry of V by comparing the Cotton effects associated with the enolised β-diketone chromophore of this compound with those of tenuazonic acid (VIII).[6] Stickings[6] showed that tenuazonic acid may be regarded as formally derived from L-isoleucine.

The CD spectrum of tenuazonic acid showed three Cotton effects, *viz.* at 240 mμ ($\Delta\varepsilon$ −2·34), 280 mμ ($\Delta\varepsilon$ −2·8) and 320 mμ ($\Delta\varepsilon$ *ca* −0·4). Due to the intense absorption of the indole chromophore the CD spectrum of V could only be determined in the



VIII

region above 270 mμ. In this region the compound showed two negative Cotton effects, *viz.* at 280 mμ ($\Delta\varepsilon$ *ca* −5·0) and 325 mμ ($\Delta\varepsilon$ *ca* −1·0). From these results it is concluded that the asymmetric centre of bissecodehydrocyclopiazonic acid has the L-configuration.

The Cotton effect at *ca* 280 mμ in V may contain a contribution from the indole chromophore. This contribution is not expected to be large since the asymmetric centre is not directly attached to the indole nucleus and does not have a fixed orientation towards it. The Cotton effect at *ca* 325 mμ in V can, however, only be attributed to the enolised β-diketone chromophore since the CD spectra of a large number of optically active compounds containing an indole moiety as the only chromophore showed no Cotton effects in the region above 300 mμ. The Cotton effects near 320 mμ in V and VIII may be due to a n → π* transition within the enolised β-diketone chromophore.

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 a MS-9 double focussing mass spectrometer. The CD curves were measured at 20° with a Jasco ORD/UV-5 instrument with CD attachment. NMR spectra were recorded at 100 Mc/s using TMS as internal reference. For preparative TLC chromatoplates were coated with Merck's Silica Gel G containing a fluorescent indicator (thickness of silica gel layer *ca* 1 mm).

The isolation of cyclopiazonic acid imine

Penicillium cyclopium strain 1082 from the culture collection of the Microbiological Research Group, C.S.I.R., Pretoria, was used in this investigation. The fungus was grown at 25° in shake culture (150 rpm) in a synthetic medium (100 ml) contained in 500 ml Erlenmeyer flasks. The synthetic medium had the following composition: glucose (60 g), $NaNO_3$ (4·2 g), $MgSO_4 \cdot 7H_2O$ (0·5 g), KCl (0·5 g), K_2HPO_4 (1 g), $Na_2B_4O_7 \cdot 10H_2O$ (0·7 mg), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0·5 mg), $CuSO_4 \cdot 5H_2O$ (0·3 mg), $MnSO_4 \cdot H_2O$ (0·11 mg), $ZnSO_4 \cdot 7H_2O$ (17·6 mg) and $FeSO_4 \cdot 7H_2O$ (10 mg), deionised water (1 l.). The flasks were inoculated with a standard homogenised culture inoculum prepared from the mycelium of a two-day-old culture of *P. cyclopium* Westl. grown on the above synthetic medium.

Seven days after the start of the fermentation the cultures (from 10 flasks) were filtered, the filtrate acidified (pH 2) and extracted with $CHCl_3$. The mycelium was continuously extracted with $CHCl_3$. The combined $CHCl_3$ extracts were extracted with a saturated $NaHCO_3$ aq. The aqueous phase was acidified with 4N HCl and extracted with $CHCl_3$. Chromatography of the extract yielded cyclopiazonic acid (580 mg), m.p. 245–246° (from $CHCl_3$—MeOH), lit.[1] m.p. 245–246°.

The neutral fraction of the extract was distributed between 95% MeOH (50 ml) and hexane (50 ml). The MeOH was evaporated and the residue (174 mg) chromatographed on silica (10 g). The column was developed with $CHCl_3$ (300 ml) and 50:1 $CHCl_3$—MeOH (200 ml). Elution with 25:1 $CHCl_3$—MeOH yielded *cyclopiazonic acid imine* (II) (52 mg). After crystallisation from MeOH this had m.p. 277–278°, λ_{max} 224, 244 (sh), 275 (sh), 286 (sh) and 293 mμ (log ε 4·58, 4·03, 4·16, 4·32 and 4·35, respectively; ν_{max} 3480, 3300 (br), 1680, 1640 and 1618 cm^{-1}. The high resolution mass spectrum showed: *m/e* 335·1627 (M^+, $C_{20}H_{21}N_3O_2$ requires: M, 335·1634), 196·1125 ($C_{14}H_{14}N$ requires: 196·1126), 180·0894 ($C_9H_{12}N_2O_2$ requires: 180·0899), and 154·0648 ($C_{11}H_8N$ requires: 154·0657) with relative abundance 41, 43, 100 and 23, respectively.

The isolation of bissecodehydrocyclopiazonic acid

Penicillium cyclopium Westl. strain 1082 was grown on shake culture in a medium which differed from the synthetic medium described above in that the amount of $ZnSO_4 \cdot 7H_2O$ was reduced to $7{\cdot}04 \times 10^{-2}$ mg per l. Seven days after the start of the fermentation the cultures (from 10 flasks) were filtered, the filtrate acidified (pH 2) and extracted with $CHCl_3$. The mycelium was continuously extracted with $CHCl_3$. The combined $CHCl_3$ extracts were extracted with sat $NaHCO_3$ aq. The aqueous phase was acidified with 4N HCl, extracted with $CHCl_3$, and the $CHCl_3$ evaporated. The residue (204 mg) was chromatographed on cellulose powder (20 g) impregnated with 50:3 $HCONH_2$—$(COOH)_2$. The column was developed with hexane (500 ml) and 1:6 hexane—C_6H_6 (300 ml). Elution with 1:3 hexane—C_6H_6 (150 ml) yielded cyclopiazonic acid (42 mg) identified, after crystallisation from $CHCl_3$—MeOH by direct comparison (m.p., mixed m.p. 245–246°, infrared spectra) with an authentic sample.

Elution of the column with 1:2 hexane—C_6H_6 (200 ml) yielded *bissecodehydrocyclopiazonic acid* (V; 84 mg). After crystallisation from $CHCl_3$–Et_2O and then MeOH it had m.p. 168–169°, λ_{max} 225, 276 and 296 (sh) mμ (log ε 4·55, 4·28 and 4·07, respectively), CD Δε (280 mμ) *ca* −5·0, (325 mμ) *ca* −1·0, ν_{max} 3478, 3200–2600, 1708 (m), 1640 (m) and 1618 (s) cm^{-1}. The high resolution mass spectrum showed: *m/e* 338·1627 (M^+, $C_{20}H_{22}N_2O_3$ requires: M, 338·1630), 198·1288 ($C_{14}H_{16}N$ requires: 198·1283), 185·1208 ($C_{13}H_{15}N$ requires: 185·1204), 156·0822 ($C_{11}H_{10}N$ requires 156·0813), 155·0723 ($C_{11}H_9N$ requires: 155·0727), and 154·0650 ($C_{11}H_8N$ requires: 154·0651) with relative abundance 10, 100, 50, 25, 8 and 20, respectively. (Found: C, 70·8; H, 6·4. $C_{20}H_{22}N_2O_3$ requires: C, 71·0; H, 6·55%). The compound gave an orange-red $FeCl_3$ colour reaction.

Conversion of cyclopiazonic acid into the imine (II)

A soln of cyclopiazonic acid (100 mg) in dioxan (10 ml) was treated with aqueous ammonia (10 ml, S.G. 0·88). The mixture was left at room temp for 6 hr. The mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ was evaporated and the residue chromatographed on silica (10 g). The column was developed with $CHCl_3$ (200 ml). Elution with 50:1 $CHCl_3$—MeOH gave cyclopiazonic acid imine (72 mg). After crystallisation from MeOH it had m.p. 277–278°. It was identical (m.p., mixed m.p., IR- and mass spectra) with cyclopiazonic acid imine isolated from *P. cyclopium* Westling.

Reduction of cyclopiazonic acid imine with sodium borohydride

To a soln of II (50 mg) in MeOH (6 ml) was added a soln of $NaBH_4$ (200 mg) in 0·025N NaOH aq (6 ml). The mixture was left at room temp for 2 days, diluted with water (25 ml) and extracted with $CHCl_3$ (3 × 25 ml). The $CHCl_3$ soln was extracted with 1N HCl (2 × 10 ml). The $CHCl_3$ was washed with water, dried (Na_2SO_4) and evaporated. The residue (61 mg) was separated on a preparative chromatoplate using 50:3 $CHCl_3$—MeOH as mobile phase. The two main absorbing bands at R_f 0·29 and 0·18 were eluted with MeOH. The band of R_f 0·18 yielded an *hydroxy amide* (25 mg) which, after crystallisation from MeOH had m.p. 292–294°, λ_{max} 223, 275 (sh), 281 and 293 mμ (log ε 4·50, 3·77, 3·78 and 3·68, respectively), ν_{max} 1682 cm^{-1}. The mass spectrum showed: *m/e* 324 (65), 196 (74), 169 (100) and 154 (88). [Found: M^+, 324·1844. $C_{20}H_{24}N_2O_2$ requires: M, 324·1838].

The band of R_f 0·29 yielded another *hydroxy amide* (18 mg) which, after crystallisation from Et_2O had, m.p. 238°, λ_{max} 224, 274 (sh), 281 and 293 mμ (log ε 4·53, 3·78, 3·80 and 3·65, respectively), ν_{max} 1682 cm^{-1}. The mass spectrum showed: *m/e* 324 (100), 196 (74), 169 (74) and 154 (84). [Found: M^+, 324·1825. $C_{20}H_{24}N_2O_2$ requires: M, 324·1838].

The above aqueous extract was neutralised with $NaHCO_3$ and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ yielded a residue (19 mg) which was separated on a preparative chromatoplate coated with Merck's neutral alumina using 100:3 $CHCl_3$—MeOH as mobile phase. The main absorbing band at R_f 0·55 was eluted with MeOH. Evaporation of the MeOH yielded a product (6·5 mg) which could not be induced to crystallise. However, the product, designated the *amino amide*, moved as one spot when examined by TLC. It had λ_{max} 223, 274 (sh), 281 and 292 mμ (log ε 4·51, 3·78, 3·79 and 3·66, respectively), ν_{max} 1680 cm^{-1}. The high resolution mass spectrum showed: *m/e* 339·1941 (M^+, $C_{20}H_{25}N_3O_2$ requires: M, 339·1947), 296·1528 ($C_{18}H_{20}N_2O_2$ requires: 296·1525), 154·0652 ($C_{11}H_8N$ requires: 154·0657), and 44·0500 (C_2H_6N requires: 44·0500) with relative abundance 50, 12, 100 and 68, respectively.

Hydrogenation of bissecodehydrocyclopiazonic acid

Bissecodehydrocyclopiazonic acid (V; 20 mg) in MeOH (20 ml) was hydrogenated over Pd-C (20 mg, 30%) until 1 mole of H_2 had been taken up. The soln was filtered and the solvent evaporated. Crystallisation

of the residue from MeOH yielded the *indole derivative* (VI), m.p. 178–179°, λ_{max} 223, 275 (sh), 280 and 293 mμ (log ε 4·52, 3·79, 3·80 and 3·68), ν_{max} 3478, 3200–3600, 1705 (m), 1639 and 1616 cm^{-1}. The high resolution mass spectrum showed *m/e* 340·1779 (M^+, $C_{20}H_{24}N_2O_3$ requires: M, 340·1787), 283·1076 ($C_{16}H_{15}N_2O_3$ requires: 283·1083), 200·1431 ($C_{14}H_{18}N$ requires: 200·1439), 144·0821 ($C_{10}H_{10}N$ requires: 144·0813) and 57·0707 (C_4H_9 requires: 57·0704) with relative abundance 18, 4, 70, 100 and 85.

Ozonolysis of bissecodehydrocyclopiazonic acid

Bissecodehydrocyclopiazonic acid (V; 34 mg) in AcOH (5 ml) at 4° was ozonised to completion. Zn dust (100 mg) was added and the mixture stirred at 60° for 1 hr. Steam distillation into 2,4-dinitrophenylhydrazine in 2N HCl gave acetone 2,4-dinitrophenylhydrazone (13 mg), m.p. 124° from aqueous EtOH, identified by mixed m.p. and mass spectrum.

Tenuazonic acid

Tenuazonic acid (VIII) was isolated from culture filtrates of *Alternaria tenius* Auct. as previously described.[7] It showed CD: Δε (320 mμ) *ca* −0·4, (280 mμ) −2·8, (240 mμ) −2·34. [Found: M^+, 197·1044. Calc. for $C_{10}H_{15}NO_3$: M, 197·1052].

REFERENCES

1. C. W. Holzapfel, *Tetrahedron* **24**, 2101 (1968)
2. D. C. Wilkins and C. W. Holzapfel, unpublished results
3. G. O. Dudek and R. H. Holm, *J. Am. Chem. Soc.* **84**, 2691 (1962)
4. J. C. Schabort, *International Symposium on the Control of the Human Environment* Abst., p. 62. Johannesburg (1969)
5. E. Houghton and J. E. Saxton, *J. Chem. Soc.* (C) 1003 (1969)
6. C. E. Stickings, *Biochem. J.* **72**, 332 (1959)
7. T. Rosett, R. H. Sankhala, C. E. Stickings, M. E. U. Taylor and R. Thomas, *Ibid.* **67**, 390 (1957)