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THE CONSTITUTION OF THE ANTIBIOTIC TRICHOVIRIDIN

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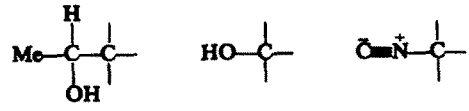
Abstract—The antibiotic trichoviridin is shown to be an isonitrile diepoxide with the relative stereochemistry (**44**).

During our antibiotic screening programme, we have isolated from a culture of an unidentified *Trichoderma* species a colourless, optically active antibiotic, $C_9H_9NO_4$, m.p. 90–91°, $[\alpha]_D^{25}$ -42.3° (*c* 2.65, MeOH). Comparison of its properties and spectral data clearly indicated that this compound was identical with trichoviridin.[1] The structural examination of trichoviridin was briefly reported recently, but only a partial structure, including a cyanhydrin residue, was suggested.[1] However, our results and their interpretation differ in some important respects from those reported.[1] We had no difficulty in determining its high resolution mass spectrum and we have established that, although trichoviridin is rather unstable, it is not a cyanhydrin and it does not contain three ethereal O atoms.

The structural investigation of trichoviridin has been rather challenging in that treatment with many reagents has resulted in either decomposition, polymerisation, or the formation of an intractable mixture of products. Only one degradative reaction was informative. It was therefore necessary to rely almost entirely upon its physical examination (UV, IR, and laser Raman spectroscopy; ^{1}H-NMR and ^{13}C-NMR spectrometry) (Table 1). The IR spectrum ($CHCl_3$) showed three significant absorption bands: ν 3560 (strong), 3440 (broad), and 2140 (strong) cm^{-1} and the absence of absorption in the C=C and C=O regions.

The ^{1}H-NMR spectrum of trichoviridin could be easily assigned (Table 1) to one OH group (δ 3.07, singlet), an AMX system (δ_A 4.09, δ_M 3.61, δ_X 3.48; J_{AM} = 2.6, J_{AX} = 1.5 and J_{MX} = 2.5 Hz) and an A'M'X$_2'$ system ($\delta_{A'}$ 2.38, $\delta_{M'}$ 3.76, $\delta_{X'}$ 1.17; $J_{A'M'}$ = 9, $J_{A'X'}$ = 0 and $J_{M'X'}$ = 6.3 Hz). On the basis of deuteriation results (Table 1, footnote b), and our observation that treatment of trichoviridin with acetic anhydride (in the NMR tube) resulted in the disappearance of one signal ($\delta_{A'}$) with a downfield shift (0.9 ppm) of the multiplet ($\delta_{M'}$ 3.76) giving a sharp quartet ($\delta_{M'}$ 4.66), then the functional groups (**1** and **2**) could be proposed containing secondary ($\delta_{A'}$ 2.38) and tertiary (δ 3.07) OH groups.



1 **2** **3**

The ^{13}C-NMR spectrum of trichoviridin, $C_9H_9NO_4$, as normally determined showed signals[2] which could be associated with only six C atoms (Table 1, i, ii, iii, iv, v, and vii). However, addition of the paramagnetic tris(acetylacetonato)-chromium(III)[3] shortened relaxation times and then two additional signals (δ 68.4 and 168.2 ppm; Table 1, vi and viii) could be observed. Off-resonance decoupling experiments[2] identified the eight C atoms of trichoviridin as primary (one) (Table 1, i), tertiary (four) (Table 1, ii–v), and quaternary (three) (Table 1, vi–viii). The highly deshielded quaternary C (δ 168.2 ppm) demonstrated the presence of an isonitrile group[2,4] (Table 1, viii). This assignment was firmly supported by (a) the IR spectrum (ν 2140 cm^{-1})[5] of trichoviridin and (b) its laser Raman spectrum which showed only *one* band (ν 2141 cm^{-1}) in the region ν 2000–2500 cm^{-1}. This, incidentally, excluded the presence of cumulene or acetylene groupings.

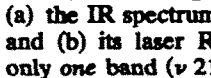
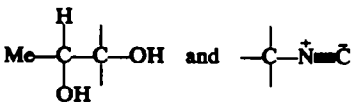
The broadening of the signal (δ 68.4; Table 1, vi) is due to ^{13}C–^{14}N spin coupling involving the isonitrile group[4] thus requiring the functional grouping (**3**). Excluding the C atom of the isonitrile group, the number of additional quaternary C atoms in trichoviridin is only two (Table 1, vi and vii). It follows therefore that two of the three quaternary C atoms indicated in the partial structures (**1**, **2**, and **3**) must be coincident. Combination of **2** and **3** to give the grouping  must be excluded on chemical grounds. Thus, the partial

Table 1. Functional groups present in trichoviridin, $C_8H_9NO_4$, with associated NMR spectral data (^1H and ^{13}C; $CDCl_3$ solutions)

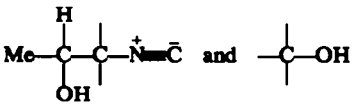
	Assignment	Chemical shifts (ppm) relative to internal tetramethylsilane (^1H)	(^{13}C)
(i)	CH_3—	1.17	17.3
(ii)	H—C—	3.76	66.9
(iii)	H—C—	3.48, 3.61, 4.09	55.1, 59.8, 60.2
(iv)	H—C—		
(v)	H—C—		
(vi)	—C—		68.4 (broad)[a]
(vii)	—C—		77.5
(viii)	—N⁺≡C⁻		168.2 (broad)[a]
(ix)	HO—[b]	2.38[b]	—
(x)	HO—[b]	3.07[b]	—
(xi)	—O—	—	—
(xii)	—O—	—	—

[a]Recorded in presence of paramagnetic tris(acetylacetonato)-chromium(III)
[b]Identified by exchange with deuterium oxide.

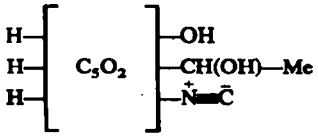
structures (**1**, **2**, and **3**) may now be replaced by either (**4**+**5**) or (**6**+**7**). The pairs of partial structures (**4**+**5**) and (**6**+**7**) plus the other groupings designated in the Table lead to the partial structure (**8**) for trichoviridin, $C_8H_9NO_4$.



4 **5**



6 **7**



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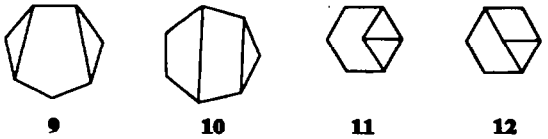
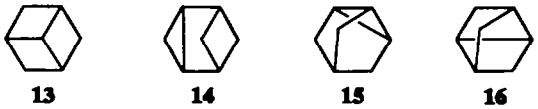
The partial structure (**8**) for trichoviridin consists of an undefined residue of *seven* atoms (C_5O_2) associated with *six* ligands. The two O atoms in the C_5O_2 residue must be ethereal and the only three quaternary C atoms (Table 1, vi, vii, and viii) present in trichoviridin have already been located in either the partial structure (**4**+**5**) or in the partial structure (**6**+**7**). *No quaternary C atoms in addition to the above three are present*, so, on logical grounds, this limits the number of possible *tricyclic* skeletons for the residue of seven atoms (C_5O_2) to only ten (**9**–**18**).

We may now address ourselves to the problem of placing the two ethereal O atoms within the tricyclic skeletons (**9**–**18**). Two conditions have to be observed: (i) the relative thermal stability of trichoviridin excludes the presence of strained 4-membered epidioxide rings and (ii) if the two O atoms of the C_5O_2 residue are located in an acetal grouping (O—C—O) then the acetal C atom must be in a 3-membered ring. Condition (ii) has to be observed because the ^{13}C chemical shift of the C atoms (Table 1, ii–vii) all lie in the range 55.1–77.5 ppm: the ^{13}C chemical shifts of acetal C atoms

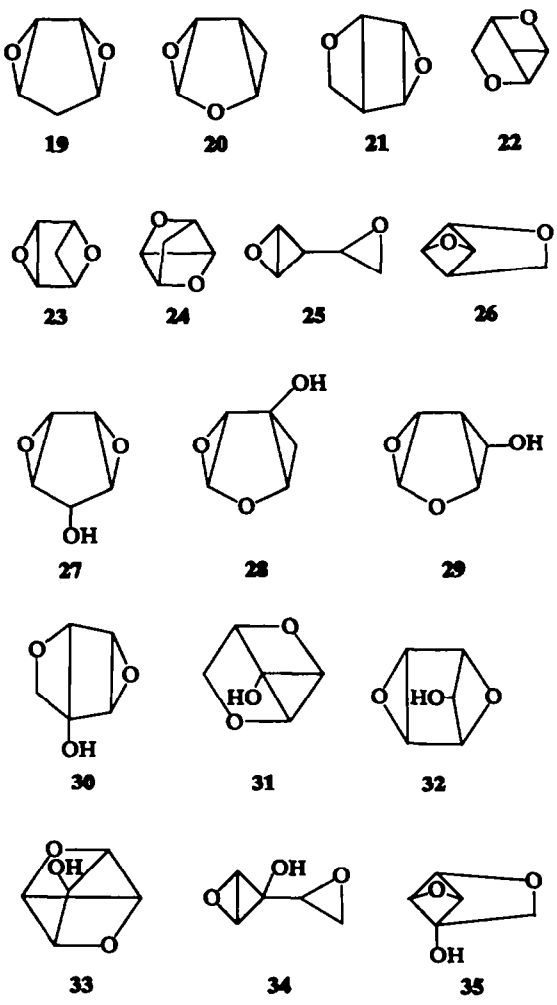


9 **10** **11** **12**



13 **14** **15** **16**

17 **18**



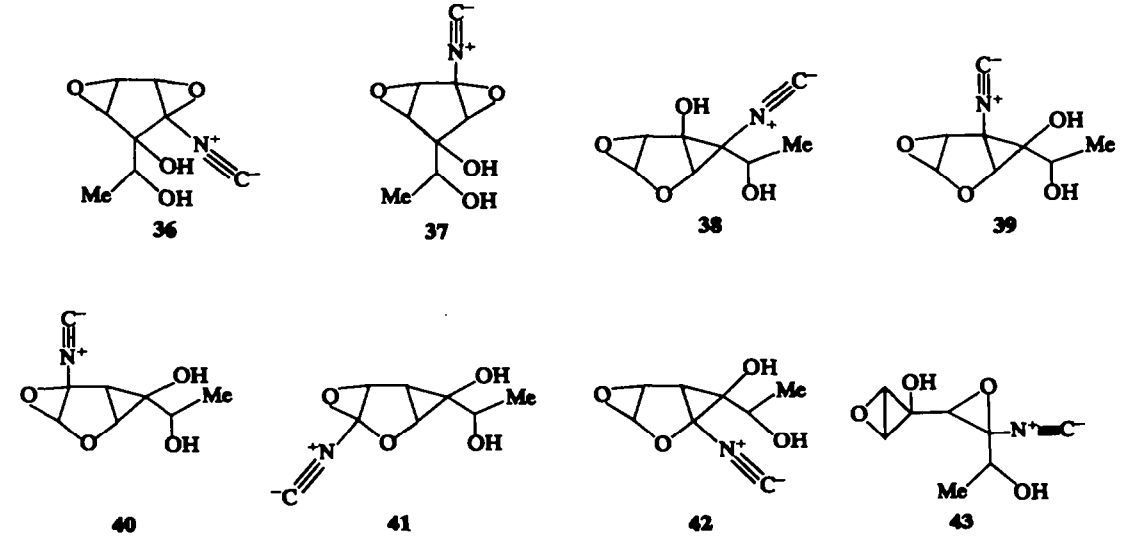
are normally in the range 80–120 ppm.[2] Applying conditions (i) and (ii) to the placing of the two ethereal O atoms in the tricyclic skeletons (**9–18**) leads to only eight possible di-ether skeletons (**19–26**). Only one acetal (**20**) meets condition (ii).

The placing of the tertiary OH group associated with either of the partial structures (**4** or **7**) could now be considered in relation to the eight di-ether tricyclic skeletons (**19–26**). This led to only nine possible hydroxyl-di-ether skeletons (**27–35**) because strained cyclic hemi-acetals, such as 2-hydroxy-epoxides or 2-hydroxy-oxetanes, are certainly excludable.

On the basis of their ^{13}C chemical shifts[2] and off-resonance decoupling results, five of the eight ^{13}C signals (Table 1, i–viii) could be assigned in relation to the partial structures (**4**+**5**) or (**6**+**7**) as follows: (i) $\underline{C}H_3$; (ii) secondary $\underline{C}H(OH)$; (vi) quaternary C bonded to isonitrile function (the broadening is due to spin coupling with ^{14}N nucleus[4]); (vii) tertiary $\underline{C}$—OH; (viii)—$\overset{+}{N}\equiv\overset{-}{C}$. The three signals (iii, iv, and v) due to three tertiary CH groups are at such high field (δ 55.1, 59.8, and 60.2) that none of these three CH groups can be bonded to O unless their three C atoms were located in 3-membered rings.[2] Thus, any structure within the group (**27–35**) can be excluded in which one or more C atoms are bonded to O if the oxygenated C atoms are *not* accommodated within 3-membered rings. This requirement excludes formulae **30**, **31**, **32**, **33**, and **35**.

Eight possible structures (**36–43**) can now be derived for trichoviridin by examining the consequences of coincidence of atoms which are common to each of the four remaining possible hydroxy-di-ether skeletons, (**27**, **28**, **29**, and **34**), and the partial structures (**4**+**5**) or (**6**+**7**).

The six structures (**38**, **39**, **40**, **41**, **42**, and **43**) can be excluded on various grounds including the fact that they all contain the tertiary OH group bonded to a cyclopropane ring. These six structures (**38–43**) can therefore be excluded because the ^{13}C chemical shift (δ 77.5 ppm) of the quaternary C atom (Table 1, vii) bonded to the tertiary OH is well outside the range associated with the ^{13}C upfield shift (δ 40–60 ppm) characteristic of cyclopropanols.[2] Thus, by a combination of logical structural analysis, coupled with a detailed interpretation of the ^1H- and ^{13}C-NMR spectra (Table 1), it

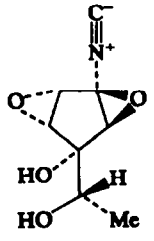


was possible to reduce the number of constitutional possibilities for trichoviridin to two: (**36** and **37**). The ^{1}H- and ^{13}C-NMR spectra show excellent and satisfying correlation with these two constitutions particularly with respect to the ^{1}H- and ^{13}C-chemical shifts to be expected for the two epoxide rings.[6,7]

These two possible constitutions (**36** and **37**) differ only in the relative position of the isonitrile group. At first sight one would have expected to have been able to distinguish between **36** and **37** on the basis of the assignment of the AMX system (δ_A 4.09, δ_M 3.61, and δ_X 3.48; $J_{AM}=2.6$, $J_{AX}=1.5$, and $J_{MX}=2.5$ Hz) which can be recognised in the ^{1}H-NMR spectrum of trichoviridin. In the constitution **36**, this AMX system would have to be associated with the grouping —CH—CH—CH—, whereas in the constitution **37** this sequence is interrupted by a quaternary C atom. Unfortunately, the ^{1}H–^{1}H coupling constants do not distinguish between these two arrangements because they could well be associated with long range ^{1}H-coupling (1, 3 and 2, 4) already observed in the close model, *syn*-1,2:3,4-di-epoxycyclopentane.[8]

Further chemical enquiry to distinguish between the constitutions **36** or **37** for trichoviridin was precluded by the very small amounts available and its reluctance to provide useful information by chemical degradation—in fact the only informative degradation we achieved was its periodate oxidation to yield acetaldehyde!

The constitution **37** for trichoviridin was finally established by direct X-ray crystallographical determination. This also established the relative stereochemistry (**44**).



Trichoviridin **44**

Trichoviridin as a mould metabolite is structurally novel in a number of respects and pathways which could be involved in its biosynthesis are not obvious. The only other mould metabolites already identified as isonitriles are xanthocillin and its methyl ethers,[9] but examination of their biosyntheses was not rewarding.[10] Recently several terpenoid isonitriles[5,11,12,13] have been isolated from marine sponges and the interesting discovery has been made that they co-occur with the structurally corresponding formamide and isothiocyanate derivatives.[5]

Our chemical and X-ray crystallographical investigation of trichoviridin was completed in 1976.[14] Subsequently a preliminary report on the elucidation of the structure and the absolute configuration of substance 142-B was published.[15] Substance 142-B (m.p. 102–104°) was believed to be identical with trichoviridin (m.p. 93–95°).[1] Clearly the X-ray investigation by Nobuhara, Tazima, Shudo, Itai, Okamoto, and Iitaka was proceeding simultaneously with ours, but it must be emphasised that they succeeded in determining the absolute configuration **44** using the anomalous dispersion effect of the O atoms. However, their structural argument is different from ours and has been presented only as a preliminary publication.[15]

The X-ray crystallographical investigation of trichoviridin (**44**). The crystals of trichoviridin available for X-ray studies were of poor quality; oscillation and Weissenberg photographs showed blurred and multiple spots suggesting the presence of disorder and/or multiple crystals. Nevertheless, it was thought worthwhile to attempt a structure determination while realising that the crystallographic quality of the results would be low. The cell parameters were thus refined on a Hilger-Watt four-circle diffractometer and reflection intensities were measured out to $\theta=24°$, although there were very few observed reflections with $\theta>18°$. Using MoK$_\alpha$ radiation (graphite monochromator) a total of 667 reflections were counted of which 326 had a nett count $\geq 2\sigma$ and were deemed observed. LP corrections were applied but absorption was neglected.

Crystal data. $C_8H_9NO_4$, M = 173.1, monoclinic, a = 10.70(2), b = 7.25(2), c = 5.54(2) Å, β = 90.5(3)°, U = 430 Å^3, D_c = 1.34 g cm^{-3}, D_m = 1.35 g cm^{-3}, Z = 2, F(OOO) = 192. Space group $P2_1$ from systematic absences. MoK$_\alpha$ radiation = 0.71069 Å, μ = 1.24 cm^{-1}.

The structure was solved fairly routinely by MULTAN, an E-map revealing all but one atom (the C of the isonitrile group) which was found readily by Fourier methods. Refinement proceeded normally by full-matrix least-squares methods and at convergence with isotropic temperature factors the conventional R was 13.0%. The distinction between O and C in the molecule was made by consideration of the temperature factors. Anisotropic refinement converged with R = 9.4%, in the final stages a weighting scheme of the form $W = 1/1+\{(F_o-P2)/P1\}^2$ with P1 = 5.0 and P2 = 11.0 was used. A final difference map showed no significant peaks other than some possibly due to hydrogen but because of the poor quality of the data, inclusion of hydrogen in the computations was not thought justifiable. Computations (apart from MULTAN) were carried out using the Oxford "Crystals" system.

Figure 1 shows a perspective drawing of trichoviridin, Table 2 gives the fractional coordinates of the atoms, Table 3 gives the bond distances and angles, and Table 4 the thermal parameters. Table 4 together with a listing of observed and calculated structure factors is available as Supplementary Publication No. SUP 0000.†

The results obtained (Fig. 1 and Tables 2 and 3) are in agreement with those recently reported in a

†This information is available on request from The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW. Any request should be accompanied by the full literature citation for this paper.

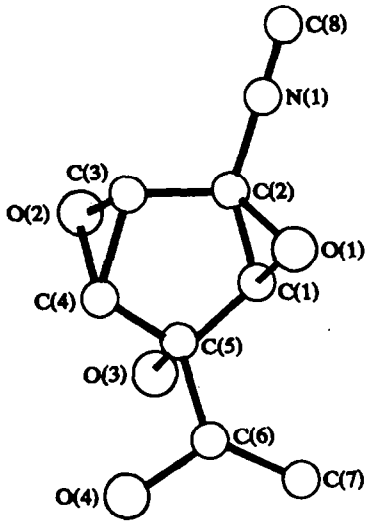


Fig. 1. Atom numbering scheme and conformation of trichoviridin (44).

Table 2. Fractional coordinates (×10³) with standard deviations in parentheses

Atom	x/a	y/b	z/c
C(1)	299(1)	147(6)	121(3)
C(2)	366(2)	−25(6)	178(4)
C(3)	296(3)	−193(6)	87(5)
C(4)	177(3)	−103(6)	−22(6)
C(5)	181(2)	95(6)	−10(3)
C(6)	170(2)	188(5)	−264(5)
C(7)	183(2)	391(6)	−256(4)
C(8)	475(3)	446(7)	469(5)
O(1)	410(2)	104(4)	−11(3)
O(2)	180(2)	−177(4)	230(3)
O(3)	77(1)	174(4)	123(2)
O(4)	61(1)	125(5)	−377(3)
N(1)	453(2)	−28	360(4)

preliminary communication.[15] In this independent work,[15] CuK_α radiation was used, which, together with the possession of a better crystal, enabled the Japanese workers to determine the absolute configuration (44) of trichoviridin.

EXPERIMENTAL

Trichoviridin (44). An unidentified *Trichoderma* species (Leo AK 5139) cultivated on a synthetic medium in shaking flask for 7 days (25°). The clarified culture broth (2.8l) was extracted with EtOAc (3×600 ml) and the extract was dried ($MgSO_4$). Evaporation under diminished pressure with the temp kept below 25° gave a partially crystalline residue (1.57 g) which was triturated with cold ether. The ethereal filtrate was concentrated (10 ml) under diminished pressure and addition of light petroleum (50 ml) gave a crystalline ppt (1.0 g). Recrystallisation from ether–light petroleum gave trichoviridin as cream crystals, m.p. 90–91° (lit.[1] m.p. 93–95°) (Found: C, 52.46; H, 4.95; N, 7.65; $M^{+\cdot}$, 183.0532. Calc. for $C_8H_9NO_4$: C, 52.46; H, 4.99; N, 7.66%; M, 183.0532); $[\alpha]_D^{25}$ −42.3°(c, 2.65, MeOH); λ_{max} 220 nm (ε 2500) (EtOH).

Acknowledgements—We wish to thank Dr. V. Fawcett, Department of Chemistry, University of Bradford, for the determination of the laser Raman spectrum of trichoviridin and the Science Research Council for grants for the purchase of ^1H and ^{13}C NMR spectrometers and an AEI MS9 mass spectrometer.

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Table 3. Bond lengths in Å and bond angles in degrees, with standard deviations in parentheses

C(1)—C(2)	1.47(4)	C(4)—O(2)	1.50(4)
C(1)—O(1)	1.43(2)	C(4)—C(5)	1.44(4)
C(1)—C(5)	1.50(3)	C(5)—C(6)	1.56(3)
C(2)—O(1)	1.48(3)	C(5)—O(3)	1.46(3)
C(2)—N(1)	1.37(3)	C(6)—O(4)	1.40(3)
C(3)—C(3)	1.52(4)	C(6)—C(7)	1.48(4)
C(3)—O(2)	1.48(3)	N(1)—C(8)	1.23(3)
C(3)—C(4)	1.54(4)		
C(5)—C(1)—C(2)	107(2)	C(3)—C(4)—C(5)	112(3)
C(5)—C(1)—O(1)	113(2)	C(3)—C(4)—O(2)	58(2)
O(1)—C(1)—C(2)	62(2)	O(2)—C(4)—C(5)	108(3)
C(1)—O(1)—C(2)	61(2)	C(4)—C(5)—C(1)	107(2)
C(1)—C(2)—C(3)	112(2)	C(4)—C(5)—O(3)	113(2)
C(1)—C(2)—O(1)	58(1)	C(4)—C(5)—C(6)	113(3)
C(1)—C(2)—N(1)	120(3)	C(1)—C(5)—C(6)	113(2)
O(1)—C(2)—C(3)	116(2)	C(1)—C(5)—O(3)	108(2)
O(1)—C(2)—N(1)	109(2)	O(3)—C(5)—C(6)	104(2)
C(3)—C(2)—N(1)	124(3)	C(5)—C(6)—O(4)	109(2)
C(2)—C(3)—C(4)	101(3)	C(5)—C(6)—C(7)	113(2)
C(2)—C(3)—O(2)	100(2)	O(4)—C(6)—C(7)	115(3)
O(2)—C(3)—C(4)	59(2)	C(8)—N(1)—C(2)	172(3)
C(3)—O(2)—C(4)	62(2)		

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