Structure of Cyclizidine (Antibiotic M146791): *X*-Ray Crystal Structure of an Indolizidinediol Metabolite bearing a Unique Cyclopropyl Side-chain

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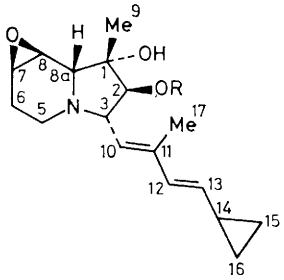
An unusual indolizidinediol with an α,β:γ,δ-unsaturated cyclopropyl side-chain has been isolated from a new *Streptomyces* species, and the structure established as (**1**) by *X*-ray crystallographic and spectroscopic methods.

A previously undescribed *Streptomyces* species NCIB 11649, isolated† from a hedgerow soil sample originating from Stretford, Greater Manchester, was grown under shake-flask aerobic fermentation conditions, and was found to exhibit low activity against *Botrytis allii*. Ethyl acetate extraction of the whole broth (37 l) at pH 7, and removal of solvent under reduced pressure gave a slightly yellow solid (8.3 g), with a strong, mousey odour. Recrystallisation from ethyl acetate, after treatment with animal charcoal, furnished an analytically pure, odourless compound (4.29 g) (**1**), m.p. 176—



(**1**) R = H
(**2**) R = COMe

† By Mr. G. Norris.

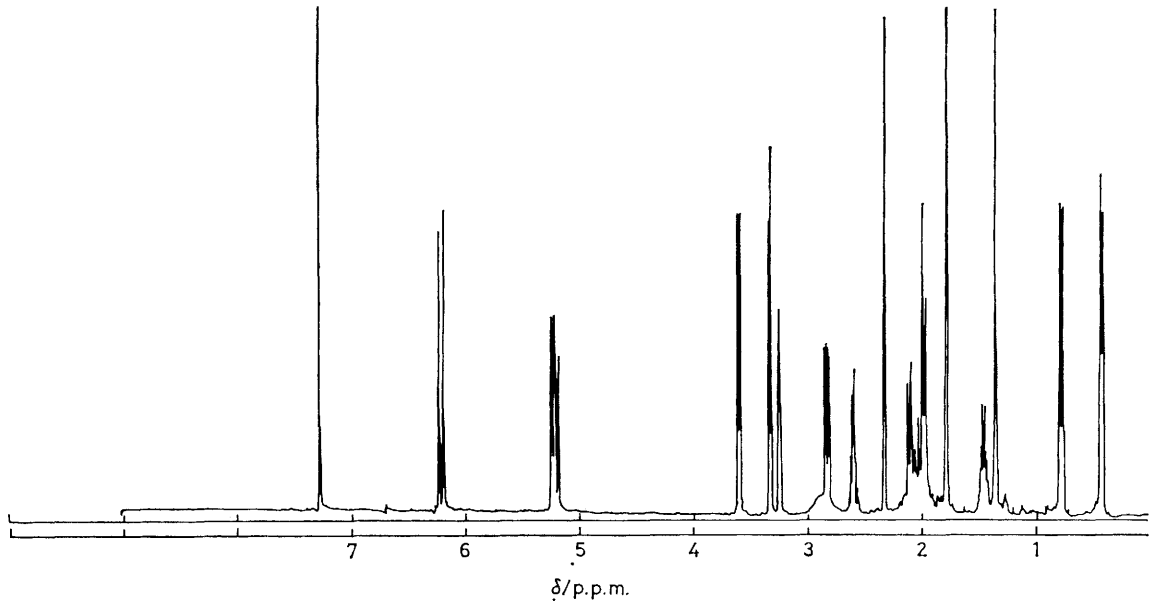


Figure 1. ^{1}H N.m.r. spectrum of cyclizidine (**1**) in $CDCl_3$ at 400 MHz.

178 °C, to which the trivial name cyclizidine has been given. The same solvent yielded crystals suitable for *X*-ray crystallographic studies (see below). Alternatively, cyclizidine could be crystallised from ether as colourless needles,‡ m.p. 184 °C; $[\alpha]_D^{23.5}$ − 46.3° (*c* 2.0, MeOH). Mass spectroscopy established the molecular formula as $C_{17}H_{25}NO_3$ ($M^{\cdot+}$, 291.1837; calc. 291.1834) and showed major fragments at *m/z* 217, 188, 161 (base peak), 150, and 98. The i.r. spectrum showed hydroxy [ν_{max} (Nujol) 3420 and 3350 cm^{-1}] and olefinic absorptions (1645w and 958s cm^{-1}). The absence of carbonyl stretching frequencies, together with the presence of a strong chromophore [u.v. λ_{max} (EtOH) 251 nm (ε 28 400)] indicated an olefinic system, resembling a conjugated triene rather than a diene (*ca.* 217 nm).

The ^{1}H n.m.r. spectrum ($CDCl_3$) of (**1**) was particularly striking, and the 400 MHz spectrum is reproduced in Figure 1. Two complex signals due to the cyclopropyl methylene groups were rapidly recognisable at δ 0.44 and 0.80. Decoupling experiments showed a fifth cyclopropyl proton at δ 1.50, and removed a 9.70 Hz coupling from the four line pattern of an olefinic proton at δ 5.20. The latter also showed a *trans*-double bond coupling of 15.9 Hz to the olefinic proton at δ 6.21. Using Gaussian enhancement,[1] long range coupling of 0.6 Hz was observed from the olefinic proton at δ 6.21 to the cyclopropyl methine group (allylic) and to the third olefinic proton at δ 5.21. The major coupling in this signal was of 9.4 Hz to the proton (on C-3) at δ 2.82, but also observed was an allylic coupling to the vinylic methyl group at δ 1.81. The remaining coupling of 4.6 Hz on the δ 2.82 signal was to the doublet at δ 3.62.

Decoupling experiments and resolution enhancement of the 'singlet' at δ 2.34 showed small splittings (<1 Hz) to δ 3.34 and 3.26, the latter also having splittings of 1.8 and 1.5 Hz to two other protons in the δ 1.90—2.20 range. An epoxide-type coupling constant of 4.1 Hz was observed for the signals at δ 3.34 and 3.26.

The nature of the hydroxy-groups in cyclizidine was revealed by conversion (acetic anhydride–pyridine) into a crystalline secondary monoacetate (**2**) $C_{19}H_{27}NO_4$, *m/z* 333 ($M^{\cdot+}$); m.p. 63—65 °C, $[\alpha]_D^{23.5}$ − 83.6° (*c* 2.0, MeOH). This esterification caused the expected downfield shift of the doublet (*J* 4.6 Hz) from δ 3.62 in (**1**) to δ 4.75 in (**2**). The i.r. spectrum confirmed the retention of a non-acetylated (tertiary) hydroxy-group.

‡ Satisfactory elemental analyses were obtained for cyclizidine and its acetate.

Table 1. ^{13}C-Chemical shifts (δ, relative to Me_4Si in $CDCl_3$).

δ/p.p.m. (**1**)	δ/p.p.m. (**2**)	Multiplicity[a]	Assignment/carbon	
—	170.2	s	acetate CO	
137.9	138.1	s	>C=	11
133.8	134.0	d		
131.6	131.6	d	3 × HC=	10, 12, 13
127.6	126.4	d		
86.0	86.1	d	CHOR	2
78.1	77.2	s	COH	1
69.1	70.2	d	2 × epoxide CH	7, 8
67.9	64.8	d		
51.5	51.3	d	2CH–N	3, 8a
51.0	51.0	d		
41.8	41.9	t	$2CH_2$	5, 6
25.8	25.7	t		
—	20.6	q	acetyl CH_3	
17.0	17.9	q	CH_3	9
14.0	14.0	d (or q)	cyclopropyl	
12.9	12.8	d (or q)	CH and CH_3	14, 17
7.2	7.2	t (2C)	2 × cyclopropyl CH_2	15, 16

[a] Using SFORD conditions.

The decoupled ^{13}C n.m.r. spectra of (**1**) and (**2**) were recorded and chemical shifts relative to tetramethylsilane are given in Table 1.

Although the nature of the side-chain (unprecedented in a natural product) was deduced by spectroscopic means, the complete structure and relative stereochemistry of cyclizidine were finally elucidated by *X*-ray crystallography.

Crystal data: $C_{17}H_{25}NO_3$, M = 291.4, monoclinic, space group *C*2, a = 27.575(3), b = 5.475(1), c = 11.458(1) Å, β = 108.91(9)°, U = 1636.3 Å^3, $F(000)$ = 632, D_c = 1.18 g cm^{-3}, Z = 4, μ(Mo-K_α) = 0.87 cm^{-1}.

1258 independent reflections ($I > 2.5\sigma_I$) were measured on an Enraf–Nonius CAD-4 automatic diffractometer. The structure was elucidated by direct phasing techniques

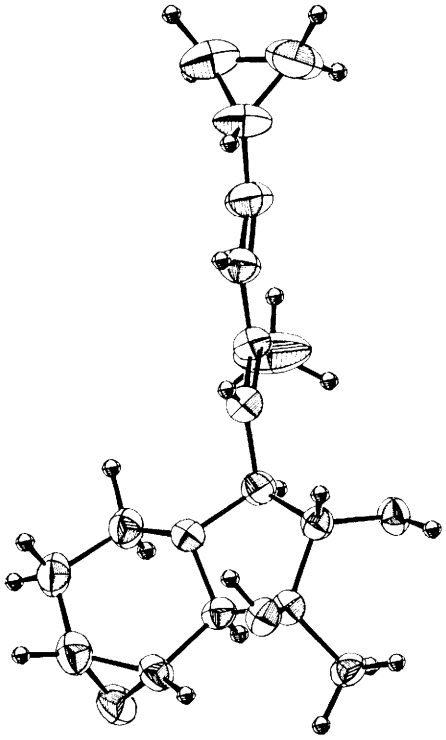


Figure 2. Perspective view of the crystal structure of cyclizidine (**1**).

(MULTAN) and refined by least squares calculations to a final R of 0.05.§

Figure 2 gives a perspective view of the molecule and illustrates the more energetically favoured bisected conformation of the vinyl cyclopropane moiety[2] which lies perpendicular to the conjugated double bonds of the fully extended side-chain. The 5- and 6-membered rings of the indolizidine moiety adopt envelope and twist-boat conformations respectively. All bond lengths and angles are close to expected values with the shortest interatomic distance between neighbouring molecules (2.82 Å) suggesting a hydrogen bond between O(19) and O(20).

Physiologically active indolizidines have been isolated from a variety of sources: fungal (slaframine, 1β-acetoxy-6β-amino-8aα-indolizidine[3]); plant (swainsonine, 8aβ-indolizidine-1α,2α,8β-triol,[4] and castanospermine, 8aβ-indolizidine-1α,6β,7α,8β-tetraol[5]); animal (251D, 8β-hydroxy-8α-methyl-6-(2-methylhexylidene)-8aα-indolizidine[6]). Although cyclizidine (**1**) proved not to be responsible for the antifungal activity, it did show non-selective immunostimulatory properties. Furthermore its acetate (**2**) caused a reduction in frequency of beats of cultured heart cells, an effect seen with certain β-blocking drugs. The difference in chemical shifts of the equatorial (δ 2.60) and axial (δ 1.9—2.2) protons at C-5 in cyclizidine is comparable with that observed in castanospermine.

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§ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

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