Structure of Myxovirescin A, a New Macrocyclic Antibiotic from Gliding Bacteria†

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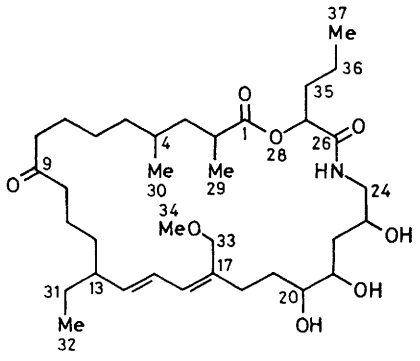
The structure elucidation of myxovirescin A, a new 28-membered macrocyclic antibiotic, is described.

Myxovirescin A was isolated from the fermentation broth of *Myxococcus virescens* strain v 48 and of various other species of gliding bacteria in the course of a screening program for antibiotics. It inhibits the growth of *E. coli* and other enterobacteria; preliminary studies on its mode of action indicate that it interferes with cell wall synthesis.[1] The previously isolated antibiotic complex[1] was separated into several closely related components by reversed phase chromatography using a methanol–water gradient. The structure elucidation of the main component, myxovirescin A, is reported here.

Myxovirescin A (**1**) consists of a mixture of two epimers, indicated by the doubling of a number of signals in the ^1H and ^{13}C n.m.r. spectra. Myxovirescin A_1 was obtained in a pure state by recrystallistion from dichloromethane–heptane, m.p. 43—44 °C [(**1**) had m.p. 95 °C, diethyl ether–heptane]; $[\alpha]_D^{20}$ + 27.3° [(**1**) + 19°, both in methanol]. A_1 showed a single set of n.m.r. signals (Table 1); λ_{max} 238 nm, log ϵ 4.32 (methanol); ν_{max} (chloroform) 3500—3300 (NH,OH), 1740 (ester CO),



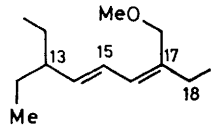
(**1**)

† Presented at: Progress in Natural Product Chemistry, International Symposium, Nottingham, July 1982.

Table 1. ^{13}C and ^{1}H N.m.r. data for myxovirescin A_1.[a]

Carbon	^{13}C	^{1}H	Carbon	^{13}C	^{1}H
1	175.9 s	—	20	73.5 d	3.58 ddd
2	37.2 d	2.63 ddq	21	71.5 d	3.88 ddd
3	40.9 t	1.60 m	22	36.0 t	1.60 m
4	30.4 d	1.29 m			1.54 m
5	36.5 t	1.35 m	23	68.4 d	4.00 m
6	26.4 t	1.40 m	24	45.4 t	3.55 dd
7	23.8 t	1.50 m			3.23 dd
8	42.4 t	2.40 t	25	—	6.70 dd
9	212.2 s	—	26	171.0 s	—
10	43.1 t	2.35 m	27	73.6 d	5.19 dd
11	22.1 t	1.50 m	29	17.3 q	1.15 d
12	34.6 t	1.44 m	30	19.6 q	0.85 d
13	45.1 d	1.90 m	31	28.3 t	1.29 m
14	139.8 d	5.37 dd	32	11.8 q	0.84 t
15	125.8 d	6.25 dd	33	70.9 t	4.18 d
16	129.7 d	6.06 d			3.91 d
17	134.7 s	—	34	58.1 q	3.36 s
18	30.4 t	2.2 m	35	34.0 t	1.84 m
		2.3 m	36	18.2 t	1.42 m
19	30.5 t	1.70 m	37	13.7 q	0.92 t
		1.55 m			

[a] Chemical shifts in $CDCl_3$ solution from internal $SiMe_4$ at 100 MHz (^{13}C) and at 400 MHz (^{1}H) on a Bruker WM 400.



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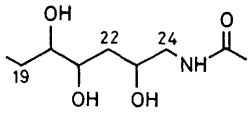

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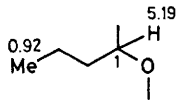

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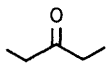

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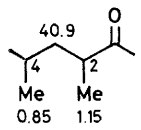
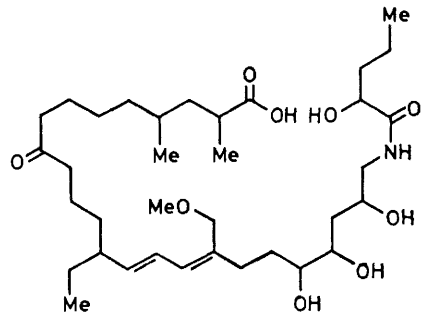


(6)

1705 (keto CO), 1655 (amide CO), and 970 and 908 cm^{-1} (olefin). High resolution m.s. (M^+, m/z 623.4395) and elemental analysis gave the molecular formula $C_{35}H_{61}NO_8$. On t.l.c. myxovirescin A appears as a red–orange spot after spraying with hydroxylamine–ferric chloride, which turns blue on standing.

The partial structures (**2**)—(**6**) were derived from extensive ^{1}H and ^{13}C n.m.r. studies, which included selective homo- and hetero-nuclear decoupling and nuclear Overhauser enhancement (n.O.e.) difference spectra.

The diene (**2**) which is responsible for the u.v. absorption of (**1**) contains *trans* double bonds in a *transoid* orientation as is evident from the coupling constants of the olefinic protons ($J_{14,15}$ 14.8 Hz; $J_{15,16}$ 11.0 Hz) and n.O.e. effects between 16-H, 14-H, and 18-H and between 15-H, 12-H, and 34-H. Irradiation at 3.38 p.p.m. (methoxy) causes an intensity increase of the 15-H signal but not of the 16-H signal, proving the partial structure (**2**).



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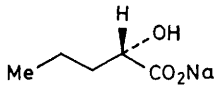

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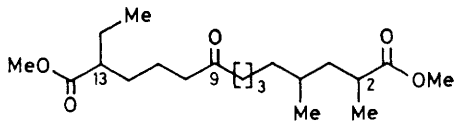

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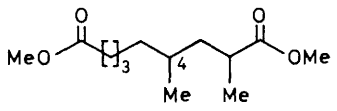

(10)

Proton decoupling experiments led to partial structure (**3**), while the unit (**4**) followed from n.O.e. and decoupling experiments. The connection of (**3**) and (**4**) was evident after observation of n.O.e. effects between the NH-proton and 1-H in subunit (**4**). Independently, the occurrence of three hydroxy-functions in (**3**) was confirmed by the formation of a triacetate (M^+ $C_{41}H_{67}NO_{11}$, isolated as an oil) upon acetylation of A_1. The n.m.r. spectra of triacetyl-A_1 exhibit downfield shifts for 20-H, 21-H, and 23-H of 1.42, 1.12, and 1.00 p.p.m. together with upfield shifts for C-19, C-22, and C-24 of 2.2, 6.2 and 3.4 p.p.m., respectively, as expected for such systems. Interestingly, the amino-triol system of (**3**) has been described for a phytotoxin from *Alternaria alternata* f.sp.lycopersici.[2]

Sodium borohydride reduction of myxovirescin A_1 yielded a tetrahydroxy-compound ($M^+C_{35}H_{63}NO_8$, mixture of epimers, isolated as an oil). In the proton n.m.r. spectrum only the CH_2 groups of (**5**) were affected, leaving the methyl branch at 1.15 p.p.m. α to a carboxylic carbonyl function as shown in subunit (**6**). A strong n.O.e. effect between 2-H in (**6**) and a high-field methyl group (δ 0.85 p.p.m.) indicates the presence of a further methyl branch at the 4-position of subunit (**6**). The low field shift of C-3 in (**6**) (δ 40.9 p.p.m.) is then rationalized by the existence of four β-carbons.

With the five partial structures (**2**)—(**6**), which are all bidentate, all atoms present in (**1**) have been assigned, except for four methylene groups. Smooth hydrolysis of (**1**) by dilute base to a carboxylic acid (**7**), M^+ $C_{35}H_{63}NO_9$ (f.d.m.s., m/z 641) reveals the presence of a lactone system in myxovirescin A. In the ^{13}C n.m.r. spectrum of (**7**) all 35 signals were observed; the i.r. spectrum lacks the ester absorption at 1740 cm^{-1}.

The order of assembly of the fragments (**2**)—(**6**) within the intact antibiotic was deduced from three degradation products (**8**), (**9**), and (**10**). Sodium (*S*)-(−)-2-hydroxyvalerate (**8**) ($[\alpha]_D^{20}$ −27°, ethanol)[3] was isolated after acid hydrolysis of (**1**) and identified by comparison with an authentic sample, prepared from L-norvaline.[4]

The keto-diester (**9**) was recovered as the main degradation product after ozonolysis of (**1**) with alkaline work-up (hydrogen peroxide–sodium hydroxide) followed by esterification with diazomethane. The two intense fragments in the m.s. (15 eV) of (**9**) at *m/z* 186 (100%) and 228 (27%), resulting from McLafferty rearrangements to both sides of the keto-group, require that the keto-function is located at C-9 in (**9**) and hence in (**1**). In addition, further McLafferty fragments at *m/z* 88 (18.1%) and 102 (13%) are indicative of the methyl branch at C-2, as well as the ethyl branch at C-13 in (**9**). The conclusion that C-9 must be the keto-position in (**1**) is also confirmed by the formation of diester (**10**) after Baeyer–Villiger oxidation of myxovirescin A_1 with *m*-chloroperbenzoic acid–trifluoroacetic acid followed by saponification and esterification.

The above reactions performed with the epimeric myxovirescin A_1/A_2 lead to epimeric mixtures for both (**9**) and (**10**) with chemical shift differences similar to those observed in the starting compounds ($\Delta\delta$ 0.02 and 0.03 p.p.m. for the α- and γ-methyl groups, respectively). Thus the epimeric centre in myxovirescin A must be either C-2 or C-4.

As the mild hydrolysis of the lactone in (**1**) causes an upfield shift of 1-H in fragment (**4**) of the order of 1.4 p.p.m., the carboxylic acid derivative (**6**) has to be connected to the oxygen in subunit (**4**). The carboxy-function of (**8**) then forms the amide bond with fragment (**3**). As compound (**9**) incorporates the four methylene groups, not assigned before, and also the fragments (**5**) and (**6**), and part of fragment (**2**), (**2**) has to be connected directly to (**3**) to give the 28 membered macrocycle (**1**). The connection of (**2**) to (**3**) was also confirmed by significant n.O.e. effects between the methoxy-group of (**2**) and the methylene protons in positions 18 and 19. From the above structural features myxovirescin A represents a new and unique member of the peptolide antibiotics.[5]

We thank Drs. L. Grotjahn and L. Witte for mass spectra, H. Steinmetz for large scale isolation of myxovirescin, and Miss K. Pfand (all GBF) for skilful technical assistance.

Received, 3rd August 1982; Com. 916

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