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ISOLATION AND SPECTROSCOPIC STRUCTURE ELUCIDATION OF SORANGICIN A, A NEW TYPE OF MACROLIDE-POLYETHER ANTIBIOTIC FROM GLIDING BACTERIA - XXX.[1]

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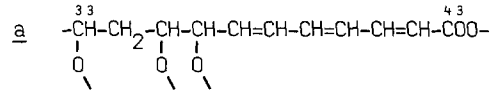
The broad spectrum antibiotic sorangicin A (1) has been isolated from *Sorangium cellulosum*, and its structure was determined spectroscopically as a 31-membered macrocyclic hydroxy-lactone carboxylic acid, containing four ether rings.

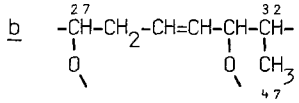
In the course of a screening programme with gliding bacteria, we have discovered a series of interesting antibiotic substances with novel structural features and a broad spectrum of biological activities and mechanisms of action.[2] Amongst these bacteria, *Sorangium cellulosum*, strain So ce 12, produces a fermentation broth with a remarkably high antibiotic activity.[3] Isolation and spectroscopic structure elucidation of sorangicin A (1), the main source of this biological activity, is described here. The minimal inhibitory concentration of 1 against Gram-negative bacteria is in the range of 3-25 µg/ml and as low as 0.01-0.3 µg/ml against Gram-positive bacteria. Studies on the mechanism of action indicate a strong inhibition of RNA polymerase.[3]

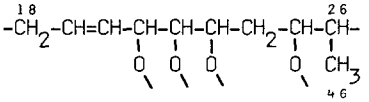
The antibiotic activity may be extracted from the fermentation broth with XAD-resin or from the cell free culture medium with ethyl acetate. The active component was enriched in the polar phase after distribution in the two-phase system methanol-heptane. Further purification was achieved by partition between ether and aqueous ammonia, followed by acidification and reextraction from the aqueous phase with dichloromethane. A final chromatographic step on reversed-phase silica gel, using methanol/aqueous 1% triethylammonium formate (pH 7) (65:35) as eluant, gave the amorphous antibiotic, which crystallised on standing from ethyl acetate. Typically this procedure yielded *ca* 560 mg of crystalline material (m.p. 105-107 °C) from 900 l fermentation broth.

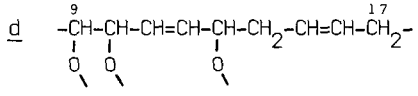
The molecular weight of 806 and elementary composition $C_{47}H_{66}O_{11}$ of 1 was obtained by negative ion f.a.b.- and high resolution e.i.-mass spectroscopy, and was affirmed by elemental analysis. From this molecular formula fifteen double bond equivalents are present, of which ten are accounted for by two carbonyl signals and eight pairs of signals in the olefinic region of the ^{13}C n.m.r. spectrum (table). Thus the remainder implies that 1 contains five rings. These considerations, together with the multiplicity of the signals in the SFORD ^{13}C spectrum, further reveal the presence of four labile protons, which, from the acidity of 1 and the formation of a triacetate, correspond to a free carboxylic acid and three hydroxyl groups in 1.

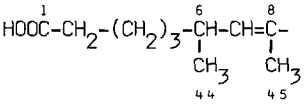
Five carbon chain segments (a-e) were deduced directly from inspection of the unambiguous cross peaks in the two dimensional ^1H homonuclear J-correlated n.m.r. (COSY) spectrum of 1 in CD_3OD:



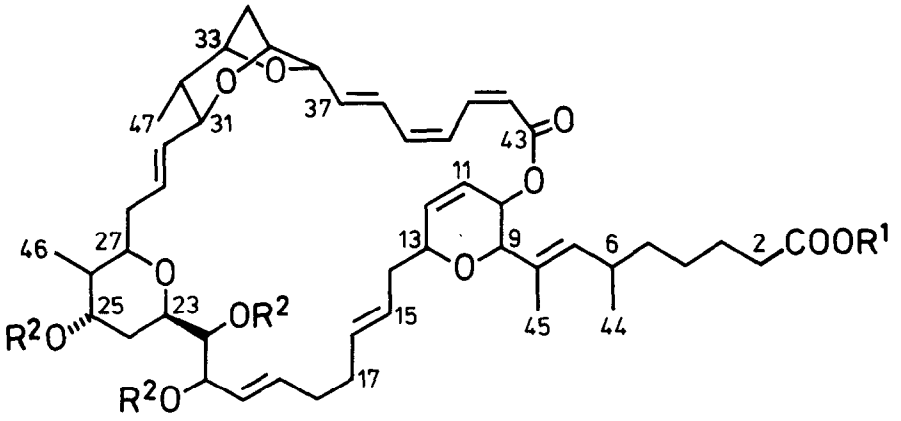






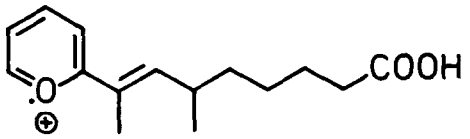


Segment a, supplemented with a carboxyl group (167.7 ppm) at C-42, forms the conjugated system, which provides the only u.v. absorption (λ_{max} = 301 nm, lg ε = 4.33) of 1. Homonuclear 1D ^1H-n.O.e. difference spectra supported these fragment structures, and gave direct evidence for the interconnection of a and b. This linkage was primary suggested by a small crosspeak of 32-H with 33-H in the COSY spectrum of 1 and its derivatives, and was corroborated by strong n.O.e's from 47-H_3 to both 31-H and 33-H.



1 $R^1 = R^2 = H$

2 R^1 = Me R^2 = Ac



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Similarly the connection of b and c, by C-26 and C-27, required further proof, as the insufficiently separated crosspeaks in the COSY spectrum from 26-H with the overlapping signals of 27-H and 25-H only showed the coupling of 26-H with 25-H. This overlap was easily resolved in the 2D ^1H n.m.r. spectrum of the triacetylated methyl ester 2; additional homonuclear decoupling demonstrated a coupling of 2.5 Hz between 26-H and 27-H in 2.

The continuation of the carbon chain with segments d and finally e is based on a combination

Table: ^{1}H- and ^{13}C-n.m.r. data of sorangicin A (1) in CD_3OD.

No	^{13}C (ppm)	^{1}H (ppm)	J (Hz)	No	^{13}C (ppm)	^{1}H (ppm)	J (Hz)
1	177.85 s	-		24	30.86 t	A1.76	(24A-24B) 14
2	35.28 t	2.30	(2-3) 7.7				(24A-25) 2.5
3	26.29 t	1.62				B1.70	(24B-25) 2.5
4	28.20 t	A1.30		25	71.07 d	3.87	
		B1.36		26	38.51 d	1.59	
5	38.51 t	A1.25		27	74.87 d	3.89	
		B1.42		28	37.12 t	A2.32	
6	32.96 d	2.43				B2.17	
7	134.15 d	5.34	(7-45) ca.1.2	29	133.00 d	5.54	(28A-29)(28B-29)
			(6-7) ca.10				4.5, 9.5
8	131.24 s	-		30	132.78 d	5.42	(29-30) 15
9	74.36 d	4.28	(9-7) 1				(30-31) 8.5
10	66.90 d	5.35	(9-10) 2	31	81.17 d	3.87	(31-32) 9.2
			(10-11) 5.8	32	42.15 d	1.46	
11	123.79 d	6.05	(11-12) 10.0	33	81.03 d	4.32	(32-33) 0.5
			(11-13) 2.1				(33-34A) 6.5
12	136.85 d	6.17	(12-13) 3.0	34	39.85 t	A2.09	(34A-34B) 11.5
13	75.33 d	4.43					(34A-35) 2.5
14	35.45 t	A2.43				B1.97	(34B-35) 1.6
		B2.17		35	77.59 d	4.45	
15	128.34 d	5.58		36	82.26 d	4.61	(35-36) ca. 4
16	133.64 d	5.58		37	134.88 d	6.26	(36-37) 4.5
17	33.35 t	A2.14					(37-38) 15.4
		B2.24		38	127.84 d	7.03	(36-38)(38-40) 1.2
18	33.97 t	A2.17		39	137.57 d	6.48	(38-39) 11.0
		B2.24					(39-40) 10.0
19	134.36 d	5.79	(18-19) ca.6	40	126.98 d	7.24	(40-41) 12.0
20	130.16 d	5.64	(19-20) 15.5	41	139.05 d	7.19	(41-42) 10.5
			(20-21) 7.5	42	119.69 d	5.66	(39-42) 2
21	74.42 d	4.19	(19-21) 0.5	43	167.68 s	-	
			(21-22) 4.5	44	21.67 q	0.93	(6-44) 7.2
22	77.77 d	3.52	(22-23) 7.3	45	14.30 q	1.68	
23	75.05 d	3.73	(23-24A) 3	46	10.89 q	0.93	(26-46) 7.2
			(23-24B) 11	47	15.36 q	0.86	(32-47) 6.8

Footnote: The ^{13}C-^{1}H correlation was obtained from heteronuclear ^{13}C-^{1}H shift correlated 2D spectra. The abbrevations after the shift refer to the multiplicity in the SFORD ^{13}C spectrum. Spectra were recorded on Bruker WM 400 and AM 300 n.m.r. spectrometers.

of arguments. The allylic 6-methine and the 2-methylene are the only aliphatic protons in the COSY spectrum possessing crosspeaks with the overlapping signals of three methylene groups in the higher field region (1.5-1.2 ppm). Together with the saturated carboxylic acid function, required to account for the C-1 signal at 177.8 ppm in the ^{13}C n.m.r. spectrum, these structural elements constitute segment e, the end of a carbon chain. This unit was however difficult to prove unambiguously by n.m.r., but could be confirmed by high resolution e.i.-m.s. of 1, where the only predominant fragment ion was observed at m/z 249. From its elementary composition $C_{15}H_{21}O_3$ and the knowledge of 1, retrospectively structure 3 can be derived for it. Simultaneously 3 includes the connection between e and d (C-8 to C-9), which primary arose from n.O.e.'s between the surrounding 9-, 10- and 45-protons. From these arguments the remaining open carbon-ends of c and d, C-17 and C-18, must be linked together, thus completing the carbon skeleton of 1, a single carbon chain with carboxylic functions at both termini.

From the low field shift of the 10-H signal (5.35 ppm), the unsaturated ester carboxyl at fragment a was attached to the corresponding C-10 (66.9 ppm), forming a macrocyclic lactone.

The pronounced low field shifts of 25-H, 22-H and 21-H (4.91, 5.09 and 5.56 ppm in 2) upon acetylation allowed the distinction between the three hydroxyl and eight ether positions, and hence implicated the four missing ring closures. The ether rings were closed as depicted in 1, by taking into consideration the n.O.e.'s between 31-H and 37-H, the ^{13}C shifts and the vicinal proton coupling constants, which are characteristically small in the furan and in the dihydropyran ring.

The configurations of the double bonds, apart from the $\Delta^{15,16}$- and $\Delta^{7,8}$-bond, are self-evident from the magnitude of the vicinal coupling constants. Although the identical chemical shifts of 15-H and 16-H conceal any stereochemical information, the E-configuration of the respective double bond can be assigned from the ^{13}C shift of C-17.[4] Examination of the n.O.e. difference spectra of the partially overlapping signals for 7-H and 10-H upon irridiation at 9-H and 11-H indicated the E-configuration of the $\Delta^{7,8}$-double bond.

In the 2,6-dioxabicyclo[3.2.1]octane system the relative configuration is derived from the pronounced n.O.e.'s between 37-H and 31-H and the coupling constant $J_{31,32}$ = 9.2 Hz. The axial position of the hydroxyl group at C-25 and the equatorial carbon chain at C-23 is evident from the vicinal coupling constants of 23-H and 25-H with the AB system of the adjoining methylene group.

The structure of sorangicin A (1) is consistent with the results of feeding experiments with ^{13}C labelled acetates. According to the ^{13}C n.m.r. spectrum, the backbone of 1 is constructed from 21 acetate units and an extra carbon, C-1.

We thank Miss *B. Jaschok* for her skilful technical assistance.

References and notes

1 Antibiotics from Gliding Bacteria XXIX, B. Kunze, W. Kohl, G. Höfle, and H. Reichenbach, J. Antibiot., in press.
2 R. Jansen, H. Irschik, H. Reichenbach, and G. Höfle, Liebigs Ann. Chem. 1985, 822 and lit. cited therein.
3 H. Irschik, R. Jansen, G. Höfle K. Gerth, and H. Reichenbach, J. Antibiot. in preparation.
4 Assuming additivity of effects and using the known shifts of hexa-1,5-diene, E- and Z-hex-3-ene and but-1-ene[5] the shifts of C-17 in the E- and Z-configuration are calculated as *ca* 33 and 27 ppm respectively. A similar calculation for C-14 is complicated by the presence of the pyran ring.
5 Ref. compounds CNMR 8249, CNMR 1921, CNMR 1922 and CNMR 4821 from W. Bremser, L. Ernst, B. Franke, R. Gerhards and A. Hardt, Carbon-13 NMR Spectral Data, Verlag Chemie, Weinheim 1981.

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