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STRUCTURE OF CONCANAMYCIN A

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Summary: The structure of concanamycin A was proposed on the basis of PMR analyses of concanamycin A and its ozonolysis products.

In the preceding paper, concanamycin A (1) was subjected to alkaline degradation to afford the anhydroaglycone P1 and the sugar S1, and their structures were described.[1] In order to elucidate the mode of binding of these components, ozonolysis of (1) was performed and a degradation product containing S1 and a fragment of P1 was obtained. In this paper, we describe the structures of the ozonolysis products and concanamycin A itself.

Concanamycin A (1) crystallized from aqueous methanol as colorless crystals; m.p. 162-163.5 °C, $C_{46}H_{75}NO_{14}$, IB-MS M^+ m/z 865, λ_{max}^{MeOH} 245 (ε 40500), 284 nm (ε 19300), $\nu_{max}^{CHCl_3}$ 3560-3500, 3420, 1718, 1690, 1585, 1455, 1385, 1363 cm^{-1}, $[\alpha]_D^{25}$ -21.7° (c 1, methanol). Ozonolysis of (1) in methanol followed by reduction with dimethyl sulfide afforded three main products, Oz1 (2a), Oz2 (2b), and Oz3 (3).

Oz1 (2a); $C_{14}H_{26}O_4$, GC-MS m/z 226 (M - MeOH), $\nu_{max}^{CHCl_3}$ 3520, 1710, 1465, 1370 cm^{-1}, $[\alpha]_D^{25}$ -124.2° (c 1.29, acetone); was subjected to PMR analysis (Table 1), and the structure was determined as depicted in Fig. 2. Clearly, the carbon chain of Oz1 corresponds to that from C-5 to C-12 of P1. The coupling constants of vicinal protons in the pyranose type structure revealed the relative configurations as shown in Fig. 2, indicating the equatorial configuration of the anomeric proton (α-anomer).

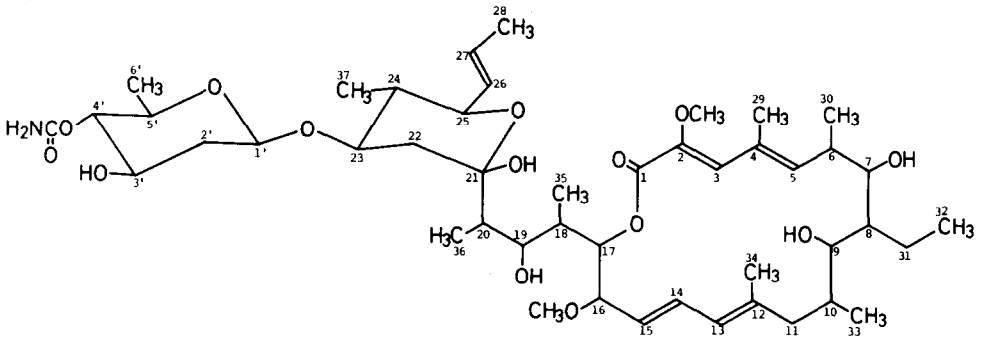
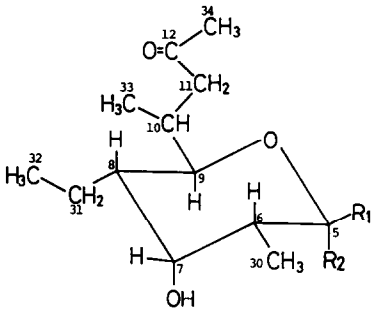


Fig. 1 Structure of concanamycin A (1)
The absolute configuration of the aglycone part has not been determined.

Table 1 PMR parameters of Oz1 (2a) and Oz2 (2b)

	Oz1 (2a)			Oz2 (2b)		
Proton	δ(ppm)	a)	J(Hz)	δ(ppm)	a)	J(Hz)
H-5	4.57	d	3.0	4.30	d	8.6
5-OMe	3.33	s		3.48	s	
H-6	1.77	m	7.2, 3.0, 3.0	1.53	m	8.6, 7.0, 2.5
Me-30	1.07	d	7.2	1.02	d	7.0
H-7	3.74	br.s		3.93	t	2.5
H-8	1.38	m		1.44	m	
H_2-31	1.39	m		1.28 m 1.41 m		
Me-32	0.94	t	7.0	0.94	t	7.3
H-9	3.53	dd	10.0, 2.0	3.50	dd	10.0, 2.2
H-10	2.39	m	7.0, 7.0, 2.0	2.32	m	7.5, 6.9, 6.3, 2.2
Me-33	0.87	d	7.0	0.89	d	6.9
H_2-11	2.56	d	7.0	2.53 dd 17.0, 7.5 2.60 dd 17.0, 6.3		
Me-34	2.14	s		2.15	s	

PMR spectra were measured in $CDCl_3$ with addition of D_2O using TMS as an internal standard. a) Multiplicity.



(2a) R_1 = H, R_2 = OCH_3
(2b) R_1 = OCH_3, R_2 = H

Fig. 2 Structures of Oz1 (2a) and Oz2 (2b)
Numbering of the carbon atoms corresponds to that of concanamycin A (1).

Oz2 (2b); $C_{14}H_{26}O_4$, GC-MS m/z 240 (M-H_2O), $\nu_{max}^{CHCl_3}$ 3640, 3540-3400, 1710, 1463, 1370 cm^{-1}, $[\alpha]_D^{25}$ +25.6° (c 0.52, acetone); showed a closely similar PMR spectrum to that of Oz1 (Table 1). From the large coupling constant between H-5 and H-6, Oz2 was deduced to be the β-anomer of Oz1.

Oz3 (3); $C_{27}H_{43}NO_{15}$, FD-MS m/z 644 (M+Na^+), $\nu_{max}^{CHCl_3}$ 3560-3500, 3420, 1770, 1740, 1720, 1585, 1460, 1385, 1330 cm^{-1}, $[\alpha]_D^{25}$ -54.7° (c 0.72, acetone); was found to be the key compound containing S1 and a fragment of P1 as described below. Extensive ^1H-^1H spin decoupling experiment of Oz3 was performed referring to the structures of S1 and P1. All the resonances were assigned as shown in Fig. 3, which revealed the presence of following three partial structures (see Fig. 4).

Structure A (C-15∿C-20): The carbon chain of this moiety corresponds to that from C-15 to C-20 of P1. Since a hemiketal ring was formed by the 19-hydroxyl and the 15-aldehyde, the relative configurations were deduced from the coupling constants of vicinal protons as shown in Fig. 3. When H-15 was irradiated, an NOE enhancement was observed for 16-OCH_3, but not for H-19, indicating the equatorial configuration of H-15 as depicted in Fig. 4. Monomethyl oxalyl ester attached to C-17 was derived from the α-methoxy-α,β,γ,δ-unsaturated lactone moiety. The upfield shifted resonance of H-20 (δ 2.02 ppm) compared with that of P1 (δ 2.92 ppm) suggests that C-21 is not present as a ketone form as in P1.

Structure B (C-21∿C-26): Decoupling experiment revealed the carbon chain from C-22 to C-25,

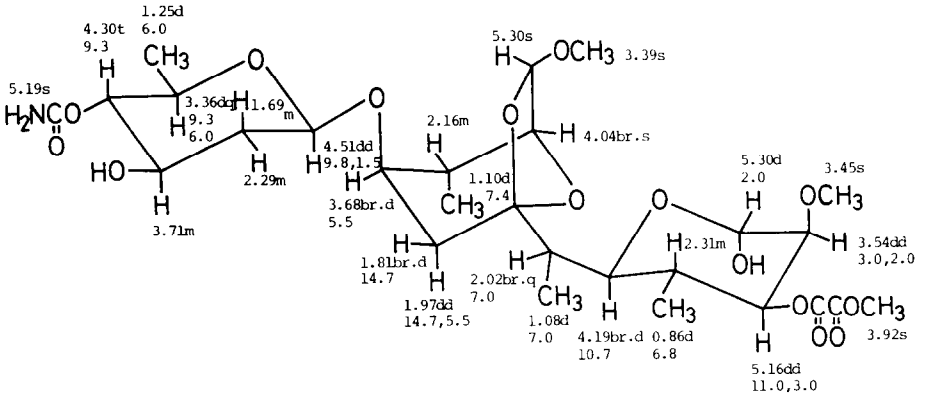


Fig. 3 Structure and PMR parameters of Oz3 (3)

Upper, chemical shift and multiplicity; lower, coupling constant. PMR spectrum was measured in $CDCl_3$ with addition of D_2O using TMS as an internal standard.

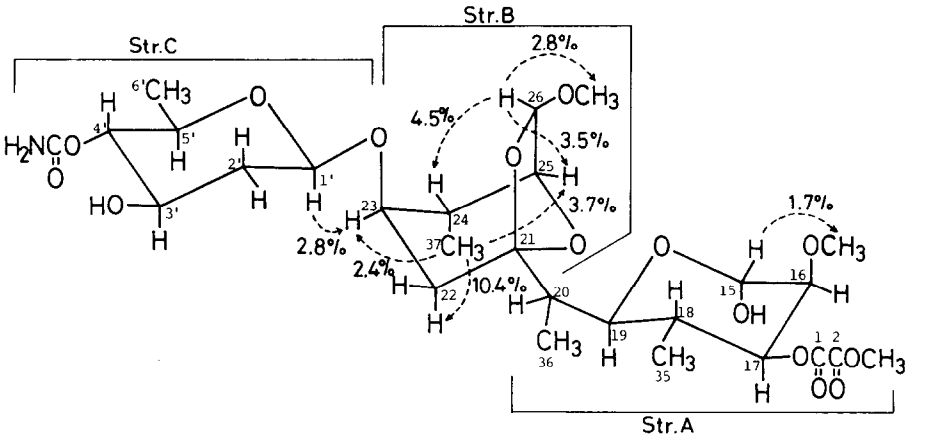


Fig. 4 Summarized result of NOE experiment of Oz3 (3)

Numbering of the carbon atoms corresponds to that of concanamycin A (1).

indicating the presence of the 22-methylene and the 23-methine instead of the double bond (C-22, 23) in P1. In the PMR spectrum, the aldehyde proton which was expected to be formed by the fission of the terminal allylic group was not observed, but in the CMR spectrum, four signals of acetal and/or ketal carbons were observed (δ 92.1, 98.6, 103.9, and 110.9 ppm). Since two of these signals are due to C-15 in the structure A and C-1' in the structure C (see below), other two signals should be assigned to C-21 and C-26 in the structure B. Thus, a bicyclic acetal-ketal structure formed from the 21-ketone, the 25-hydroxyl, and the 26-aldehyde was suggested. Although the coupling of H-26 (δ 5.30 ppm, s) with H-25 was not detected, this structure was confirmed by the NOE enhancements observed for H-24, H-25, and 26-OCH_3 on irradiation of H-26, establishing the relative configuration of the acetal carbon as depicted in Fig. 4. NOE enhance ments were also observed for axial H-22, H-23 and H-25 when CH_3-37 was irradiated, indicating that CH_3-37 and axial H-22 has the 1,3-diaxial relationship and that the 6-membered ketal ring

has a chair conformation. From the coupling constant (J=5.5 Hz) between H-23 and axial H-22, H-23 was deduced to have the equatorial configuration.

Structure C (C-1'∿C-6'): All the resonances of this moiety were almost identical to those of the β-anomer of the sugar S1, and an NOE enhancement was observed between H-1' and H-23. This indicates the β-glycoside linkage of S1 to C-23 of the aglycone moiety.

The presence of free hydroxyl group at C-15 and C-3' was confirmed from the couplings of H-15 and H-3' with hydroxyl protons in the PMR spectrum measured without addition of D_2O. Thus, the structure of Oz3 (3) was determined as depicted in Fig. 3.

Structural elucidation of concanamycin A (1) was performed by PMR analysis referring to the structures of the anhydroaglycone P1, the sugar S1, and the ozonolysis product Oz3. Close resemblance of the resonances of the lactone moiety (C-2∿C-19) to those of P1 and the UV spectrum (284 nm) demonstrate the same lactone ring structure in (1) as that in P1. The upfield shifted proton resonances of H-20 (δ 1.75 ppm) compared with that of P1, and a carbon resonance (δ 99.6 ppm, s) indicate that C-21 does not take a carbonyl form. Decoupling experiment revealed the carbon chain from C-22 to C-28, in which the double bond (C-26,27) was deduced to have the E configuration from the large coupling constant (J=15.2 Hz) between H-26 (δ 5.31 ppm) and H-27 (δ 5.54 ppm). A large coupling constant (J=10.0 Hz) between H-24 (δ 1.25 ppm) and H-25 (δ 3.97 ppm) revealed their trans-diaxial relationship. This indicates that the 21-ketone and the 25-hydroxyl form a 6-membered hemiketal ring and that this ring adopts the inverted chair conformation from that of Oz3. Almost identical proton resonances of the sugar moiety to those of the β-anomer of S1 indicate that S1 is linked to C-23 of the aglycone moiety (H-23, δ 3.76 ppm) by a β-glycoside bond. The anhydroaglycone P1 is considered to be formed by β-elimination of the sugar S1 from concanamycin A when treated with NaOH.

From all the results described above, the structure of concanamycin A (1) has been established as depicted in Fig. 1. Thus, concanamycin A is a novel 18-membered macrolide antibiotic[2] consisting of an α,β,γ,δ-unsaturated lactone ring[3], a long side chain which forms an intramolecular hemiketal ring, and 4-O-carbamyl-2-deoxy-D-rhamnose.

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