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ALKALINE DEGRADATION PRODUCTS OF CONCANAMYCIN A

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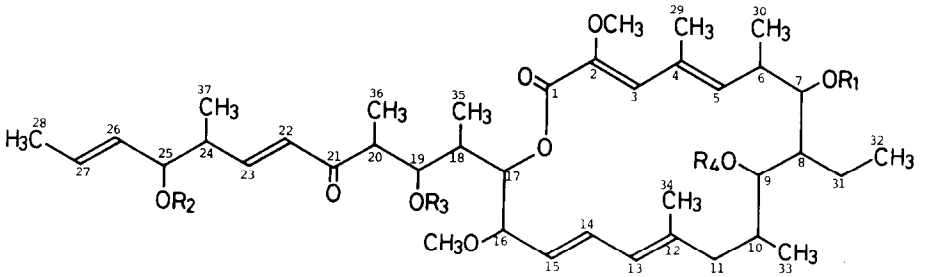
Summary: The structures of alkaline degradation products of concanamycin A were elucidated by analysis of their 270 MHz PMR spectra.

Concanamycins A, B, and C, produced by *Streptomyces diastatochromogenes* S45, inhibit the proliferation of the mouse splenic lymphocytes stimulated by concanavalin A.[1] In order to elucidate the structure of the main component, concanamycin A, several chemical transformations were performed. We wish to describe here the structures of its alkaline degradation products.

Treatment of concanamycin A with 0.03N NaOH in methanol afforded two products, an anhydroaglycone P1[2] (1a) and a sugar S1 (2a). The anhydroaglycone P1 (1a); M^+ m/z 674, λ_{max}^{MeOH} 240 (ε 46500), 282 nm (ε 17200), $\nu_{max}^{CHCl_3}$ 3600-3400, 1690, 1620, 1455, 1363 cm^{-1}; was acetylated with acetic anhydride in pyridine to give di- (1b), tri- (1c), and tetraacetate (1d). The molecular formula of the triacetate was established as $C_{45}H_{68}O_{12}$ by high resolution mass spectrometry (M^+ m/z 800.47056, Calcd. for $C_{45}H_{68}O_{12}$ m/z 800.47109), from which that of P1 was deduced to be $C_{39}H_{62}O_9$. CMR spectra of (1c) in $CDCl_3$ revealed all the carbon atoms of the assigned formula as follows; 14 quartets, 11.1, 11.6, 11.8, 14.4, 15.6, 16.4, 17.0, 17.7, 20.7, 20.9, 21.0, 21.1, 55.8, and 59.1 ppm, 2 triplets, 21.8 and 44.8 ppm, 21 doublets, 34.2, 34.7, 36.4, 41.2, 44.1, 44.8, 72.8, 74.5, 75.3, 77.1, 79.7, 83.5, 123.5, 126.7, 127.3, 128.7, 130.0, 131.0, 133.1, 137.5, and 147.2 ppm, 8 singlets, 132.2, 141.1, 142.8, 163.7, 170.1, 170.2, 170.7, and 200.8 ppm.



(1a) R_1, R_2, R_3, R_4 = H
(1b) R_1, R_2 = $COCH_3$, R_3, R_4 = H
(1c) R_1, R_2, R_3 = $COCH_3$, R_4 = H
(1d) R_1, R_2, R_3, R_4 = $COCH_3$

Fig. 1 Structures of P1 (1a) and its acetyl derivatives

Table 1 PMR parameters of the anhydroaglycone P1 (1a)

Proton	δ(ppm)	a)	J(Hz)	Proton	δ(ppm)	a)	J(Hz)
2-OMe	3.64	s		H-15	5.24	dd	15.1, 8.8
H-3	6.42	s		H-16	3.79	t	8.8
Me-29	1.98	br.s		16-OMe	3.23	s	
H-5	5.79	br.d	10.5	H-17	5.17	dd	8.8, 1.5
H-6	2.73	m	10.5, 7.0, 2.5	H-18	2.11	m	9.0, 7.0, 1.5
Me-30	1.07	d	7.0	Me-35	0.94	d	7.0
H-7	3.81	dd	8.0, 2.5	H-19	3.72	dd	9.0, 3.8
H-8	1.50	m		H-20	2.92	dq	7.0, 3.8
H_2-31	1.24	m		Me-36	1.19	d	7.0
Me-32	0.89	t	7.4	H-22	6.27	dd	15.9, 0.9
H-9	3.25	br.d	11.0	H-23	6.85	dd	15.9, 7.7
H-10	2.21	m		H-24	2.40	m	7.7, 7.0, 7.0, 0.9
Me-33	1.04	d	7.0	Me-37	1.04	d	7.0
H_2-11	1.96	m		H-25	3.91	t	7.0
Me-34	1.82	br.s		H-26	5.46	ddq	15.5, 7.0, 1.5
H-13	5.79	br.d	10.5	H-27	5.69	dq	15.5, 6.5
H-14	6.50	dd	15.1, 10.5	Me-28	1.71	dd	6.5, 1.5

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

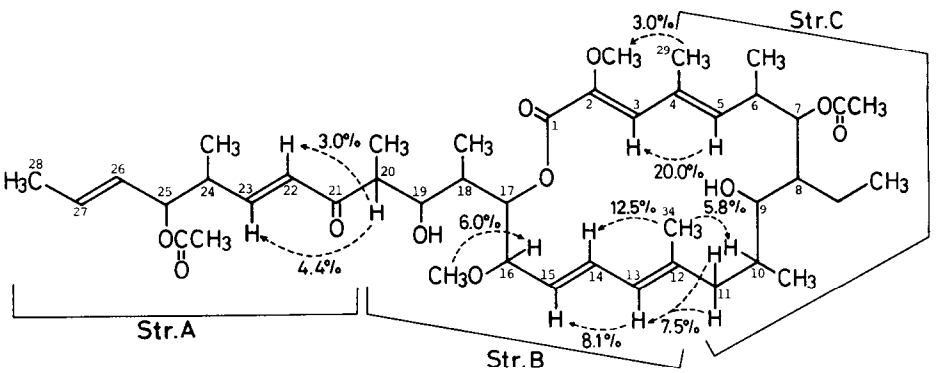


Fig. 2 Summarized result of NOE difference experiment of the diacetate (1b)

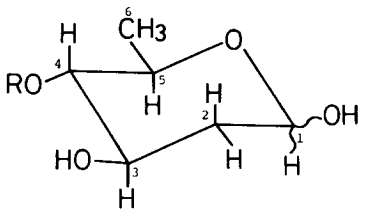
Extensive ^1H-^1H spin decoupling experiments of P1 and the three acetyl derivatives allowed to assign all the resonances of P1 as shown in Table 1, and to reveal the presence of three partial structures A (C-21∿C-28), B (C-12∿C-20), and C (C-4∿C-11), (see Fig. 2). Conjugation of a ketone with the double bond (C-22,23) was indicated by the chemical shifts of H-22 (δ 6.27 ppm) and H-23 (δ 6.85 ppm). From the large coupling constants (J=15.5, 15.9, and 15.1 Hz) between the protons on the disubstituted double bonds (C-26,27, C-22,23, and C-14,15), they were deduced to have the E configurations. Allylic couplings between the olefinic and the vinylic methyl protons (H-13···CH_3-34 and H-5···CH_3-29) served to build the partial structures B and C. Downfield shifted resonances (1.24-1.46 ppm) of H-7, H-9, H-19, and H-25 of the tetraacetate (Id) compared with those of P1 indicated the positions of the acetylated hydroxyl groups.

In order to elucidate the relations between the three partial structures, NOE difference experiment[3] of the diacetate (Ib) was performed. Upon irradiation of H-20 proton, NOE enhancements were observed for H-22 and H-23 protons. NOE enhancements were also observed for H-10 and

H-13 protons when CH_3-34 and H_2-11 protons were irradiated respectively. Thus, the structures A, B, and C could be alligned as depicted in Fig. 2. The position of the methoxyl group (16-OCH_3) and the E configurations of the trisubstituted double bonds (C-12,13 and C-4,5) were also deduced from the result shown in Fig. 2. Furthermore, the structure C could be extended to C-2, to which a methoxyl group is attached, from the NOE enhancement observed between CH_3-29 and the methoxyl group.

Considering the molecular formula of P1, only O=C-O group has remained to be assigned. As the UV spectrum of P1 (282 nm) indicates the presence of an α,β,γ,δ-unsaturated carbonyl, the CO_2 group can be placed between C-2 and C-17 to form a lactone ring, which is consistent with the chemical shifts of H-3 (δ 6.42 ppm) and H-17 (δ 5.17 ppm). Thus, the structure of P1 (1a) has been established as an 18-membered α,β,γ,δ-unsaturated lactone with a long side chain as depicted in Fig. 1.

The sugar S1 (2a) is an amorphous powder; ν_{max}^{KBr} 3400-3200, 1670, 1410 cm^{-1}, $[\alpha]_D^{25}$ +66.4° (c 1, acetone). The molecular formula of S1 was established as $C_7H_{13}NO_5$ by elementary analysis (C 43.92, H 6.69, O 41.80, N 7.25 %; Calcd. for $C_7H_{13}NO_5$, C 43.98, H 6.85, O 41.84, N 7.33 %). The CMR spectrum (δ 19.2; 40.0, 42.2; 68.4, 68.6; 70.8, 72.5; 80.6, 81.1; 93.5, 95.7; 161.3 ppm in D_2O) showed S1 to be an anomeric mixture in D_2O. The presence of an amide group was deduced from the IR and the CMR spectra and the positive color reaction with the chlorine-pyrazolinone-cyanide reagent. By elaborate PMR analysis of S1 in D_2O, all the protons of α- and β-anomers were assigned as shown in Table 2. Treatment of S1 with saturated $Ba(OH)_2$ in water afforded $BaCO_3$ and 2-deoxy-D-rhamnose[4] (2b; $[\alpha]_D^{25}$ +57.2°, c 1, acetone). Thus, S1 was confirmed to be 4-O-carbamyl-2-deoxy-D-rhamnose[5] (2a) as depicted in Fig. 3.



(2a) R = $CONH_2$

(2b) R = H

Fig. 3 Structures of S1 (2a) and 2-deoxy-D-rhamnose (2b).

The structure of concanamycin A will be described in the following paper.[6]

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Table 2 PMR parameters of the sugar S1 (2a)

	α-Anomer			β-Anomer		
Proton	δ(ppm)	a)	J(Hz)	δ(ppm)	a)	J(Hz)
H-1	5.34	br.d	3.5	4.92	dd	10.0, 2.0
Hax-2	1.78	m	13.2, 12.0, 3.5	1.58	m	12.5, 12.0, 10.0
Heq-2	2.16	dd	13.2, 5.0	2.29	m	12.5, 5.0, 2.0
H-3	4.04	m	12.0, 9.4, 5.0	3.85	m	12.0, 9.4, 5.0
H-4	4.30	t	9.4	4.25	t	9.4
H-5	4.00	dq	9.4, 6.2	3.56	dq	9.4, 6.2
Me-6	1.17	d	6.2	1.20	d	6.2

PMR spectrum was measured in D_2O using DSS as an internal standard. a) Multiplicity.

concanamycin A and for high resolution mass spectrometry, respectively.

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