THE STRUCTURE OF CP-96,797, A POLYETHER ANTIBIOTIC RELATED TO K-41A AND PRODUCED BY *Streptomyces* sp.

JOHN P. DIRLAM, JON BORDNER, WALTER P. CULLEN, MARK T. JEFFERSON and LAURA PRESSEAU-LINABURY

Central Research Division, Pfizer Inc, Groton, CT 06340, U.S.A.

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In the course of screening actinomycetes for novel antimicrobial substances, a new strain of *Streptomyces* sp. was found to produce a new polyether antibiotic, CP-96,797 (**1**). This paper describes the isolation, properties and structure of **1**, which is related to K-41A[1].

The 100-liter fermentation of *Streptomyces* sp. ATCC 55028 and isolation of crude **1**-Na as an ethyl acetate soluble oil were carried out in a manner described elsewhere[2]. This material was purified by HPLC on a silica gel column using methylene chloride-ethanol (3:2). The eluates were examined by TLC on silica gel plates developed with chloroform-2-propanol (19:1), then sprayed with 3% vanillin dissolved in ethanol-phosphoric acid (3:1). Upon heating to 80°C the polyether antibiotic appeared as a pink spot at Rf 0.33. Fractions containing **1**-Na were combined and evaporated to dryness. The crude antibiotic was taken up in chloroform, extracted with dilute phosphoric acid and then with pH 9.0 phosphate buffer. The organic phase was dried (Na_2SO_4), evaporated, and the residue crystallized from hexane to afford 4.1 g of **1**-Na.

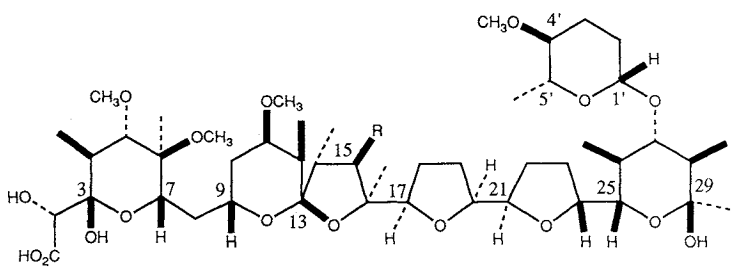
The free acid form of **1** was prepared by vigorously shaking a chloroform solution of **1**-Na with an equal volume of hydrochloric acid at pH 2 in a separatory funnel. The phases were separated and the chloroform layer was washed with water and then evaporated under vacuum to give **1**.

The physico-chemical properties of **1** and **1**-Na are given in Table 1. Spectroscopic data were consistent with $C_{47}H_{80}O_{17}$ for free acid **1**, and $C_{47}H_{79}O_{17}Na$ for sodium salt **1**-Na. For example, in the positive FAB-MS, diagnostic cationized molecules *m/z* 939 $(M+Na)^+$ and 961 $(M+2Na-H)^+$ were detected for **1**-Na. Furthermore, **1**-Na gave a base peak at *m/z* 877, 62 daltons less than the corresponding metal-adduct molecular ion, which is common for polyethers having a β-hemiketal carboxylic acid group ($(M+Na-CO_2-H_2O)^+$)[3].

The ^{13}C, ^1H and DEPT[4] NMR data for **1**-Na, recorded on a Bruker AM-500 spectrometer, revealed the following groups: CH_3 (9), CH_2 (9), CH (5), CH_3O (4), O–CH (13), C–O (2), O–CH–O

Table 1. Physico-chemical properties of CP-96,797 free acid (**1**) and Na-salt (**1**-Na).

Property	**1**	**1**-Na
MP (°C)	111~113	193~195
$[\alpha]_D^{25}$ (*c* 1.0, MeOH)	−6.8°	−9.7°
Empirical formula	$C_{47}H_{80}O_{17}$	$C_{47}H_{79}O_{17}Na$
MW	917.15	939.13
Elemental analysis		
Calcd:		C 60.11, H 8.48
Found:		C 59.81, H 8.63
IR (KBr) cm^{-1}	3220, 2940, 2880, 2820, 1740 ($-CO_2H$), 1460, 1380, 1160, 1070, 960	3420, 3280, 2980, 2940, 1620 ($-CO_2Na$), 1460, 1360, 1165, 1120, 1080
Solubility		
Soluble:	Organic solvents	Organic solvents
Insoluble:	H_2O	H_2O



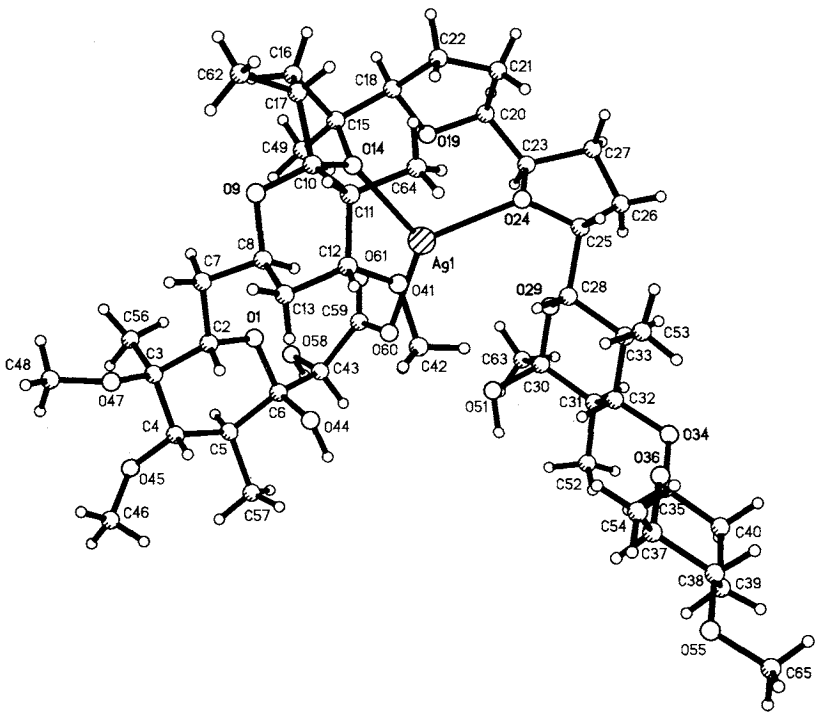
CP-96,797 (**1**) R=H
K-41A R=OCH_3

Table 2. ^{13}C and ^{1}H NMR chemical shift data for the Na-salts of CP-96,797 and K-41A in C_6D_6.

Carbon	CP-96,797		K-41A[a]	Carbon	CP-96,797		K-41A[a]
	^{13}C shift[b]	^{1}H shift[c]	^{13}C shift		^{13}C shift[b]	^{1}H shift[c]	^{13}C shift
1 COONa	179.70 (0)	—	179.77 (0)	25 O–CH	74.65 (1)	4.18	74.61 (1)
2 O–CH	72.43 (1)	4.43	72.50 (1)	26 CH	39.71 (1)	1.45	39.80 (1)
3 O–C–O	99.70 (0)	—	99.76 (0)	27 O–CH	82.97 (1)	3.78	82.93 (1)
4 CH	39.36 (1)	2.71	39.39 (1)	28 CH	48.22 (1)	1.71	48.24 (1)
5 O–CH	86.34 (1)	3.65	86.90 (1)	29 O–C–O	98.86 (0)	—	98.87 (0)
6 C–O	78.73 (0)	—	78.77 (0)	4-Me	12.52 (3)	1.39	12.51 (3)
7 O–CH	67.63 (1)	4.16	67.57 (1)	6-Me	10.92 (3)	1.44	10.97 (3)
8 CH_2	33.23 (2)	1.83, 1.94	33.30 (2)	12-Me	12.57 (3)	0.74	12.56 (3)
9 O–CH	61.69 (1)	4.19	61.94 (1)	14-Me	13.23 (3)	0.91	11.71 (3)
10 CH_2	31.25 (2)	0.85, 2.00	31.27 (2)	16-Me	28.47 (3)	1.67	28.71 (3)
11 O–CH	79.89 (1)	2.79	79.85 (1)	26-Me	13.86 (3)	1.28	13.84 (3)
12 CH	37.01 (1)	1.46	37.04 (1)	28-Me	13.23 (3)	1.21	13.23 (3)
13 O–C–O	108.28 (0)	—	107.16 (0)	29-Me	27.14 (3)	1.57	27.18 (3)
14 CH	39.69 (1)	—	46.44 (1)	5-OMe	60.80 (3)	3.44	60.77 (3)
15 CH_2 or O–CH	40.95 (2)	1.41, 1.48	94.87 (1)	6-OMe	50.92 (3)	3.32	50.92 (3)
				11-OMe	59.29 (3)	3.41	59.40 (3)
16 C–O	82.85 (0)	—	83.69 (0)	15-OMe	—	—	59.86 (3)
17 O–CH	85.50 (1)	3.29	83.79 (1)	Deoxysugar (Deo)[d]:			
18 CH_2	24.16 (2)	1.27, 1.41	25.91 (2)	1′ O–CH–O	102.95 (1)	4.45	102.99 (1)
19 CH_2	22.79 (2)	1.25, 1.52	23.26 (2)	2′ CH_2	30.94 (2)	1.59, 1.84	30.94 (2)
20 O–CH	80.23 (1)	3.51	79.62 (1)	3′ CH_2	27.62 (2)	1.04, 1.79	27.38 (2)
21 O–CH	79.49 (1)	4.42	79.39 (1)	4′ O–CH	80.48 (1)	2.62	80.59 (1)
22 CH_2	29.12 (2)	0.97, 1.49	29.24 (2)	5′ O–CH	74.60 (1)	3.23	74.67 (1)
23 CH_2	24.21 (2)	2.02	24.25 (2)	5′-Me	18.80 (3)	1.42	18.78 (3)
24 O–CH	80.87 (1)	4.17	81.12 (1)	4′-OMe	56.15 (3)	3.09	56.20 (3)

[a] Assignments are based on data given in ref 5; no ^{1}H shift values were reported.
[b] In ppm from TMS in C_6D_6 solution; number of attached protons are in parentheses.
[c] In ppm from TMS in C_6D_6 solution.
[d] 4′-*O*-Methylamicetose.

Fig. 1. Crystal structure of CP-96,797 Ag-salt (**1**-Ag).



(1), O–C–O (3), and –COONa (1) (Table 2). These groups accounted for all the hydrogens in **1**-Na except for three exchangeable ones, which were assumed to be free hydroxy functions on carbons with ^{13}C shifts at δ_C 99.70, 98.86 and 72.43 ppm, based on deuterium induced upfield shifts observed in the ^{13}C NMR spectrum of **1**-Na. Therefore, δ_C 99.70 and 98.86 ppm were assigned to hemiketal carbons, and δ_C 108.28 ppm was assigned to a ketal carbon by process of elimination.

Using DEPT, COSY and HETCOR experiments in the manner previously described[2] for ionophore CP-84,657, the spectrum of **1**-Na was systematically assigned (Table 2). From the data obtained for **1**-Na, it was apparent that the structure was very similar to K-41A[1], for which ^{13}C NMR values have been published[5]. Namely, **1**-Na is missing the methoxy group at C-15. A comparison of the ^{13}C NMR data obtained for **1**-Na and K-41A Na-salt, both in benzene-d_6 solvent, is given in Table 2. Although more than 120 polyether ionophores have been discovered, to our knowledge this is only the second one found that is closely related to K-41A; the other is K-41B[5]. The relative K-41B is also modified at the C-15 position, but it is substituted with a 4-methylamicetose moiety and is thus a diglycoside polyether.

The absolute stereochemistry of **1** was determined by X-ray crystallography using a single crystal of the silver salt of **1**. A computer generated perspective drawing of **1**-Ag (Fig. 1) clearly supports the above proposed structure of **1** based on analysis of the spectroscopic data.

Compound **1**-Na showed good activity against a number of Gram-positive bacteria, as well as the spirochete, *Serpulina* (*Treponema*) *hyodysenteriae* (the causative agent of swine dysentery), but was inactive against Gram-negative bacteria. It afforded anticoccidial activity against *Eimeria tenella* in chickens at levels between 60 and 90 mg/kg in feed. Most interestingly, **1**-Na demonstrated anthelmintic ctivity versus *Trichostrongylus colubriformis* in a rat assay by oral administration at 20 mg/kg.

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