Notes

THE STRUCTURE OF CP-80,219, A NEW POLYETHER ANTIBIOTIC RELATED TO DIANEMYCIN

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In the course of screening actinomycetes for novel antimicrobial substances, a new strain of *Streptomyces hygroscopicus* was found to produce a new polyether antibiotic, CP-80,219 (**1**). This paper describes the isolation, properties and structure of **1**, which is related to dianemycin (**2**).[1]

The fermentation of *S. hygroscopicus* sp. ATCC 53626 and isolation of crude **1**-Na as a hexane soluble oil (150 g) was carried out as described elsewhere.[2] This material was purified by HPLC on a Waters μBondapak C18 column using methanol-water (90:10), followed by HPLC on a silica gel column using methylene chloride-ethanol (96:4). The eluates were examined by TLC on silica gel plates developed with chloroform-2-propanol (95:5), then sprayed with 3% vanillin dissolved in ethanol-phosphoric acid (2:1). Upon heating to 80°C the polyether antibiotic appeared as a pink spot at Rf 0.19. Fractions containing **1**-Na were combined and evaporated to dryness to afford 33 g of the desired product.

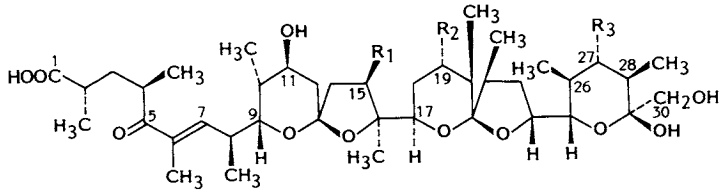
The free acid form of **1** was prepared by vigorously shaking a chloroform solution of **1**-Na with an equal volume of hydrochloric acid at pH 2 in a separatory funnel. The phases were separated and the chloroform layer was washed with water and then evaporated under vacuum to give **1**.

The physico-chemical properties of **1** and **1**-Na are given in Table 1. Spectroscopic data and elemental analysis were consistent with $C_{47}H_{78}O_{14}$ for the free acid **1**, and $C_{47}H_{77}O_{14}Na$ the sodium salt **1**-Na. For example, in the positive FAB-MS, diagnostic cationized molecules *m/z* 890 $(M+Na)^+$ and 912 $(M+2Na-H)^+$ were detected for **1**-Na. The ^{13}C and ^1H NMR spectral data for **1**-Na, including a polarization transfer (DEPT)[3] experiment, revealed the following groups: $CH_3 \times 11$, $CH_2 \times 9$, $CH \times 8$, $CH_3O \times 1$, $O-CH_2 \times 1$, $O-CH \times 8$, $C-O \times 1$, $O-CH-O \times 1$, $O-C-O \times 3$, $-\overset{|}{C}= \times 1$, $-CH= \times 1$, $-COONa \times 1$, and C=O (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 δ_C 99.5, δ_C 70.8, and δ_C 65.4 ppm based on deuterium induced upfield shifts observed in the ^{13}C NMR spectrum of **1**-Na. Therefore, δ_C 99.5 was assigned to a hemiketal carbon, and δ_C 110.8 and δ_C 106.8 ppm to ketal carbons by process of elimination.

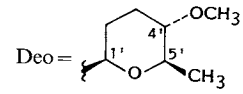
In our efforts to elucidate the structures of unknown ionophores, we have found that it is helpful to estimate the number of rings (R) and the number of oxygen links (E). This is done by the NMR method developed by WHIPPLE *et al.*,[4] and we have previously illustrated its use for another ionophore,[5] *i.e.*, CP-84,657. In the present case, where there are four exchangeable protons (including Na^+ for **1**-Na), the estimated number of rings

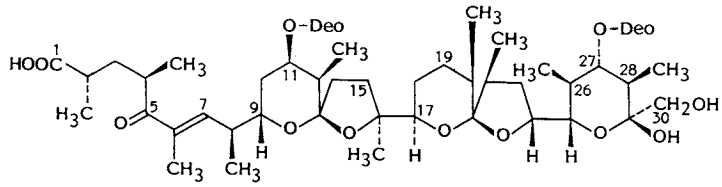
Table 1. Physico-chemical properties of CP-80,219 free acid (**1**) and Na-salt (**1**-Na).

Property	**1**	**1**-Na
MP (°C)	98~102	176~180
$[\alpha]_D^{25}$ (*c* 1.0, MeOH)	+27.1°	+45.9°
Empirical formula	$C_{47}H_{78}O_{14}$	$C_{47}H_{77}O_{14}Na$
MW	867.13	889.12
Elemental analysis		
Calcd for	$C_{47}H_{78}O_{14} \cdot H_2O$: C 63.78, H 9.11	$C_{47}H_{77}O_{14}Na \cdot H_2O$: C 62.23, H 8.78
Found:	C 64.05, H 9.07	C 62.61, H 8.61
Solubility soluble:	Hexane, $CHCl_3$, MeOH	Hexane, $CHCl_3$, MeOH
Solubility insoluble:	H_2O	H_2O
IR ($CHCl_3$) cm^{-1}	1711 (COOH), 1661 (C=O)	1657 (C=O), 1551 (COONa)
UV λ_{max}^{MeOH} nm (log E)	231 (4.15)	233 (4.08)



CP-80,219 (**1**) R_1=H R_2=H R_3=O–Deo
Dianemycin (**2**) R_1=H R_2=O–Deo R_3=H
Endusamycin (**3**) R_1=O–Deo R_2=H R_3=H





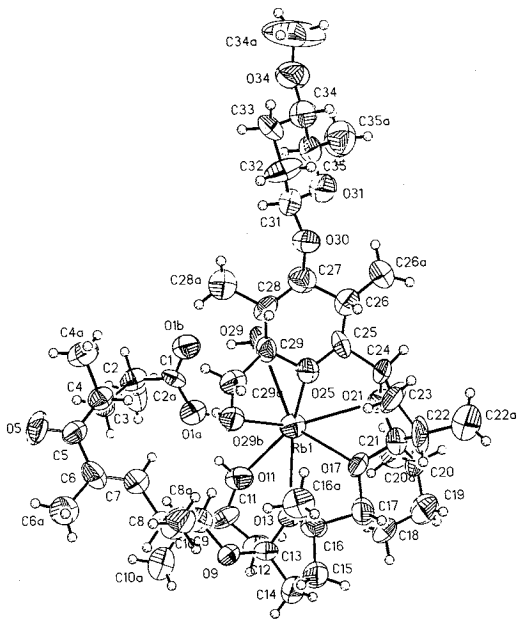
A-130B (**4**)

(R) is 6, and the number of oxygen links (E) is 8 for **1**-Na.

With a knowledge of R, E, and the empirical formula of **1**-Na, coupled with other information such as the presence of an α,β-unsaturated ketone, two ketal and one hemiketal functions, and a sugar moiety, a comparison with known polyether antibiotics can be readily made. Indeed, among the known ionophores, **1**-Na is similar to a group of antibiotics related to dianemycin (**2**). A comparison of the ^{13}C and ^{1}H NMR chemical shifts obtained for **1**-Na and the sodium salt of endusamycin (**3**-Na), a member of the dianemycin group of compounds that was discovered in these laboratories and for which a complete NMR analysis has been recently published,[6] is shown in Table 2. Many of the ^{13}C and ^{1}H signals of **1**-Na essentially correspond to those of **3**-Na. However, some marked changes in the ^{13}C chemical shifts between **1**-Na and **3**-Na were observed that are centered at C-15 and C-27, which were consistent with the deoxysugar (Deo) moiety at C-15 in **3**-Na and C-27 in **1**-Na.

Although there are numerous polyether antibiotics related to dianemycin (**2**) that contain a single Deo moiety,[6,7] to our knowledge none of these structures has the Deo substituent at C-27. However, A-130B (**4**), which possesses two deoxysugars, does have a Deo group at the C-27 position, as well as one at C-11. The δ_C values for the Deo moiety at C-27 for **1**-Na are in good agreement with those observed[8] for **4**-Na. For example, C-26 is δ_C 40.0, C-27 is δ_C 82.6, and C-28 is δ_C 45.4 ppm in **4**-Na when recorded in C_6D_6. As listed in Table 2, values of 39.5, 82.2, and 44.2 ppm, respectively, were obtained for **1**-Na in $CDCl_3$ solvent.

Fig. 1. Crystal structure of CP-80,219 Rb-salt (**1**-Rb).



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

Compound **1**-Na showed good activity against a number of Gram-positive bacteria, as well as the spirochete, *Treponema hyodysenteriae*. No activity was observed versus a number of Gram-negative

Table 2. ^{13}C and ^{1}H NMR chemical shift data for CP-80,219 Na-salt (**1**-Na) and endusamycin Na-salt (**3**-Na)[6] in $CDCl_3$.

Carbon	Functionality	**1**-Na		**3**-Na	
		^{13}C Shift	^{1}H Shift	^{13}C Shift	^{1}H Shift
1	COONa	183.7	—	183.8	—
2	CH	40.3	2.47	39.9	2.47
3	CH_2	41.6	0.98, 1.73	41.5	1.01, 1.72
4	CH	37.8	3.42	37.5	3.49
5	C=O	206.2	—	206.3	—
6	C=	133.6	—	133.7	—
7	CH=	144.9	6.69	144.5	6.70
8	CH	36.0	2.60	35.9	2.64
9	O–CH	69.9	4.58	69.7	4.68
10	CH	36.0	1.92	36.0	1.83
11	O–CH	70.8	3.87	70.2	3.87
12	CH_2	34.1	1.63, 1.92	33.7	1.76, 1.85
13	O–C–O	106.8	—	104.2	—
14	CH_2	39.8	1.76, 2.02	44.9	1.88, 2.30
15	CH_2 or O–CH	32.5	1.77, 2.01	84.3	4.48
16	C–O	86.3	—	85.3[a]	—
17	O–CH	80.4	3.29	78.9	3.51
18	CH_2	18.6	1.30, 1.50	19.3	1.50, 1.71
19	CH_2	26.8	1.66	27.0	1.61
20	CH	30.0	2.00	30.0	1.97
21	O–C–O	110.8	—	110.6	—
22	CH	35.7	2.56	35.3	2.59
23	CH_2	29.8	1.37, 2.32	29.8	1.39, 2.29
24	O–CH	78.9	4.41	78.5	4.37
25	O–CH	72.2	3.97	72.9	3.88
26	CH	39.5	1.25	32.9	1.31
27	O–CH or CH_2	82.2	3.47	36.5	1.36, 1.49
28	CH	44.2	1.47	35.9	1.50
29	O–C–OH	99.5	—	98.6	—
30	CH_2OH	65.4	3.28, 4.04	65.2	3.27, 4.00
2-CH_3		19.7	1.03	19.4	1.02
4-CH_3		14.3	1.08	14.4	1.09
6-CH_3		11.2	1.75	11.1	1.77
8-CH_3		16.8	1.06	16.9	1.07
10-CH_3		10.0	0.74	9.9	0.74
16-CH_3		26.8	1.42	24.8	1.49
20-CH_3		13.2[b]	1.02[b]	12.9	1.04
22-CH_3		15.0	0.97	14.7	0.97
26-CH_3		13.2[b]	1.01[b]	17.4	0.84
28-CH_3		12.4[b]	1.02[b]	16.6	0.90
		Deoxysugar (Deo)[c] at:			
		C-27		C-15	
1′	O–CH–O	102.9	4.38	101.5	4.36
2′	CH_2	30.5	1.46, 1.93	30.4	1.46, 1.83
3′	CH_2	27.4	1.25, 2.18	26.8	1.31, 2.19
4′	O–CH	80.3	2.76	79.9	2.83
5′	O–CH	74.7	3.20	74.6	3.30
4′-OCH_3		56.8	3.33	56.8	3.35
5′-CH_3		18.3	1.22	18.2	1.27

[a] A chemical shift of δ 69.7 ppm was inadvertently reported previously (ref 6); the observed shift is δ 85.3 ppm.
[b] Assignments may be interchanged.
[c] 4-Methylamicetose.

aerobes including *Escherichia coli*. It afforded anticoccidial activity against *Eimeria tenella* in chickens between 30 and 120 mg/kg in feed.

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