Notes

THE ISOLATION AND STRUCTURE OF CP-120,509, A NEW POLYETHER ANTIBIOTIC RELATED TO SEMDURAMICIN AND PRODUCED BY MUTANTS OF *Actinomadura roseorufa*

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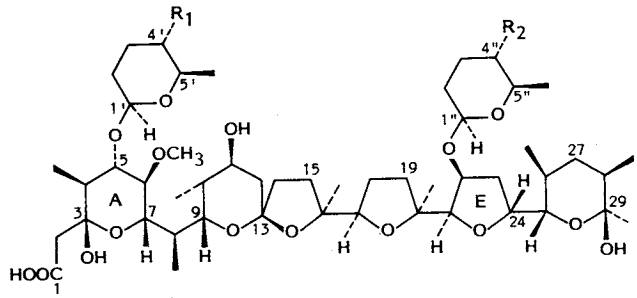
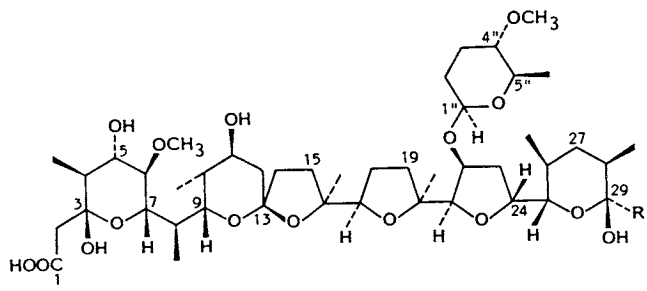
Following the discovery of the semi-synthetic anticoccidial ionophore semduramicin[1,2] (UK-61,689), a mutation program was undertaken to produce this polyether antibiotic by direct fermentation[3]. The producing culture of the antibiotic UK-58,852, *Actinomadura roseorufa* ATCC 53666, was chosen as the parental strain to be mutagenically treated to induce a semduramicin-producing mutant[4,5]. The two compounds differ from each other in that the A-ring sugar moiety of UK-58,852 is replaced by a hydroxyl group in semduramicin. Recently, we described the formation of two new polyether antibiotics, CP-91,243 and CP-91,244, isolated from the fermentation broths of two mutants of the parental strain[6]. These new antibiotics were co-produced with UK-58,852. Ultimately, the direct fermentation of semduramicin, free of UK-58,852, CP-91,243 and CP-91,244, was accomplished using other mutants of this same parental strain[3]. Interestingly, an unknown minor metabolite was observed together with semduramicin. We now report the isolation and characterization of this minor metabolite, the novel polyether CP-120,509 (**1**).

The production of semduramicin by direct fermentation of *A. roseorufa* ATCC 53664 (obtained



UK-58,852 $R_1=R_2=OCH_3$
CP-91,243 $R_1=R_2=OH$
CP-91,244 $R_1=OH$, $R_2=OCH_3$



Semduramicin $R=CH_3$
CP-120,509 (**1**) $R=CH_2OH$

Scheme 1. Isolation and purification of CP-120,509.

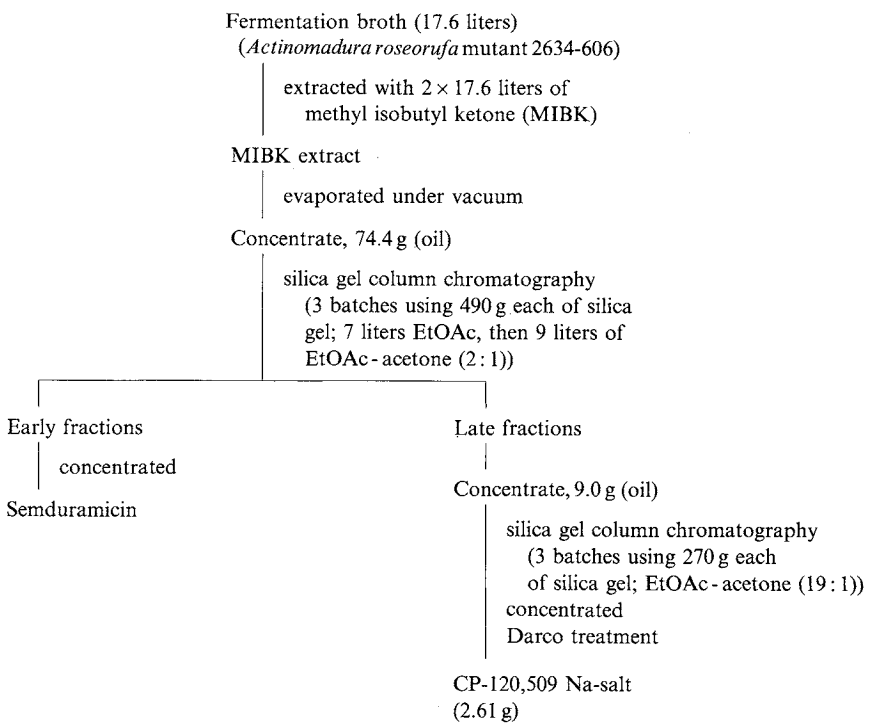


Table 1. Physico-chemical properties of CP-120,509 free acid (**1**) and Na-salt (**1**-Na).

Property	**1**	**1**-Na
MP	80~82°C	143~145°C
$[\alpha]_D^{25}$ (*c* 1.0, $CHCl_3$)	+32.3°	+28.3°
Empirical formula	$C_{45}H_{76}O_{17}$	$C_{45}H_{75}O_{17}Na$
MW	889.10	911.07
Elemental analysis		
Calcd:	C 60.79, H 8.62	C 59.33, H 8.30
Found:	C 60.18, H 9.06	C 59.11, H 8.87
IR ($CHCl_3$) cm^{-1}	3360, 2940, 2900, 2845, 1725 ($-CO_2H$), 1460, 1380, 1110, 1060, 990, 970	3060, 2940, 2895, 2840, 1590 ($-CO_2Na$), 1460, 1400, 1375, 1110, 1050, 990, 970
Solubility		
Soluble	Organic solvents	Organic solvents
Insoluble	H_2O	H_2O

from the mutation of strain ATCC 53666 with 1-methyl-3-nitro-1-nitrosoguanidine) was performed as described elsewhere[3]. Although this fermentation was free of the parent antibiotic UK-58,852, a minor metabolite (*ca.* 2% relative to semduramicin based on HPLC analysis) was detected that was more polar than semduramicin. Since we sought to determine the nature of this minor metabolite, we were fortunate to find that another mutant of ATCC 53666, designated mutant 2634-606, produced this same polar metabolite in a greater quantity relative to semduramicin (18:82 ratio, respectively, by HPLC analysis). From the isolation method shown in Scheme 1, 17.6-liters of fermentation broth using this latter mutant afforded 2.61 g of CP-120,509 Na-salt (**1**-Na). TLC analysis with silica gel plates using $CHCl_3$-methanol (9:1) gave an Rf value of 0.52 for CP-120,509 and 0.56 for semduramicin. The antibiotics were visualized by spraying the TLC plate with vanillin-EtOH-

Table 2. ^{13}C and ^{1}H NMR chemical shift data for the Na-salts of CP-120,509 and semduramicin in $CDCl_3$.

Carbon	CP-120,509		Semduramicin[a]	
	^{13}C shift[b]	^{1}H shift[c]	^{13}C shift[b]	^{1}H shift[c]
1 COONa	178.51 (0)	—	179.09 (0)	—
2 CH_2	44.97 (2)	2.22, 2.62	45.38 (2)	2.17, 2.49
3 O–C–O	97.81 (0)	—	97.70 (0)	—
4 CH	45.33 (1)	1.47	45.28 (1)	1.48
5 O–CH	75.20 (1)	3.62	74.74 (1)	3.71
6 O–CH	82.42 (1)	2.98	81.95 (1)	3.11
7 O–CH	67.55 (1)	3.73	66.80 (1)	3.73
8 CH	33.93 (1)	1.88	33.74 (1)	1.98
9 O–CH	68.60 (1)	3.97	67.61 (1)	4.23
10 CH	34.51 (1)	1.84	33.54 (1)	1.81
11 O–CH	69.83 (1)	3.95	70.03 (1)	3.92
12 CH_2	33.21 (2)	1.66, 1.94	33.77 (2)	1.62, 1.90
13 O–C–O	106.80 (0)	—	107.45 (0)	—
14 CH_2	39.16 (2)	1.74, 1.94	38.89 (2)[d]	1.73, 1.97
15 CH_2	33.54 (2)	1.72, 1.96	33.39 (2)[d]	1.76, 1.98
16 C–O	84.41 (0)	—	84.51 (0)	—
17 O–CH	82.45 (1)	3.45	82.28 (1)	3.53
18 CH_2	28.44 (2)	1.48, 1.67	26.83 (2)	1.47, 1.71
19 CH_2	32.17 (2)	1.40, 2.63	32.25 (2)	1.50, 2.40
20 C–O	83.43 (0)	—	84.15 (0)	—
21 O–CH	87.19 (1)	3.81	86.96 (1)	4.03
22 O–CH	80.76 (1)	4.10	80.92 (1)	4.16
23 CH_2	33.21 (2)	2.12, 2.30	32.47 (2)	2.23
24 O–CH	77.98 (1)	4.52	80.22 (1)	4.49
25 O–CH	74.76 (1)	3.83	73.01 (1)	3.93
26 CH	31.60 (1)	1.34	33.11 (1)	1.23
27 CH_2	35.26 (2)	1.32, 1.46	36.40 (2)[d]	1.32, 1.42
28 CH	35.97 (1)	1.45	39.81 (1)	1.43
29 O–C–O	97.69 (0)	—	96.89 (0)	—
4-Me	12.30 (3)	1.04	12.10 (3)	1.03
6-OMe	60.17 (3)	3.53	59.03 (3)	3.52
8-Me	10.76 (3)	1.07	11.05 (3)	1.08
10-Me	10.50 (3)	0.83	10.43 (3)	0.84
16-Me	27.31 (3)	1.41	27.56 (3)	1.49
20-Me	24.67 (3)	1.18	23.25 (3)	1.12
26-Me	16.69 (3)	0.82	17.51 (3)	0.87
28-Me	16.15 (3)	0.81	16.99 (3)	0.91
29-CH_2OH	65.01 (2)	3.23, 3.96	—	—
29-Me	—	—	26.05 (3)	1.29
	Deoxysugar (Deo)[e]			
1′ O–CH–O	103.30 (1)	4.41	103.22 (1)	4.41
2′ CH_2	30.53 (2)	1.53, 1.77	30.55 (2)	1.53, 1.80
3′ CH_2	26.87 (2)	1.32, 2.18	26.92 (2)	1.31, 2.18
4′ O–CH	79.82 (1)	2.80	79.83 (1)	2.81
5′ O–CH	74.60 (1)	3.31	74.57 (1)	3.31
4′-OMe	56.85 (3)	3.35	56.86 (3)	3.36
5′-Me	18.38 (3)	1.26	18.38 (3)	1.24

[a] For details of spectral assignment of semduramicin Na-salt see ref 6.

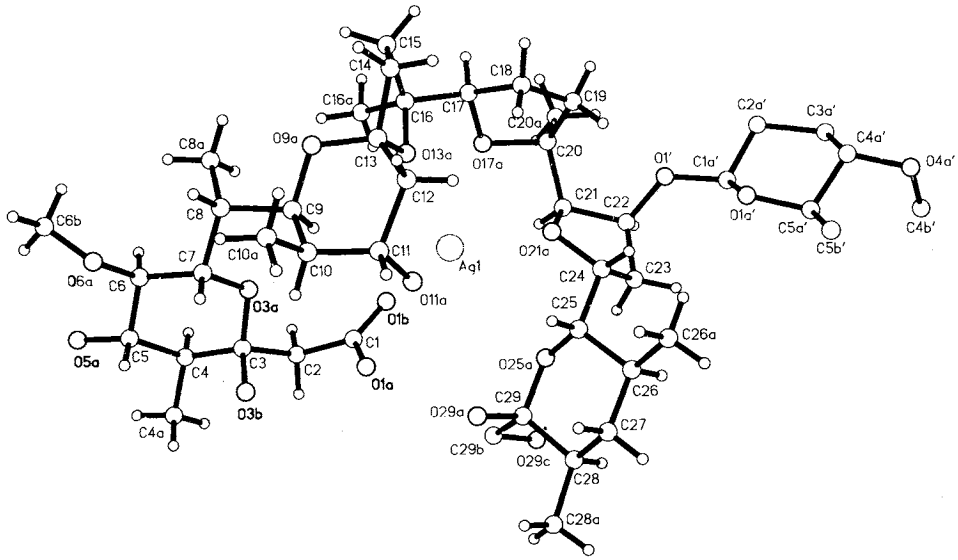
[b] In ppm from TMS in $CDCl_3$ solution; number of attached protons are in parentheses.

[c] In ppm from TMS in $CDCl_3$ solution.

[d] Previous assignment (ref 6) has been confirmed in a recent study of the biosynthesis of semduramicin, *i.e.*, incorporation of [1-^{13}C]propionate, [1-^{13}C]acetate, or [1,2-$^{13}C_2$]acetate: J. P. DIRLAM, H. A. I. MCARTHUR & E. B. WHIPPLE, unpublished results.

[e] 4′-*O*-Methylamicetose.

Fig. 1. Crystal structure of CP-120,509 Ag-salt (1-Ag).



H_2SO_4 reagent followed by heating at 100°C.

The physico-chemical properties of **1** and **1**-Na are given in Table 1. Spectroscopic data and elemental analysis were consistent with $C_{45}H_{76}O_{17}$ for free acid **1**, and $C_{45}H_{75}O_{17}Na$ for sodium salt **1**-Na. For example, in the low resolution positive FAB-MS, a diagnostic cationized molecule *m/z* 911 $(M+Na)^+$ was detected for **1**-Na, where M is defined as the mass of the free acid (calcd for $C_{45}H_{76}O_{17}+Na$: 911.4980). Furthermore, **1**-Na gave a base peak at *m/z* 849, 62 daltons less than the corresponding metal-adduct molecular ion, which is common for polyethers having a β-hemiketal carboxylic acid group $((M+Na-CO_2-H_2O)^+)$[7]. Thus, the molecular formula for **1**-Na was assumed to contain one oxygen (16 daltons) more than that for semduramicin Na-salt ($C_{45}H_{75}O_{16}Na$).

The ^{13}C, ^1H and DEPT[8] NMR data for **1**-Na, recorded on a Bruker AM-500 spectrometer, revealed the following groups: CH_3 (8), CH_2 (11), CH (5), CH_3O (2), O–CH (12), C–O (2), O–CH–O (1), O–C–O (3), and –COONa (1) (Table 2). It was apparent that the structure of **1**-Na was very similar to semduramicin Na-salt, but **1**-Na was missing the methyl group at δ_C 26.05 ppm (29-Me) and contained an additional methylene group at δ_C 65.01. From a consideration of the chemical shift of the new methylene group, hydroxymethyl substitution at C-29 seemed a likely possibility.

Using ^{13}C DEPT, COSY and HETCOR experiments in the manner previously described[9] for ionophore CP-84,657, the spectrum of **1**-Na was systematically assigned (Table 2), except for three methylene units (C-14, C-15 and C-27) which were assigned by analogy with semduramicin Na-salt, where a detailed NMR analysis had already been performed[6]. The observed shifts for the terminal hydroxymethyl at C-29 (δ_C 65.01; δ_H 3.23 and 3.96) are in excellent agreement with those reported for the terminal hydroxymethyl at C-25 in monensin (δ_C 64.83; δ_H 3.23 and 3.93)[10].

The structure of **1** was later confirmed by X-ray crystallography using a single crystal of the silver salt of **1**. All diffractometer data were collected at −100°C. A computer generated perspective drawing of **1**-Ag (Fig. 1) clearly supports the above proposed structure of **1**. Interestingly, another X-ray structure of **1**-Ag was determined in the same experiment owing to a disorder of the sugar moiety. This second structure (not shown) was identical with the one shown in Fig. 1 with the exception that the substituents O1', methoxy (O4a',C4b') and methyl (C5b') on the sugar ring were all in axial positions (these substituents are all equatorial in Fig. 1). The two structures, both of which had the sugar group in a chair conformation, were present in nearly a 1:1 ratio.

A small quantity of **1**-Na was isolated from the fermentation of *A. roseorufa* ATCC 53664, in which **1**-Na was detected at only 2% of the semduramicin level. The NMR spectral data obtained using this

sample were in good agreement with those reported in Table 2. The high resolution positive FAB-MS showed a cationized molecule with an exact mass at *m/z* 911.4980 ($(M+Na)^+$; calcd for $C_{45}H_{76}O_{17}$+Na: 911.4980). Finally, both samples were analyzed by reversed-phase HPLC on a Partisil C_8 column (10 μm; 4.6×250 mm) using RI detection; elution was by MeCN-H_2O (95:5) with a flow rate of 1.0 ml/minute. A retention time of 4.83 minutes was found for the two samples of **1**-Na. Using these HPLC conditions, the following retention times were determined: CP-91,243 (4.41 minutes), semduramicin (5.13 minutes), CP-91,244 (5.66 minutes) and UK-58,852 (7.78 minutes).

CP-120,509 exhibited *in vitro* antibiotic activity against certain Gram-positive bacteria, and the spirochete, *Serpulina* (*Treponema*) *hyodysenteriae* (the causative agent of swine dysentery), but was inactive against Gram-negative bacteria. It afforded excellent anticoccidial activity against *Eimeria acervulina* in chickens at levels between 30 and 60 mg/kg in feed; however, it was devoid of activity versus *Eimeria tenella* at levels as high as 60 mg/kg in feed. In the same test, semduramicin was superior to CP-120,509 against *E. acervulina* at 15 mg/kg in feed.

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