# CP-91,243 AND CP-91,244, NOVEL DIGLYCOSIDE POLYETHER ANTIBIOTICS RELATED TO UK-58,852 AND PRODUCED BY MUTANTS OF *Actinomadura roseorufa*

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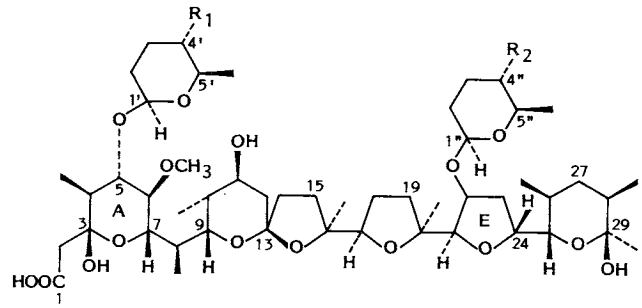
Interest in polyether antibiotics has remained at a high level for over 20 years, owing largely to the commercial importance of this class of drugs in veterinary medicine. For example, monensin[1], lasalocid[1] and salinomycin[2] are marketed as anticoccidial agents for poultry, and are used as growth permittants in cattle or swine. Narasin[1] and maduramicin[3] are also used as anticoccidial agents.

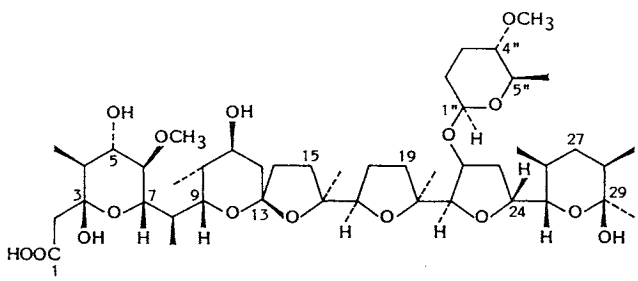
Following the discovery of the attractive, semisynthetic anticoccidial ionophore semduramicin[4,5] (UK-61,689), a mutation program was undertaken that was designed to produce this polyether antibiotic by direct fermentation[6]. The producing culture of the antibiotic UK-58,852, was chosen as the parental strain to be mutagenically treated to induce a semduramicin producing mutant[7,8]. The two compounds differ from each other in that the A-ring glycone of UK-58,852 has been replaced by a hydroxyl group in semduramicin (Fig. 1).

This paper describes the unexpected formation of two new polyether antibiotics, CP-91,243 and CP-91,244 (Fig. 1), isolated from the fermentation broths of two mutants (ATCC 53869 and ATCC 53870) of *Actinomadura roseorufa* (ATCC 53666). The isolation, characterization, and biological testing of these new antibiotics, which were coproduced with the parent antibiotic UK-58,852,

Fig. 1. The structures of UK-58,852, CP-91,243, CP-91,244 and semduramicin.



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

† A part of this work has been presented at the 2nd S.I.M. Int. Conf. on Biotech. Prod. of Microb. with Novel Pharmacolog. and Agrobio. Activities, Abstract No. P-14, p. 31, Sarasota, FL, October 14~17, 1990.

are discussed.

CP-91,243, CP-91,244 and UK-58,852 were produced by microorganisms obtained from the mutation of a strain of *A. roseorufa* ATCC 53666 with 1-methyl-3-nitro-1-nitrosoguanidine, as described elsewhere[9]. Using the isolation method shown in Scheme 1, a 15-liter fermentation broth using *A. roseorufa* ATCC 53870 afforded 1.20 g of CP-91,243, 480 mg of CP-91,244 and 190 mg of UK-58,852. A second mutant culture (ATCC 53869) also yielded CP-91,243 (280 mg), CP-91,244 (850 mg) and UK-58,852 (1.50 g). TLC analysis with silica gel plates using $CHCl_3$-methanol (9 : 1) gave the following Rf values: 0.2 for CP-91,243, 0.4

Scheme 1. Isolation and purification of CP-91,243, CP-91,244 and UK-58,852.

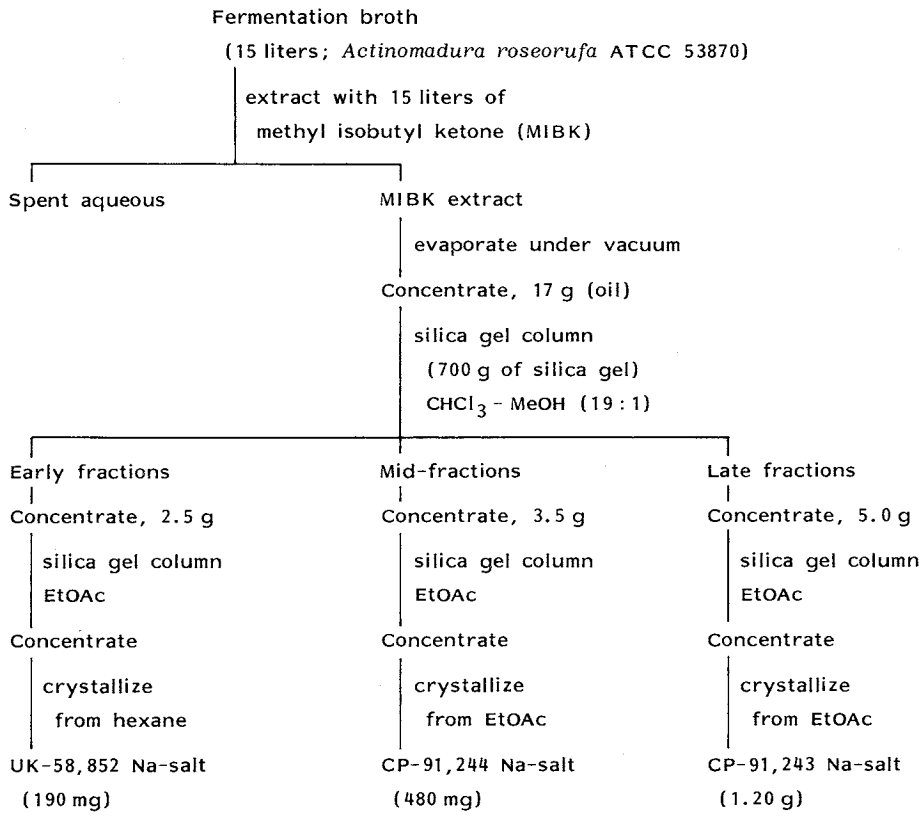



Table 1. Physico-chemical properties of CP-91,243 Na-salt and CP-91,244 Na-salt.

| Property | CP-91,243 Na-salt | CP-91,244 Na-salt |
|---|---|---|
| MP (°C) | 178~180 | 154~157 |
| $[\alpha]_D^{25}$ (*c* 1.0, MeOH) | −7.6° | −4.8° |
| Empirical formula | $C_{50}H_{83}O_{18}Na$ | $C_{51}H_{85}O_{18}Na$ |
| MW | 995.1 | 1,009.1 |
| Elemental analysis | | |
| Calcd for: | $C_{50}H_{83}O_{18}Na \cdot H_2O$<br>C 59.22, H 8.47 | $C_{51}H_{85}O_{18}Na$<br>C 60.65, H 8.59 |
| Found: | C 59.22, H 8.23 | C 60.41, H 8.58 |
| IR (KBr) $cm^{-1}$ | 3280, 2980, 2910, 2865, 1583 (–$CO_2Na$), 1455, 1375, 1240, 1160, 1130, 1120, 1060, 980, 940 | 3260, 2975, 2930, 2865, 1610 (–$CO_2Na$), 1455, 1380, 1160, 1120, 1100, 1065, 980, 940 |
| Solubility | | |
| Soluble: | Organic solvents | Organic solvents |
| Insoluble: | $H_2O$ | $H_2O$ |

Table 2. $^{13}C$ and $^{1}H$ NMR chemical shift data for the Na-salts of CP-91,243, CP-91,244, UK-58,852 and semduramicin in $CDCl_3$.

| Carbon | CP-91,243 | | CP-91,244 | | UK-58,852 | | Semduramicin | |
|---|---|---|---|---|---|---|---|---|
| | $^{13}C$ Shift[a] | $^{1}H$ Shift[b] | $^{13}C$ Shift[a] | $^{1}H$ Shift[b] | $^{13}C$ Shift[a] | $^{1}H$ Shift[b] | $^{13}C$ Shift[a] | $^{1}H$ Shift[b] |
| 1 | 179.33 (0) | — | 179.25 (0) | — | 179.19 (0) | — | 179.09 (0) | — |
| 2 | 45.06 (2) | 2.10, 2.47 | 45.55 (2) | 2.15, 2.49 | 45.74 (2) | 2.11, 2.47 | 45.38 (2) | 2.17, 2.49 |
| 3 | 97.91 (0) | — | 97.91 (0) | — | 97.16 (0) | — | 97.70 (0) | — |
| 4 | 44.50 (1) | 1.37 | 44.62 (1) | 1.44 | 44.79 (1) | 1.44 | 45.28 (1) | 1.48 |
| 5 | 81.72 (1) | 3.67 | 81.68 (1) | 3.79 | 81.73 (1) | 3.74 | 74.74 (1) | 3.71 |
| 6 | 82.35 (1) | 3.09 | 82.44 (1) | 3.16 | 82.58 (1) | 3.13 | 81.95 (1) | 3.11 |
| 7 | 67.53 (1) | 3.63 | 67.37 (1) | 3.67 | 67.39 (1) | 3.65 | 66.80 (1) | 3.73 |
| 8 | 33.25 (1) | 1.92 | 33.41 (1) | 1.98 | 33.54 (1) | 1.96 | 33.74 (1) | 1.98 |
| 9 | 67.79 (1) | 4.08 | 67.69 (1) | 4.21 | 67.73 (1) | 4.18 | 67.61 (1) | 4.23 |
| 10 | 33.62 (1) | 1.76 | 33.58 (1) | 1.79 | 33.66 (1) | 1.73 | 33.54 (1) | 1.81 |
| 11 | 69.90 (1) | 3.84 | 70.04 (1) | 3.91 | 70.19 (1) | 3.88 | 70.03 (1) | 3.92 |
| 12 | 33.62 (2) | 1.55, 1.86 | 33.78 (2) | 1.60, 1.88 | 33.90 (2) | 1.60, 1.87 | 33.77 (2) | 1.62, 1.90 |
| 13 | 107.32 (0) | — | 107.48 (0) | — | 107.59 (0) | — | 107.45 (0) | — |
| 14 | 38.85 (2) | 1.66, 1.93 | 38.89 (2) | 1.68, 1.98 | 38.96 (2) | 1.67, 1.92 | 38.89 (2) | 1.73, 1.97 |
| 15 | 33.35 (2) | 1.71, 1.90 | 33.41 (2) | 1.75, 1.98 | 33.48 (2) | 1.69, 2.01 | 33.39 (2) | 1.76, 1.98 |
| 16 | 84.74 (0) | — | 84.53 (0) | — | 84.55 (0) | — | 84.51 (0) | — |
| 17 | 82.21 (1) | 3.49 | 82.27 (1) | 3.54 | 82.43 (1) | 3.50 | 82.28 (1) | 3.53 |
| 18 | 26.90 (2) | 1.42, 1.65 | 26.80 (2) | 1.46, 1.69 | 26.85 (2) | 1.42, 1.63 | 26.83 (2) | 1.47, 1.71 |
| 19 | 32.25 (2) | 1.44, 2.34 | 32.22 (2) | 1.48, 2.39 | 32.31 (2) | 1.41, 2.37 | 32.25 (2) | 1.50, 2.40 |
| 20 | 84.13 (0) | — | 84.15 (0) | — | 84.20 (0) | — | 84.15 (0) | — |
| 21 | 86.95 (1) | 3.93 | 86.96 (1) | 4.03 | 87.08 (1) | 4.01 | 86.96 (1) | 4.03 |
| 22 | 80.97 (1) | 4.13 | 80.89 (1) | 4.15 | 80.94 (1) | 4.12 | 80.92 (1) | 4.16 |
| 23 | 32.32 (2) | 2.17 | 32.45 (2) | 2.25 | 32.54 (2) | 2.21 | 32.47 (2) | 2.23 |
| 24 | 79.94 (1) | 4.43 | 80.22 (1) | 4.49 | 80.34 (1) | 4.46 | 80.22 (1) | 4.49 |
| 25 | 73.36 (1) | 3.80 | 72.98 (1) | 3.92 | 73.05 (1) | 3.90 | 73.01 (1) | 3.93 |
| 26 | 32.93 (1) | 1.17 | 33.11 (1) | 1.22 | 33.21 (1) | 1.19 | 33.11 (1) | 1.23 |
| 27 | 36.32 (2) | 1.25, 1.30 | 36.41 (2) | 1.30, 1.40 | 36.54 (2) | 1.28, 1.35 | 36.40 (2) | 1.32, 1.42 |
| 28 | 39.62 (1) | 1.40 | 39.82 (1) | 1.42 | 39.97 (1) | 1.38 | 39.81 (1) | 1.43 |
| 29 | 96.95 (0) | — | 96.88 (0) | — | 96.93 (0) | — | 96.89 (0) | — |
| 6-$OCH_3$ | 59.72 (3) | 3.43 | 59.66 (3) | 3.49 | 59.58 (3) | 3.45 | 59.03 (3) | 3.52 |
| 4-$CH_3$ | 12.23 (3) | 0.96 | 12.38 (3) | 1.01 | 12.40 (3) | 0.99 | 12.10 (3) | 1.03 |
| 8-$CH_3$ | 10.88 (3) | 0.98 | 11.06 (3) | 1.05 | 11.04 (3) | 1.01 | 11.05 (3) | 1.08 |
| 10-$CH_3$ | 10.21 (3) | 0.78 | 10.34 (3) | 0.83 | 10.40 (3) | 0.79 | 10.43 (3) | 0.84 |
| 16-$CH_3$ | 27.34 (3) | 1.43 | 27.58 (3) | 1.48 | 27.65 (3) | 1.44 | 27.56 (3) | 1.49 |
| 20-$CH_3$ | 23.19 (3) | 1.07 | 23.22 (3) | 1.11 | 23.24 (3) | 1.08 | 23.25 (3) | 1.12 |
| 26-$CH_3$ | 17.28 (3) | 0.79 | 17.47 (3) | 0.85 | 17.48 (3) | 0.82 | 17.51 (3) | 0.87 |
| 28-$CH_3$ | 16.77 (3) | 0.83 | 17.28 (3) | 0.90 | 16.96 (3) | 0.86 | 16.99 (3) | 0.91 |
| 29-$CH_3$ | 26.00 (3) | 1.23 | 26.02 (3) | 1.26 | 26.06 (3) | 1.24 | 26.05 (3) | 1.29 |
| Deoxysugars (Deo)[c]: | | | | | | | | |
| 1′ | 102.47 (1) | 4.63 | 102.40 (1) | 4.68 | 102.41 (1) | 4.65 | — | — |
| 2′ | 31.13 (2) | 1.37, 1.94 | 31.40 (2) | 1.56, 1.91 | 31.10 (2) | 1.48, 1.87 | — | — |
| 3′ | 31.29 (2) | 1.49, 1.85 | 31.55 (2) | 1.44, 2.04 | 27.39 (2) | 1.25, 2.13 | — | — |
| 4′ | 71.03 (1) | 3.11 | 71.53 (1) | 3.22 | 80.58 (1) | 2.76 | — | — |
| 5′ | 75.68 (1) | 3.14 | 75.63 (1) | 3.20 | 74.44 (1) | 3.24 | — | — |
| 4′-$OCH_3$ | — | — | — | — | 56.78 (3) | 3.30 | — | — |
| 5′-$CH_3$ | 17.83 (3) | 1.17 | 18.03 (3) | 1.24 | 18.28 (3) | 1.19 | — | — |
| 1″ | 103.33 (1) | 4.37 | 103.19 (1) | 4.40 | 103.22 (1) | 4.38 | 103.22 (1) | 4.41 |
| 2″ | 30.92 (2) | 1.51, 1.70 | 30.54 (2) | 1.51, 1.78 | 30.62 (2) | 1.51, 1.75 | 30.55 (2) | 1.53, 1.80 |
| 3″ | 30.77 (2) | 1.37, 1.94 | 26.91 (2) | 1.30, 2.17 | 26.99 (2) | 1.25, 2.14 | 26.92 (2) | 1.31, 2.18 |
| 4″ | 70.50 (1) | 3.12 | 79.84 (1) | 2.75 | 79.95 (1) | 2.76 | 79.83 (1) | 2.81 |
| 5″ | 75.90 (1) | 3.19 | 74.57 (1) | 3.29 | 74.67 (1) | 3.24 | 74.57 (1) | 3.31 |
| 4″-$OCH_3$ | — | — | 56.82 (3) | 3.33 | 56.82 (3) | 3.30 | 56.86 (3) | 3.36 |
| 5″-$CH_3$ | 17.97 (3) | 1.19 | 18.36 (3) | 1.23 | 18.40 (3) | 1.20 | 18.38 (3) | 1.24 |

[a] In ppm from TMS in $CDCl_3$ solution; number of attached protons in parentheses.
[b] In ppm from TMS in $CDCl_3$ solution.
[c] 4-Methylamicetose.

for CP-91,244 and 0.7 for UK-58,852. The antibiotics were visualized by spraying with vanillin-EtOH-$H_2SO_4$ reagent and heating the TLC plate to 100°C.

The physico-chemical properties of CP-91,243 Na-salt and CP-91,244 Na-salt are given in Table 1. Spectroscopic data and elemental analyses were consistent with $C_{50}H_{83}O_{18}Na$ for the Na-salt of CP-91,243, and $C_{51}H_{85}O_{18}Na$ for the Na-salt of CP-91,244. For example, in the positive FAB-MS, diagnostic cationized molecules *m/z* 996 ($(M+Na)^+$) and 1,010 ($(M+Na)^+$) were detected for CP-91,243 and CP-91,244, respectively. Furthermore, both antibiotics gave base peaks 62 daltons less than the corresponding metal-adduct molecular ion, which is common for polyethers having a $\beta$-hemiketal carboxylic acid group ($(M+Na-CO_2-H_2O)^+$)[10].

In our efforts to elucidate the structures of CP-91,243 Na-salt and CP-91,244 Na-salt by NMR, we used UK-58,852 and semduramicin, which is devoid of a deoxy (Deo) sugar on the A-ring, as model compounds. Spectra were recorded on a Bruker WM-250 spectrometer (modified to incorporate a pulse programmer and Aspect-3000 data system) and a Bruker AM-500 spectrometer. Using $^{13}C$ DEPT, COSY and HETCOR experiments in a manner previously described[11] for the structure elucidation of ionophore CP-84,657, the structure of semduramicin Na-salt was systematically assigned (Table 2), except for three methylene units which were based partly on a comparison with the unambiguous assignments reported for monensin A[12]. The resulting shifts for C-14 ($\delta_C$ 38.89), C-15 ($\delta_C$ 33.41) and C-27 ($\delta_C$ 36.41) correspond to values of $\delta_C$ 39.28, 33.25 and 35.75, respectively, in monensin A. Most of the assignments given in Table 2 for the other model compound, UK-58,852, were obtained independently, including the Deo sugars on the A- and E-rings.

The $^{13}C$ and $^1H$ NMR spectral data for CP-91,243 Na-salt revealed that two methoxy groups were absent from the parent antibiotic UK-58,852, while in the CP-91,244 Na-salt, only one methoxy group was missing. The structure of CP-91,244 Na-salt was independently assigned, whereas the assignments for CP-91,243 Na-salt were determined by analogy with the other structures studied (Table 2). Based on these NMR results, CP-91,243 Na-salt bears hydroxy groups at the 4′ and 4″ positions of the Deo moieties, whereas CP-91,244 Na-salt is *O*-demethylated at only the 4′-position (Fig. 1).

As summarized in Table 2, excellent agreement was observed for the $^{13}C$ and $^1H$ chemical shift assignments of all four polyether antibiotics. Furthermore, the $^{13}C$ chemical shifts found for the *O*-demethylated Deo sugars in CP-91,243 Na-salt and CP-91,244 Na-salt are similar to reported values[13]. In view of the results obtained in the present study, the relative and absolute stereochemistry for CP-91,243 and CP-91,244 are assumed to be the same as that shown for UK-58,852 in Fig. 1, which was previously determined by X-ray crystallography[7].

Both CP-91,243 and CP-91,244 exhibited *in vitro* antibiotic activity against certain Gram-positive bacteria, and the spirochete, *Treponema hyodysenteriae* (the causative agent of swine dysentery), but were not active against Gram-negative bacteria. CP-91,243 afforded anticoccidial activity against *Eimeria tenella* in chickens at 60 mg/kg in feed, and the less polar CP-91,244 was about twice as active (25 mg/kg in feed). Salinomycin[2], a commercial anticoccidial agent that was used as a positive control drug, gave activity at 60 mg/kg in feed.

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