Notes

ELLORAMYCINS B, C, D, E AND F: MINOR CONGENERS OF THE ELLORAMYCIN PRODUCER *STREPTOMYCES OLIVACEUS*†

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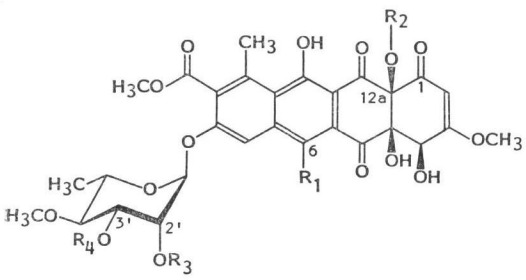
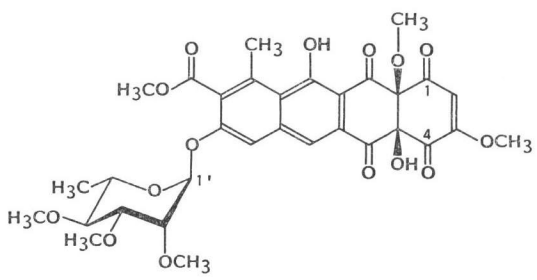
Elloramycin (**1**) is a new anthracycline-like antibiotic with a permethylated L-rhamnose in a phenolic α-glycosidic linkage[2,3]. Structure-activity considerations[4,5] resulted in the hypothesis that less lipophilic, *i.e.* less methylated elloramycin derivatives will result in better solubility, which is necessary for uptake by living cells. Furthermore, additional polar groups would perhaps increase the interaction with DNA resulting in enhanced antitumor activity. The high level of *O*-methylation in **1** marks an unusual biosynthetic activity of the producer strain *Streptomyces olivaceus*. We expected less methylated congeners of **1** as probable late precursors during the biosynthesis. Thus, our screening lead to five new antibiotics, called elloramycins B to F (**2**~**6**). Compounds B and F were separated by chromatography on silica gel, C, D and E were detected by HPLC and diode array detection[6] and separated by chromatography on silica gel and preparative HPLC on reversed-phase columns. The concentrations of the new elloramycins is about 0.01 mg/liter (F) and 1.3~4 mg/liter (B, C, D and E) in the culture medium.

The physico-chemical properties of the new elloramycins differ only slightly (Table 1). The similarity of the IR, UV and CD spectra indicate the same chromophore and the same stereochemistry as in **1**. The structures **2** to **6** were established by comparison of the EI mass spectra and the ^{1}H NMR spectra (Table 2) with those of elloramycin (**1**)[1]. Key fragments at *m/z* 472 (**3** and **4**), 470 (**5**) and 458 (**2**), respectively, were assigned to the aglycones. The sugar moieties were seen at *m/z* 189 (**1** and **5**) and 175 (**2** to **4**). The fragments indicate whether the aglycone or the sugar part is less methylated. The change of a methoxyl to a hydroxyl group in the sugar moiety results in a significant downfield shift of the adjacent proton (*i.e.* 2′-H for **2** and **4**, 3′-H for **3**). The missing 12a-OCH_3

Fig. 1. Structures of elloramycin (**1**), elloramycins B (**2**), C (**3**), D (**4**), E (**5**) and F (**6**).



1	R_1=H,	R_2=CH_3,	R_3=CH_3,	R_4=CH_3
2	R_1=H,	R_2=H,	R_3=H,	R_4=CH_3
3	R_1=H,	R_2=CH_3,	R_3=CH_3,	R_4=H
4	R_1=H,	R_2=CH_3,	R_3=H,	R_4=CH_3
6	R_1=OH,	R_2=CH_3,	R_3=CH_3,	R_4=CH_3



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† Metabolic products of microorganisms. 235[1].

Table 1. Physico-chemical properties of elloramycins B to F (**2**~**6**).

Elloramycin	B (**2**)	C (**3**)	D (**4**)	E (**5**)	F (**6**)
Molecular formula (MW)	$C_{30}H_{32}O_{15}$ (632.58)	$C_{31}H_{34}O_{15}$ (646.61)	$C_{31}H_{34}O_{15}$ (646.61)	$C_{32}H_{34}O_{15}$ (658.62)	$C_{32}H_{36}O_{16}$ (676.63)
EI-MS (high resolution)*	458.0849	646.1898	472.1006	658.1898	M^+ and aglycone
m/z (aboundance)	($C_{22}H_{18}O_{11}$, aglycone, 2%),	($C_{31}H_{34}O_{15}$, M^+, 3%),	($C_{23}H_{20}O_{11}$, aglycone, 0.1%),	($C_{32}H_{34}O_{15}$, M^+, 2%),	not available,
	175.0970	472 (aglycone, 64%),	175.0970	470 (aglycone, 2%),	189 (sugar, 12%)
	($C_8H_{15}O_4$, sugar, 9%)	175 (sugar, 35%)	($C_8H_{15}O_4$, sugar, 2%)	189 (sugar, 100%)	
Rf values** I	0.44	0.59	0.55	0.67	0.64
II	0.11	0.12	0.14	0.20	0.23
IR (KBr) cm^{-1}	1735, 1710, 1690, 1603	1730 (sh), 1712, 1685, 1604	1739, 1712, 1690 (sh), 1607	1738, 1711, 1682, 1608	1735, 1685, 1660, 1602
UV λ_{max}^{MeOH} nm (ε)	286 (30,700),	238 (25,500),	240 (25,100),	242 (sh, 25,800),	286 (20,300),
	386 (8,000),	286 (40,600),	286 (41,500),	285 (41,200),	412 (8,200),
	406 (8,800)	391 (11,400),	391 (11,700),	388 (11,600),	439 (6,100)
		410 (11,700)	408 (12,000)	405 (11,900)	
$\lambda_{max}^{MeOH-NaOH}$ nm (ε)	253 (29,100),	252 (33,500),	251 (36,900),	256 (37,800),	254 (20,700),
	386 (8,000),	424 (sh, 12,100),	424 (sh, 12,600),	312 (sh, 8,000),	448 (5,200),
	440 (8,800)	440 (13,000)	440 (13,700)	440 (12,200)	510 (sh, 2,800)
CD $\lambda_{extreme}^{MeOH}$ nm	400 (sh), 345, 302, 289,	400 (sh), 344, 297,	400 (sh), 344, 298, 286,	405, 390, 367, 340,	
($[\theta]^{22} \times 10^{-4}$)	264, 244	285, 260, 242	262, 244	285 (sh), 270	
	(−0.3, −0.9, +0.5,	(−0.5, −1.9, +0.5,	(−0.4, −1.8, +0.7,	(+0.6, +0.6, +0.3,	
	−0.3, +4.0, −0.7)	−0.6, +8.0, −1.0,	−0.8, +8.3, −1.0,	+1.4, −7.2, −8.8,	
		0, −1.0)	0, −1.1)	+6.0)	

* Preselected peak maching.

** I: $CHCl_3$ - MeOH (9: 1), II: EtOAc - *n*-pentane - acetic acid (55: 40: 5).

Table 2. ^{1}H NMR data of elloramycins B to F (**2**~**6**) in comparison with elloramycin (**1**) in $CDCl_3$ (δ in ppm relative to internal tetramethylsilane (J in Hz)).

H-atom	1[a]	2[b]	3[a]	4[a]	5[a]	6[b]
2-H	5.57 d (2)	5.67 d (2)	5.67 d (2)	5.57 d (2)	5.62 s	5.52 d (2)
3-OCH_3	3.80 s	3.82 s	3.81 s	3.81 s	3.98 s	3.79 s
4-H	4.74 d (2)	4.83 d (2)	4.79 d (2)	4.75 d (2)	—	4.74 d (2)
6-H	8.00 s	8.02 s	8.00 s	8.01 s	8.02 s	—
7-H	7.56 s	7.51 s	7.56 s	7.56 s	7.58 s	8.09 s
9-OCH_3	3.99 s	3.94 s	3.98 s	3.99 s	4.00 s	3.98 s
10-CH_3	2.90 s	2.83 s	2.90 s	2.90 s	2.91 s	2.85 s
11-OH*	13.81 s	13.75 s	13.92 s	13.93 s	14.04 s	13.80 s
12a-OCH_3	3.65 s	—	3.65 s	3.65 s	3.43 s	3.64 s
1′-H	5.76 d (2)	5.70 d (2)	5.77 d (2)	5.78 d (2)	5.77 d (2)	5.79 d (2)
2′-H	*ca.* 3.78**	4.16 dd (2, 2)	3.72 dd (2, 2)	4.24 dd (2, 2)	3.78 dd (2, 2)	*ca.* 3.75**
2′-OCH_3	3.60 s	—	3.60 s	—	3.57 s	3.55 s
3′-H	3.49 dd (9, 3)	*ca.* 3.5**	3.89 m	3.50 dd (9, 3)	3.52 dd (9, 3)	*ca.* 3.6**
3′-OCH_3	3.57 s	3.51 s	—	3.57 s	3.56 s	3.54 s
4′-H	3.24 dd (9, 9)	3.24 dd (9, 9)	3.10 dd (9, 9)	3.20 dd (9, 9)	3.24 dd (9, 9)	3.20 dd (9, 9)
4′-OCH_3	3.58 s	3.51 s	3.61 s	3.57 s	3.58 s	3.58 s
5′-H	*ca.* 3.6**	*ca.* 3.6**	*ca.* 3.6**	*ca.* 3.6**	*ca.* 3.6**	*ca.* 3.6**
5′-CH_3	1.27 d (6)	1.26 d (6)	1.27 d (6)	1.27 d (6)	1.27 d (6)	1.26 d (6)
OH-signals*	2.82 s 4.34 s	1.85 s	1.78 s 2.51 s 4.44 s	4.50 s	4.58 s	12.21 s (6-OH)

* Exchangeable with CD_3OD. ** Partially obscured. [a] 200 MHz. [b] 80 MHz.

Table 3. Antimicrobial spectrum of elloramycins.

Organism	MIC (μg/ml)				
	Elloramycin	B	C	D	E
Bacillus brevis	100	30	100	100	100
Micrococcus luteus	100	30	100	100	100
Arthrobacter aurescens	100	10	100	100	100
Brevibacterium flavum	>100	10	>100	>100	>100
Staphylococcus aureus	100	10	100	30	100
Streptomyces olivaceus	<0.1	0.3	<0.1	<0.1	<0.1
S. prasinus	<0.1	0.3	<0.1	<0.1	<0.1
S. violaceus-niger	<0.1	3.0	<0.1	<0.1	<0.1

signal of **2**, the missing 4-H signal of **5** and the missing 6-H signal in combination with an additional signal (δ 12.21) for a chelated hydroxyl group of **6** prove the given structures.

The antimicrobial activities of the elloramycin compounds were tested by the broth dilution method (inoculum 10^5 cells/ml, respectively spores/ml) with the results shown in Table 3. The antibiotics are strongly active against *Streptomyces* strains, including the producer strain *S. olivaceus*. As expected, the less methylated elloramycin B is more active against Gram-positive bacteria.

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