The Structures of the Antitumour Antibiotics, PD 114720 and PD 114721†

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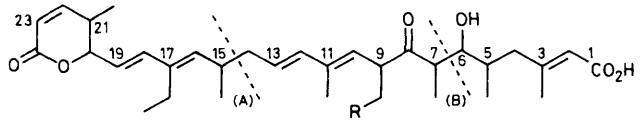
The structures of the lipophilic antitumour antibiotics, PD 114720 and PD 114721, have been established by n.m.r. and mass spectral analyses of these compounds and their derivatives.

The recent publications by Hamamoto *et al.* describing the antifungal antibiotics, leptomycins A and B,[1,2] prompt us to report our work with a similar set of antibiotics. During the course of screening for new antitumour antibiotics, an as yet unidentified actinomycete (ATCC 39366) was found that produces two previously unreported antibiotics, PD 114720 (**1**) and PD 114721 (**2**). Both of these compounds are highly cytotoxic to L1210 lymphocytic leukaemia cells (IC_{50} = 3 × 10^{-10} M) and display a wide range of antitumour activity in

† The provisional names, elactocin and hydroxyelactocin, have been assigned, respectively, to PD 114720 and PD 114721.



(1) R = H
(2) R = OH

Table 1. ^{1}H N.m.r. data for PD 114720 (**1**) and PD 114721 (**2**).[a]

Position	(1)	(2)
2	5.66 s	5.66 s
4a	2.17 m	2.15 m
4b	1.88 dd (9.0, 13.0)	1.91 dd (8.7, 13.2)
5	1.73 m	1.74 m
6	3.56 t (5.4)	3.60 m
7	2.80 m	2.78 m
9	3.64 m	3.85 m
10	5.06 d (10.1)	5.02 d (9.8)
12	5.98 d (14.6)	5.99 d (15.4)
13	5.57 m	5.61 dd (7.6, 15.4)
14	2.06 t (7.0)	2.06 m
15	2.65 m	2.65 m
16	5.21 d (9.7)	5.20 d (9.1)
18	6.62 d (15.8)	6.62 d (15.8)
19	5.70 dd (7.0, 15.8)	5.69 dd (6.9, 15.8)
20	4.97 m	4.96 m
21	2.51 m	2.52 m
22	6.93 dd (5.6, 9.7)	6.93 dd (5.6, 9.7)
23	5.97 d (9.7)	5.98 d (9.7)
3-Me	2.11 s	2.11 s
5-Me	0.77 d (6.7)	0.77 d (6.7)
7-Me	1.13 d (7.1)	1.17 d (7.1)
9-Me	1.10 d (6.6)	
9-C*H*$_2$OH		3.60 m, 3.85 m
11-Me	1.80 s	1.84 s
15-Me	0.97 d (6.6)	0.95 d (6.6)
17-C*H*$_2$Me	2.17 q (7.5)	2.18 q (7.5)
17-CH$_2$-C*H*$_3$	1.03 t (7.5)	1.03 t (7.5)
21-Me	1.05 d (7.2)	1.05 d (7.2)

[a] In $CDCl_3$ at 360 MHz. Signals (δ) are downfield from $SiMe_4$. Coupling constants in Hz are given in parentheses.

mice. A complete account of the antitumour activity will be published separately.

Compounds (**1**) and (**2**) were isolated by extraction of the fermentation beer with ethyl acetate at pH 3.5, followed by partition into MeOH–H_2O (90:10), and chromatography over silicic acid, eluting with a CH_2Cl_2–MeOH gradient. Further purification was effected by silica gel chromatography, followed by reverse-phase chromatography over C-18 silica gel. The antibiotics were obtained as low melting point solids that could not be crystallized. Each component exhibits a λ_{max} (MeOH) at 237 nm (ε 31 500) and ν_{max} ($CHCl_3$) 3600—3200, 1715, and 1700 cm^{-1}. PD 114720 (**1**) showed: $[\alpha]_D^{23}$ −105.4° (*c* 1.12, MeOH) and fast atom bombardment (f.a.b.) mass spectrum *m/z* 563 (*M* + Na); PD 114721 (**2**) showed: $[\alpha]_D^{23}$ −81.5° (*c* 1.07, MeOH) and f.a.b. mass spectrum *m/z* 579 (*M* + Na).

The ^{1}H and ^{13}C n.m.r. and mass spectral data for (**1**) and (**2**) (Tables 1—3) agree with the molecular formulae $C_{33}H_{48}O_6$ and $C_{33}H_{48}O_7$, respectively. The nature of the six oxygen atoms in (**1**) and the additional oxygen atom in (**2**) could be determined by a combination of chemical and spectral means. Thus, acetylation of PD 114720 yielded a monoacetate derivative [δ 5.1 (m, 6-H), 2.1 (s, MeCO)], while treatment with ethereal diazomethane gave a monomethyl ester [δ 3.9 (s, OMe)]. These data, in combination with the corresponding i.r. and n.m.r. spectral data, indicated the presence of carboxy and secondary hydroxy groups in (**1**). The ^{13}C n.m.r. spectrum of (**1**), in addition to displaying the signal for the carboxy carbon at δ 171, exhibits signals for additional carbonyl carbons at δ 215.1 and 164.3. These latter signals were assigned, respectively, to a saturated ketone and the carbonyl carbon of an α,β-unsaturated lactone.

The location of the additional oxygen atom in (**2**) could be determined from n.m.r. data, which indicated the replace-

Table 2. ^{13}C N.m.r. data for PD 114720 (**1**) and PD 114721 (**2**).[a]

Position	(1)	(2)
1	171.0 s	170.6 s
2	116.9 d	117.1 d
3	161.1 s	160.4 s
4	45.7 t	45.6 t
5	33.6 d	33.5 d
6	74.3 d	73.9 d
7	46.7 d	48.0 d
8	215.1 s	215.0 s
9	45.7 d	53.8 d
10	128.0 d[b]	122.1 d
11	136.4 s	139.3 s
12	135.1 d	134.8 d
13	128.1 d[b]	128.9 d
14	40.8 t	40.8 t
15	32.2 d	32.1 d
16	136.4 d	136.8 d
17	135.5 s	135.5 s
18	130.2 d	130.2 d
19	122.8 d	122.6 d
20	81.5 d	81.6 d
21	33.6 d	33.5 d
22	151.5 d	151.7 d
23	120.1 d	120.0 d
24	164.3 s	164.4 s
3-Me	18.7 q	18.6 q
5-Me	13.7 q[c]	13.6 q[d]
7-Me	20.9 q	20.9 q
9-Me	16.1 q	
9-CH$_2$OH		62.5 t
11-Me	13.1 q	13.3 q
15-Me	12.6 q[c]	12.4 q[d]
17-CH$_2$Me	26.6 t	26.6 t
17-CH$_2$*Me*	13.6 q[c]	13.5 q[d]
21-Me	12.4 q[c]	12.4 q[d]

[a] In $CDCl_3$ at 90.56 MHz. Signals (δ) are downfield from $SiMe_4$. Assignments are based on chemical shift data and selective proton decoupling experiments. Multiplicities were determined from off-resonance decoupled spectra. [b—d] Assignment of these signals may be interchanged.

Table 3. Mass spectral fragmentation for PD 114720 (**1**) and PD 114721 (**2**).

Fragment	Ions (*m/z*) observed (1)	(2)
M + H	541	—
(*M* + H) − H_2O	523[a]	539
(*M* + H) − CH_2O	—	527
(*M* + H) − $2H_2O$	505	520[b]
(*M* + H) − H_2O, − CH_2O	—	509
Retro-aldol [fragment (B)]	385	—
Fragment (B) − H_2O	367	382[c]
Fragment (B) − CH_2O	—	371
Fragment (A)	219[d]	219[d]

[a] *m/z* 523.3430; $C_{33}H_{47}O_5$ requires 523.3425. [b] *m/z* 520.3209; $C_{33}H_{44}O_5$ requires 520.3190. [c] *m/z* 382.2507; $C_{25}H_{34}O_3$ requires 382.2509. [d] *m/z* 219.1369 for (**1**), 219.1346 for (**2**); $C_{14}H_{19}O_2$ requires 219.1386.

ment of a methyl in (**1**) with a hydroxymethyl in (**2**). Specifically, the methyl signal centred at δ 1.10 in the ^{1}H n.m.r. spectrum of (**1**) is replaced by a pair of 1-proton signals at δ 3.60 and 3.85 in the spectrum of (**2**), representing the nonequivalent protons of a hydroxymethyl group. Correspondingly, in the ^{13}C n.m.r. spectrum of (**2**) a new methylene signal is observed at δ 62.5 replacing the methyl signal located at δ 16.1 in the spectrum of (**1**).

Extensive homonuclear spin decoupling experiments in the ^{1}H n.m.r. spectra of PD 114720 and PD 114721 established a number of partial structures separated by quaternary carbon atoms. These pieces could be connected by analysis of the fragmentation patterns in the electron impact–chemical ionisation mass spectra (Table 3) and allowed the assignment of complete structures (**1**) and (**2**).‡ Thus, allylic fragmentation yields an ion at *m/z* 219 ($C_{14}H_{19}O_2$) for both (**1**) and (**2**), indicating that the compounds are identical in this structural segment [fragment (A)]. Retro-aldol cleavage could be detected in the spectra of each compound, yielding fragment (B) from (**1**) and ions for fragment (B) − H_2O in the spectra of both (**1**) and (**2**). These fragmentations show that the secondary hydroxy group is attached to C-6. The mass spectrum of (**2**) also exhibits fragments corresponding to loss of formaldehyde, resulting from a second retro-aldol cleavage. This fragmentation is consistent with the attachment of the hydroxymethyl group in (**2**) at C-9.

Analysis of the data described above clearly indicates that PD 114721 (**2**) is a novel antitumour antibiotic, while the structure proposed for PD 114720 (**1**) is identical to that reported for leptomycin B.[2] The differences in physical properties between leptomycin B and (**1**), however, and the discrepancy in assignments of the ^{13}C n.m.r. signals, necessitate a direct comparison before an identity can be established.

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‡ The stereochemistry at the six chiral centres and the configuration of the three trisubstituted double bonds have not been determined with certainty.

References

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