PD 124,895 AND PD 124,966, TWO NEW ANTITUMOR ANTIBIOTICS

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The isolation and characterization of the title antibiotics, which are produced by the same *Streptomyces* sp., is described. The potent antitumor agent, PD 124,895, is an analog of hydroxyelactocin (PD 114,721). PD 124,966 is a new member of the depsipeptide family of antibiotics.

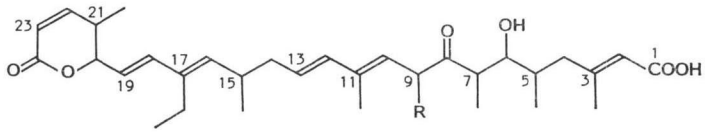
Recently we reported the isolation of the novel antitumor agents elactocin (**1**)† and hydroxyelactocin (**2**)[1,2]. During the fractionation of fermentation beers containing these antibiotics, the presence of additional elactocin analogs was detected. Reverse phase chromatography of certain fractions led to the isolation of a new antibiotic (PD 124,895) which proved to an analog (**4**) of hydroxyelactocin by examination of its NMR and mass spectral properties. From another, less polar fraction an additional antibiotic of a different structure type was obtained. This compound, PD 124,966, contains nitrogen and is a depsipeptide of still unknown structure. In addition to the compounds described above, a small quantity of another elactocin analog (PD 118,607) was isolated and shown to have the same structural formula (**3**) assigned to leptomycin A[3].

Experimental

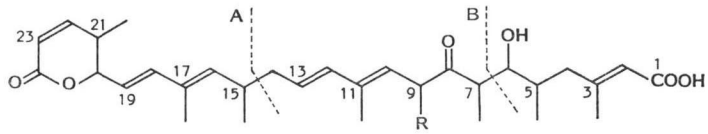
PD 124,895 (**4**)

The EtOAc extract of unfiltered fermentation beer (5,880 liters, adjusted to pH 3.5 with H_2SO_4) was concentrated to 54 liters and washed with water. The EtOAc layer was further concentrated to 15 liters and diluted with 75 liters of petroleum ether. The resulting mixture was extracted with 30 liters of MeOH - H_2O (9 : 1) followed by a second extraction with 15 liters of MeOH - H_2O (9 : 1). The aqueous methanol extracts were combined and washed with 8 liters of petroleum ether. The aqueous methanol layer was then concentrated *in vacuo* to remove MeOH and the remaining organic material was extracted into CH_2Cl_2. The CH_2Cl_2 extract was dried (Na_2SO_4), diluted to 16 liters, and chromatographed over 48 kg of silicic acid - Celite 545 (1 : 1) eluting with CH_2Cl_2 followed by CH_2Cl_2 containing 2% and then 4% MeOH. All fractionation steps were monitored by HPLC using either a μBondapak or a Novapak C-18 silica gel column and 0.05 M ammonium phosphate pH 6.8 buffer - CH_3CN (35 : 65 or 40 : 60) as the mobile phase. Retention times of the four elactocin analogs, detected at 254 nm at a flow rate of 1.5 ml/minute, are approximately 3.0, 4.1, 6.1 and 7.5 minutes for PD 124,895, PD 114,721, PD 118,607 and PD 114,720, respectively. The silicic acid - Celite column fractions containing PD 114,721 and PD 124,895 were concentrated to a residual oil which was dissolved in 2.2 liters of MeOH. One-third aliquots of this MeOH solution were chromatographed separately over 17 kg of C-18 silica gel (Sepralyte, 40 μm particle size, Analytichem International,

† Elactocin (PD 114,720; CI-940) appears to be identical to leptomycin B which was isolated by HAMAMOTO *et al.*[3]. Hydroxyelactocin (PD 114,721) was shown to possess the same structure assigned to kazusamycin by KOMIYAMA *et al.*[4].



PD 114,720 (**1**) R = CH_3
PD 114,721 (**2**) R = CH_2OH



PD 118,607 (**3**) R = CH_3
PD 124,895 (**4**) R = CH_2OH

Harbor City, CA, U.S.A.) contained in a 15 cm×183 cm stainless steel column and previously equilibrated with 60 liters of 0.05 M NH_4OAc pH 6.5 buffer - MeOH (1 : 1). The mobile phase used for this chromatographic step was 0.05 M NH_4OAc pH 6.5 buffer - CH_3CN (62 : 38). Before the second and third columns were run, the C-18 silica gel was first washed with 40 liters of CH_3CN, 30 liters of MeOH, and finally with 60 liters of 0.05 M ammonium acetate pH 6.5 buffer - MeOH (1 : 1). The fractions containing PD 124,895 were combined (86 liters) and diluted to 124 liters with water. The resulting solution was then pumped over 5.5 kg of C-18 silica gel (40 μm particle size) packed in a 10 cm×122 cm stainless steel column and previously equilibrated with 0.05 M NH_4OAc pH 6.5 buffer - MeOH (1 : 1). Elactocin congeners (PD 124,895 and a small amount of PD 114,721) were retained at the top of the column which was then developed with 0.05 M NH_4OAc pH 6.6 buffer - CH_3CN (59 : 41). After 35 liters of eluate was collected, most of the PD 124,895 was eluted in the next eight 2-liter fractions. The fractions containing PD 124,895 as the only UV-absorbing component were combined and concentrated *in vacuo*. PD 124,895 was removed from this concentrate by an extraction with CH_2Cl_2. The CH_2Cl_2 extract was washed four times with water, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The residual solid was dissolved in Et_2O and the resulting solution was filtered to remove a trace of insoluble material. The filtrate was concentrated to dryness and the residue was lyophilized from 700 ml of *tert*-butanol to yield 12.5 g of PD 124,895 as a white solid, melting indistinctly between 40~50°C. Fast atom bombardment MS (FAB-MS) *m/z* 543 (M+1); high resolution electron impact mass spectra *m/z* 368.2353 (fragment B−H_2O), 205.1241 (fragment A); UV λ_{max}^{MeOH} nm (ε) 234 (44,100); IR (KBr) 2971, 2935, 1709, 1644, 1457, 1380, 1253, 1160, 1103, 1047, 968 cm^{-1}; $[\alpha]_D$ +158° (*c* 0.76, $CHCl_3$). NMR data are listed in Table 1.

Anal Calcd for $C_{32}H_{46}O_7 \cdot 0.33H_2O \cdot 0.66C_4H_{10}O$: C 69.62, H 8.98, H_2O 0.99, $C_4H_{10}O$ 8.2.
Found: C 69.58, H 8.95, H_2O 1.31, $C_4H_{10}O$ 8.0.

PD 118,607 (3)

In earlier work impure preparations of elactocin (PD 114,720) obtained by silicic acid - Celite chromatography as described above were rechromatographed over C-18 silica gel using a stepwise gradient from MeOH - H_2O (7: 3) to MeOH - H_2O (8: 2). Several fractions of the 80% MeOH eluate contained a component that eluted prior to elactocin. These fractions were lyophilized to afford a relatively minor component (PD 118,607) as a pale yellow solid. FAB-MS *m/z* 527 (M+1). The UV and IR spectra of PD 118,607 are virtually identical to the corresponding spectra of elactocin. ^1H NMR spectral data (not listed) match the ^1H NMR data for elactocin except for the expected difference associated with the replacement of the C-17 ethyl group in elactocin by a methyl group in PD 118,607.

Table 1. NMR data for PD 114,721 and PD 124,895[a].

Position	^{13}C		^{1}H	
	PD 114,721	PD 124,895	PD 114,721	PD 124,895[b]
1	170.6 s	170.3		
2	117.1 d	116.7	5.66 s	5.68 s
3	160.4 s	161.0		
4	45.6 t	45.6	1.91 dd	2.1 m[d]
			2.15 m	
5	33.5 d	33.5	1.74 m	1.8 m
6	73.9 d	74.1	3.60 m	3.62 m
7	48.0 d	47.7	2.78 m	2.8 m
8	215.0 s	215.2		
9	53.8 d	53.7	3.85 m	3.88 m
10	122.1 d	122.0	5.02 d	5.05 d (9.4)
11	139.3 s	139.5		
12	134.8 d	134.9	5.99 d	6.02 d (15.4)
13	128.9 d	129.0	5.61 d	5.6 m
14	40.8 t	40.7	2.06 m	2.0 m[d]
15	32.1 d	32.3	2.65 m	2.7 m
16	136.8 d	138.6	5.20 d	5.25 d (9.5)
17	135.5 s	129.7		
18	130.2 d	131.1	6.62 d	6.74 d (15.8)
19	122.6 d	123.3	5.69 dd	5.7 m
20	81.6 d	81.3	4.96 m	4.99 m
21	33.5 d	33.5	2.52 m	2.5 m
22	151.7 d	151.7	6.93 dd	6.97 dd (5.7, 9.8)
23	120.0 d	120.1	5.98 d	6.01 d (9.8)
24	164.4 s	164.4		
3-CH_3	18.6 q	18.6	2.11 s	2.12 s
5-CH_3	13.6 q[c]	13.6[c]	0.77 d	0.78 d (6.5)
7-CH_3	20.9 q	20.9	1.17 d	1.18 d (7.0)
9-CH_2OH	62.5 t	62.6	3.60 m	3.62 m
			3.85 m	3.88 m
11-CH_3	13.3 q[c]	13.4[c]	1.84 s	1.82 s
15-CH_3	12.4 q	12.4	0.95 d	0.96 d (6.5)
17-CH_2CH_3	26.6 t	Absent	2.18 q	Absent
17-CH_2CH_3	13.5 q[c]		1.03 t	
17-CH_3	Absent	20.5	Absent	1.86 s
21-CH_3	12.4 q	12.4	1.05 d	1.06 d (7.2)

[a] In $CDCl_3$ at 360 MHz and 90.6 MHz for ^{1}H and ^{13}C NMR spectra of PD 114,721 and at 200 MHz and 75.4 MHz for PD 124,895. Signals (δ) are downfield from TMS.
[b] *J*-values are in parentheses.
[c] Signals may be interchanged.
[d] Overlapping signals.

PD 124,966

During the latter stages of isolating relatively large quantities of elactocin (PD 114,720), the presence of a compound with different solubility properties was noted which was not detected by the HPLC method used routinely. When a CH_3CN solution of elactocin was being prepared as a charge for a final chromatographic purification, a crystalline solid precipitated. The mixture was filtered and the precipitate was washed with cold CH_3CN, and dried *in vacuo*. This product (184 g) readily crystallized from either acetone or aqueous EtOH to yield PD 124,966 as a white crystalline solid, mp 182~184°C. FAB-MS *m/z* 841 (apparent M+H); $[\alpha]_D$ +17.7° (*c* 1.03, MeOH). PD 124,966 exhibits only end absorption in the UV but can be detected by HPLC at 205 nm using a μBondapak C-18 column and 0.05 M ammonium phosphate pH 6.5 buffer - CH_3CN (35: 65) as the mobile phase

Fig. 1. IR (KBr) spectrum of PD 124,966.

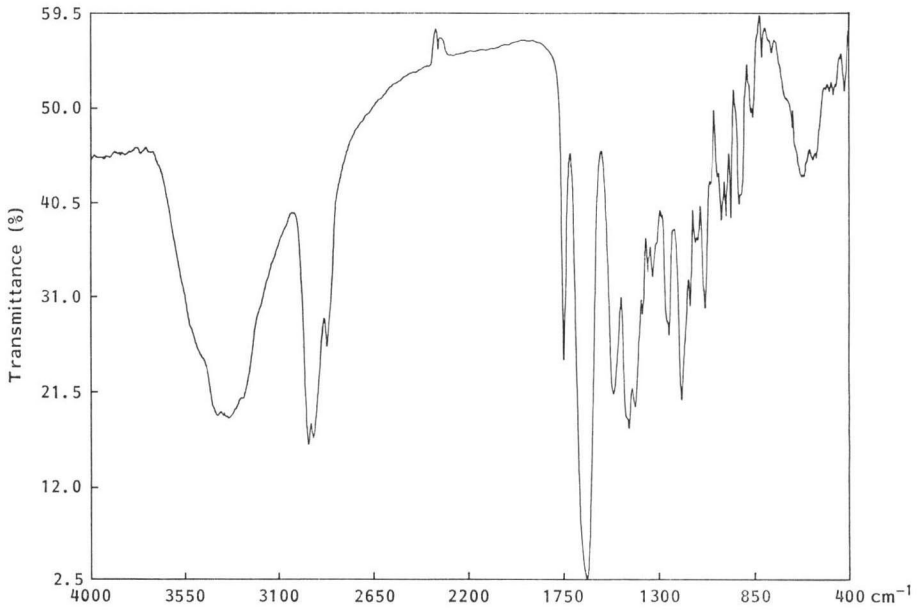
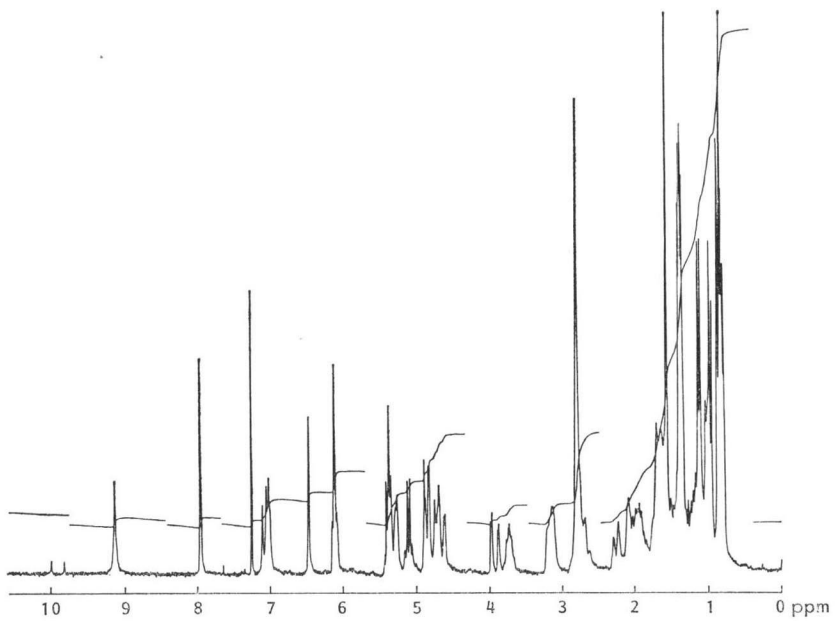


Fig. 2. ^{1}H NMR spectrum of PD 124,966 in $CDCl_3$.



(retention time at a flow rate of 1.5 ml/minute is 6.1 minutes). ^{13}C NMR (75.3 MHz, $CDCl_3$) δ 176.03, 172.52, 172.47, 171.04, 170.28, 169.17, 98.93, 78.97, 76.84, 71.75, 53.54, 52.90, 49.37, 49.04, 47.12, 46.80, 46.28, 42.13, 39.54, 38.61, 31.06, 30.97, 29.93, 27.42, 24.87, 23.89, 23.31, 21.77, 21.58, 20.38, 19.90, 19.39, 18.73, 18.18, 12.94, 11.84, 11.64. The IR and ^{1}H NMR spectra of PD 124,966 are shown in Figs. 1 and 2.

Anal Calcd for $C_{38}H_{64}N_8O_{13}\cdot 0.75H_2O$: C 53.41, H 7.73, N 13.11, O 25.75, H_2O 1.58.
Found: C 53.34, H 7.60, N 13.05, O 25.54, H_2O 1.24.

Discussion

Comparisons of the ^{13}C and ^{1}H NMR spectral data listed in Table 1 for PD 124,895 with those previously reported[1] for hydroxyelactocin (PD 114,721) show that these compounds are very similar. The absence of signals in the NMR spectrum of PD 124,895 for the C-17 ethyl group of PD 114,721 and the presence of a new signal (a singlet at 1.86 ppm) for a vinylic methyl group indicated that PD 124,895 is the C-17 methyl analog (**4**) of hydroxyelactocin. Similarly, NMR data showed that in PD 118,607 the C-17 ethyl group in elactocin (PD 114,720) is replaced by a methyl group leading to the assignment of structure (**3**) to PD 118,607. High resolution mass spectral data support these formulations, exhibiting fragments corresponding to the lactone end of the molecules which are 14 mass units less than the corresponding fragments observed in the mass spectra of **1** and **2**. PD 124,895 represents the heretofore "missing" fourth member (**4**) of the elactocin series of antibiotics containing one or two hydroxyl groups.

Table 2. *In vivo* activity of PD 124,895 against P388 lymphocytic leukemia in mice[a].

Dose[b]	T/C (%)
200	Toxic
100	153
50	139
25	130

[a] Tumor inoculated intraperitoneally on day 0.
[b] μg/kg/injection; single doses given intraperitoneally on days 1~5.

The high nitrogen content of PD 124,966 and the presence in its IR spectrum of an ester or lactone peak at 1754 cm^{-1} and a much more intense amide absorption at 1643 cm^{-1} suggest that this antibiotic is a depsipeptide. Several FAB-MS determinations using different matrices indicate a molecular weight of 840 daltons. Coupled with elemental analytical data, this corresponds to $C_{38}H_{64}N_8O_{13}$ as a possible molecular formula. Inspection of the literature did not disclose any compound identical to PD 124,966; however, cyclic peptides such as empedopeptin[5], SF-2068[6], and the SF-1902 (globomycin) components[7] appear to be closely related. PD 124,966 is an acidic compound which upon hydrolysis yields ninhydrin-positive products and a lipophilic acid. Further structural information will be reported separately.

PD 124,895 and PD 124,966 are highly cytotoxic. The IC_{50} values for PD 124,895 against L1210 and human colon adenocarcinoma (HCT-8) cells are 0.0011 μg/ml and 0.0010 μg/ml, respectively. Against the same cell lines, the IC_{50} values for PD 124,966 are 0.0034 μg/ml and 0.0087 μg/ml, respectively. PD 124,966, administered ip, was toxic to mice at 500 μg/kg and was inactive *in vivo* against P388 lymphocytic leukemia at the highest non-toxic dose. The *in vivo* antitumor activity of PD 124,895 against P388 lymphocytic leukemia is shown in Table 2.

Acknowledgments

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