PD 113,618 AND PD 118,309, NEW PACTAMYCIN ANALOGS

T. R. HURLEY, T. A. SMITKA, J. H. WILTON, R. H. BUNGE,
G. C. HOKANSON and J. C. FRENCH

Warner-Lambert/Parke-Davis Pharmaceutical Research
Ann Arbor, Michigan 48105, U.S.A.

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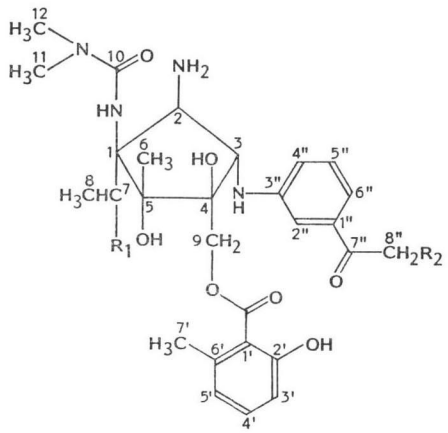
Two antibiotics were isolated from culture broths of a *Streptomyces* sp. and identified as 8″-hydroxypactamycin and 7-deoxypactamycin. The latter antibiotic was shown to be identical to cranomycin. An additional compound, 8″-hydroxypactamycate, was also isolated.

In the course of screening for new antitumor agents, a *Streptomyces* sp. (WP-4371) was found that produces a novel antibiotic with *in vitro* and *in vivo* antitumor activity. Fractionation of fermentation beers obtained from this organism yielded a new compound, PD 118,309, with a UV absorption spectrum identical to that of pactamycin (**1**)[1~3]. Examination of the ^{1}H and ^{13}C NMR spectra of these two compounds showed that PD 118,309 is 8″-hydroxypactamycin (**2**)†. The IC_{50} of PD 118,309 vs. leukemia L1210 cells is 0.014 μg/ml; the *in vivo* activity against lymphocytic leukemia P388 is presented in Table 1. During an earlier fractionation a related antibiotic, designated PD 113,618, was isolated in addition to PD 118,309. The NMR spectral data for PD 113,618 revealed that it is 7-deoxypactamycin (**3**). A comparison of the spectral properties of PD 113,618 and a sample of cranomycin[4,5], an antibiotic reported

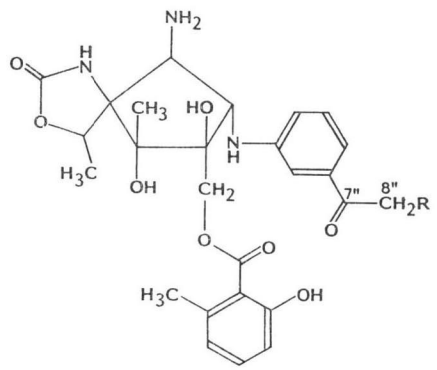
Table 1. *In vivo* activity of PD 118,309 vs. lymphocytic leukemia P388[a].

Dosage[b]	T/C (%)	
	Test-1	Test-2
1.0	175	Toxic
0.5	148	161
0.25	141	149

[a] P388 cells inoculated ip in mice on day 0.
[b] mg/kg/injection; single doses given ip on days 1~5.



Pactamycin (**1**) R_1=OH, R_2=H
8″-Hydroxypactamycin (**2**) R_1=R_2=OH
7-Deoxypactamycin (**3**) R_1=R_2=H



Pactamycate (**4**) R=H
8″-Hydroxypactamycate (**5**) R=OH

† The numbering system adopted in ref 3 is used here.

in 1964, showed that the compounds are identical†. Thus, for the first time, the structure of cranomycin can be reported to be 7-deoxypactamycin (**3**).

During the structural characterization of pactamycin, WILEY *et al.*[2] isolated another compound called pactamycate (**4**) obtained by treating pactamycin with acid. Later reports[3,6] indicate that pactamycate can be isolated directly from fermentation broths. Similarily, in the course of our fractionation procedures leading to the isolation of **2** and **3**, an additional component was detected by HPLC. The structure of this latter compound was shown to be the novel 8″-hydroxypactamycate (**5**) on the basis of its spectral properties and its identity with the product obtained by the treatment of 8″-hydroxypactamycin (**2**) with acid.

Experimental

Isolation of PD 118,309 (**2**)

Fermentation beer (550 liters)†† was adjusted to pH 3.0 with H_2SO_4, mixed with Celite 545 (23 kg) and filtered. The filtrate was adjusted to pH 8.5 with NaOH and extracted three times with EtOAc. The extracts were combined (520 liters), washed with 55 liters of H_2O and concd *in vacuo* to 2 liters. The concentrate was diluted with 10 liters of petroleum ether and then extracted three times with 0.05 M sodium citrate buffer (pH 3.1). The aqueous extracts were combined, adjusted to pH 8.5 and extracted with 2.3 liters of CH_2Cl_2, followed by a second extraction using 0.8 liter of CH_2Cl_2. The organic extracts were combined, concd *in vacuo* to 700 ml, diluted to 5 liters with petroleum ether, and extracted once with 1.4 liters of 0.05 M citrate buffer (pH 3.1). The upper organic layer was diluted with 3 liters of petroleum ether and extracted 5 times with 1.4-liter portions of pH 3.1 citrate buffer. The aqueous extracts were combined (8.4 liters), adjusted to pH 6.5 and chromatographed over 1.9 kg of C-18 silica gel (40 μm particle size, previously equilibrated with MeOH, MeOH - H_2O (1 : 1), and finally with 0.05 M NH_4OAc buffer, pH 7.5) packed in a 7 cm (i.d.) × 85 cm stainless steel column using 0.1 M NH_4OAc buffer (pH 7.5) - MeOH (53 : 47) as the mobile phase. Each fraction was analyzed by HPLC using a 0.4 × 25 cm C-18 silica gel column (5 μm particle size; Sepralyte, Analytichem International), UV detection at 240 nm, and 0.015 M NH_4OAc buffer (pH 5.5) - MeOH - MeCN (25 : 60 : 15) as the mobile phase. Under these conditions, at a flow rate of 1.0 ml/minute, the retention times of 8″-hydroxypactamycate, PD 118,309, pactamycin, and PD 113,618 are approximately 3.2, 4.6, 6.2 and 8.1 minutes, respectively. The fractions containing PD 118,309 were combined and concd *in vacuo* to remove MeOH. The aqueous concentrate (550 ml) was adjusted to pH 5.1 with HOAc and rechromatographed over 760 g of C-18 silica gel (pre-equilibrated with 0.05 M NaOAc buffer, pH 5.1) using 0.05 M NaOAc buffer (pH 5.1) - MeOH (55 : 45) as the mobile phase. Fractions shown by HPLC to contain 8″-hydroxypactamycin as the only UV absorbing component were combined and concd *in vacuo*. The resulting concentrate (590 ml) was adjusted to pH 8.0 with NaOH and extracted with $CHCl_3$ (170 ml). The $CHCl_3$ layer was washed with H_2O (3 × 30 ml), diluted with hexane (600 ml), and extracted four times with dilute HCl (pH 3.1). The aqueous extracts were combined (150 ml), adjusted to pH 5.5 with Amberlite IR-45(OH^-), and lyophilized to yield 365 mg of PD 118,309 hydrochloride as a pale yellow solid. FAB-MS *m/z* 575 (M+1); UV λ_{max}^{MeOH} nm (*a*) 240 (48.9), 316 (4.6); IR ν_{max} (KBr) cm^{-1} 3380, 2930, 1686, 1640, 1607, 1590, 1530; ^1H NMR (200 MHz, $CDCl_3$) δ 1.03 (1H, d, J=6.4 Hz, 8-H), 1.54 (3H, s, 6-H), 2.36 (3H, s, 7′-H), 2.95 (1H, s, 4- or 5-OH), 2.98 (6H, s, 11- and 12-H), 3.8 (1H, d, J=10.5 Hz, 2-H), 3.9 (1H, m, 7-H), 4.7~4.9 (4H, s and ABq, 9- and 8″-H), 5.73 (1H, d, J=10.5 Hz, 3-H), 6.61 (1H, dd, J=1.3, 8.8 Hz, 3′-H), 6.78 (1H, dd, J=1.2, 8.8 Hz, 5′-H), 6.83 (1H, ddd, J=1.1, 1.3, 7.2 Hz, 4″-H), 7.1~7.3 (4H, m, 4′, 2″, 5″ and 6″-H), 7.93 (1H, d, J=11.3 Hz, 3-NH or 7-OH). Additional NMR data are listed in Tables 2 and 3.

Anal Calcd for $C_{28}H_{38}N_4O_9 \cdot 1.08HCl \cdot 0.99H_2O$: C 53.23, H 6.55, N 8.87, Cl 6.06.
Found: C 53.44, H 6.41, N 8.73, Cl 6.40.

† We thank Dr. SHINICHI KONDO for supplying us with a sample of cranomycin for this comparison.

†† A description of the producing organism and the fermentation method will be reported separately.

Table 2. ^{13}C NMR spectral data of pactamycin analogs*.

Position	Pactamycin (1)	PD 118,309 (2)	PD 113,618 (3)
1	71.5	71.5	66.4
2	63.3	63.4	63.7
3	68.9	68.7	68.6
4	84.7	84.8	84.9
5	88.8	88.8	82.1
6	21.1	21.1	21.4
7	74.2	74.2	24.6
8	18.1	18.0	8.5
9	65.3	65.3[a]	65.5
10	159.2	159.2	158.4
11, 12	36.8	36.8	36.5
1′	112.4	112.1	113.0
2′	162.4	162.6	161.4
3′	115.5	115.7	115.5
4′	134.2	134.4[b]	134.0
5′	123.0	123.0	122.8
6′	141.2	141.1	141.1
7′	23.8	23.9	23.3
8′	172.2	172.3	170.7
1″	138.2	134.5[b]	137.8
2″	110.9	110.2	111.8
3″	146.7	146.9	147.7
4″	118.8	119.7[c]	118.1
5″	129.6	130.0	129.3
6″	118.3	117.0[c]	117.8
7″	198.7	198.7	198.5
8″	26.7	65.4[a]	26.5

* Spectra were recorded at 75.4 MHz using $CDCl_3$ as a solvent. Signals marked with the same letter may be interchanged. Data for pactamycin were taken from ref 3. A DEPT-GL experiment[7] was used to confirm the required multiplicities of the carbon atoms.

Isolation of PD 113,618 (3)

During an earlier fractionation, the EtOAc extract of filtered beer (at pH 8.5) was concd and chromatographed over silica gel using $CHCl_3$ containing 1~15% MeOH as eluents. In addition to early fractions (2~5% MeOH) containing PD 118,309, later fractions (5~10% MeOH) were collected that contained another component with UV and NMR properties similar to those of PD 118,309. The product from these latter fractions was purified by reverse phase silica gel chromatography as described above to yield PD 113,618 hydrochloride. FAB-MS *m/z* 543 (M+1); UV λ_{max}^{MeOH} nm (*a*) 240 (48.8), 314 (4.4); IR ν_{max} (KBr) cm^{-1} 3400, 1675, 1605, 1590, 1530.

Anal Calcd for $C_{28}H_{38}N_4O_7 \cdot HCl \cdot 2H_2O$: C 54.67, H 7.05, N 9.11, Cl 5.76.
Found: C 54.87, H 6.75, N 9.29, Cl 6.01.

The TLC, HPLC and IR spectral properties of PD 113,618 and an authentic sample of cranomycin[4,5] are identical. NMR data for PD 113,618 are listed in Tables 2 and 3.

8″-Hydroxypactamycate (5)

The presence of another component was detected when the purification of PD 113,618 and PD 118,309 over silica gel was monitored by HPLC. Fractions rich in this relatively polar component were combined and rechromatographed over silica gel to yield a compound which proved to be 8″-hydroxypactamycate (5), as shown by its ^1H NMR and IR spectral properties and its identity with the product derived from PD 118,309 as described below. IR ν_{max} (KBr) cm^{-1} 3400, 1740, 1685, 1610, 1590, 1530. ^1H NMR data are given in Table 3.

Table 3. ^{1}H NMR spectral data of pactamycin analogs*.

Position	PD 118,309 (2)	PD 113,618 (3)	8″-Hydroxy-pactamycate (5)
2	4.1 m	3.49 d (J=9.1 Hz)	3.79 d (J=6.3 Hz)
3	4.1 m	4.09 dd (J=9, 9 Hz)	3.99 dd (J=8.9, 6.3 Hz)
6	1.5 s (3H)	1.40 s (3H)	1.20 s (3H)
7a	5.0 m	1.90 m	4.78 q (J=6.3 Hz)
7b		2.48 m	
8	1.3 d (3H, J=6.2 Hz)	0.83 t (3H)	1.4 d (3H, J=6.3 Hz)
9a	4.1 d (J=12 Hz)	4.13 d (J=12 Hz)	4.24 d (J=11.7 Hz)
9b	4.6 d (J=12 Hz)	4.56 d (J=12 Hz)	4.45 d (J=11.7 Hz)
11, 12	2.99 s (6H)	2.86 s (6H)	
3′	6.7 d	6.68 d (J=8.3 Hz)	6.7 d
4′	[a]	[a]	[a]
5′	6.6 d	6.6 d (J=7.5 Hz)	6.6 d
7′	2.14 s (3H)	2.08 s (3H)	2.2 s (3H)
2″	7.28 s	7.26 s	7.3 s
4″	[a]	[a]	[a]
5″	[a]	[a]	[a]
6″	[a]	[a]	[a]
8″	4.7 d (2H, J=5.4 Hz)	2.42 s (3H)	4.65 d (2H, J=5.7 Hz)
3-NH	6.2	6.25	8.9 (3 or 10-NH)

* Recorded at 200 MHz in DMSO-d_6; chemical shifts are given as ppm downfield from TMS; some assignments were made on the basis of single frequency decoupling experiments. Unless otherwise noted, signals correspond to single protons.

[a] Overlapping signals appearing between 6.9~7.2 ppm.

A solution of 30 mg of PD 118,309 in 10 ml of 2.5 N HCl was heated for 1.5 hours on a steam bath. HPLC analysis of the reaction mixture at this point showed that PD 118,309 was no longer present and that 8″-hydroxypactamycate was now the major UV absorbing component (95%). The solution was adjusted to pH 9.7 with 5% Na_2CO_3 and extracted twice with 30 ml portions of EtOAc. The EtOAc extracts were combined, washed with H_2O, and evaporated to dryness *in vacuo* to yield 13 mg of 8″-hydroxypactamycate (FAB-MS m/z 530 (M+1)), possessing the same spectral properties as the product (**5**) obtained from fermentation beers.

Acetylation of PD 118,309

A solution of PD 118,309 (9 mg), pyridine (1 ml), and acetic anhydride (1.5 ml) was allowed to stand overnight at room temp. MeOH (5 ml) was then added and the mixture, after concentration, was chromatographed on a silica gel preparative TLC plate (0.5 mm thickness, E. Merck) using $CHCl_3$-MeOH (95: 5). Elution of the major UV absorbing band afforded 9 mg of a white solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (3H, d, J=6.8 Hz, 8-H), 1.48 (3H, s, 6-H), 2.09 (3H, s, 7′-H), 1.92, 2.20, 2.23, 2.24 (12H, s, s, s, s, 2-NCOCH_3, 7, 2′, 8″-COCH_3), 2.95 (6H, s, 11- and 12-H), 3.58 (1H, s, exchangeable), 3.63 (1H, dd, J=3.7, 10.3 Hz, 3-H), 3.92 (1H, s, exchangeable), 4.22 (1H, d, J=12 Hz, 9a-H), 4.92 (1H, d, J=12 Hz, 9b-H), 4.93 (1H, dd, J=7.1, 10.2 Hz, 2-H), 5.21 (2H, ABq, J=16.5 Hz, 8″-H), 5.44 (1H, d, exchangeable, J=3.6 Hz, 3-NH), 5.65 (1H, s, exchangeable), 6.32 (1H, q, J=6.8 Hz, 7-H), 6.9~7.24 (7H, m, 3′, 4′, 5′, 2″, 4″, 5″, 6″-H), 9.10 (1H, d, J=7 Hz, 2-N*H*Ac).

Acetylation of PD 113,618

PD 113,618 (7 mg) was dissolved in 1 ml of anhydrous pyridine and 0.5 ml acetic anhydride and stirred for 16 hours at room temp. MeOH (10 ml) was added and the solution was evaporated to dryness *in vacuo*. The residue was chromatographed on a preparative TLC plate (0.5 mm thickness, E. Merck) using $CHCl_3$ - MeOH (96: 4). Elution of the major UV absorbing band afforded 5 mg of a white solid. FAB-MS m/z 627 (M+H); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.91 (3H, t, J=7.5 Hz, 8-H), 1.40 (3H, s, 6-H), 1.95 (3H, s, 7′-H), 2.0, 2.25 (2H, m, 7-H), 2.26, 2.31 (6H, s, s, 2′-COCH_3, 2-

$NCOCH_3$), 2.51 (3H, s, 8″-H), 2.80 (1H, s, exchangeable), 2.94 (6H, s, 11- and 12-H), 3.67 (1H, dd, J=2.7, 10.5 Hz, 3-H), 3.68 (1H, s, exchangeable), 4.26, 4.96 (2H, AB, J=12.3 Hz, 9-H), 4.61 (1H, dd, J=6.1, 10.5 Hz, 2-H), 5.59 (1H, s, exchangeable), 5.84 (1H, d, exchangeable, J=2.7 Hz, 3-NH), 6.7~7.4 (7H, m, 3′, 4′, 5′, 2″, 4″, 5″, 6″-H), 9.46 (1H, d, J=6.1 Hz, 2-NH).

Structure Determination

8″-Hydroxypactamycin (PD 118,309) (2)

The chemical and spectral properties of PD 118,309 clearly indicate its close relationship to pactamycin (**1**). The microanalysis of PD 118,309 and the (M+1) ion observed at m/z 575 in the mass spectrum support a formula of $C_{28}H_{38}N_4O_9$, differing from pactamycin by an additional oxygen atom. The only significant difference in the ^{13}C NMR spectra of PD 118,309 (Table 2) and pactamycin[3] is the absence of an 8″-methyl signal at 26.7 ppm and the presence of a new signal at 65.4 ppm, corresponding to a CH_2O group, in the spectrum of PD 118,309. Additionally, the 8″-methyl signal at 2.10 ppm in the ^1H NMR spectrum of pactamycin[3] is replaced by an AB quartet at 4.8 ppm in the spectrum of PD 118,309 using $CDCl_3$ as solvent (see Experimental) or by a two-proton doublet at 4.7 ppm in DMSO-d_6, collapsing to a singlet upon addition of D_2O (Table 3). Signals for the 8″-protons appear as an AB quartet at 5.2 ppm in the acetylated derivative, further indicating that the 8″-methyl group of pactamycin has been hydroxylated in PD 118,309 (**2**).

7-Deoxypactamycin (PD 113,618) (3)

The mass spectrum (M+1; 543) and microanalysis of PD 113,618 are consistent with the molecular formula of $C_{28}H_{38}N_4O_7$, differing from pactamycin by one less oxygen atom. The 8-methyl signal in the ^1H NMR spectrum of PD 113,618 appears as a triplet coupled to protons at 1.9 and 2.48 ppm (instead of as a doublet in the spectrum of pactamycin), indicating that PD 113,618 is 7-deoxypactamycin (**3**). Additionally, in the ^{13}C NMR spectrum of PD 113,618, the 8-CH_3 signal appears at 8.5 ppm compared to 18.1 ppm in pactamycin. The signal for the 7-CH_2 of PD 113,618 most probably appears at 24.6 ppm, consistent with the presence of an alkyl methylene group. As expected from the proposed structure **3**, acetylation of PD 113,618 yields an *N,O*-diacetyl derivative whose FAB-MS exhibits an (M+1) ion at 627.

8″-Hydroxypactamycate (5)

The ^1H NMR and IR spectral properties of this compound were very similar to those of PD 118,309 except for the absence of a signal for the protons of a dimethylamino function and the presence of a new carbonyl absorption in the IR at 1740 cm^{-1}. These data are consistent with the loss of dimethylamine and the incorporation of the C-10 carbonyl group into a 2-oxazolidone ring to form a product which bears the same relationship to 8″-hydroxypactamycin as that which exists between pactamycate (**4**) and pactamycin (**1**). Treatment of PD 118,309 in the same manner reported for the conversion of pactamycin to pactamycate[2] afforded a product identical to the metabolite isolated from fermentation broths, proving that the latter compound, possibly an artifact arising during fractionation, is 8″-hydroxypactamycate (**5**).

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