PD 118,576: A NEW ANTITUMOR MACROLIDE ANTIBIOTIC

JOHN H. WILTON, GERARD C. HOKANSON and JAMES C. FRENCH

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

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The isolation and properties of PD 118,576, a new cytotoxic antibiotic obtained from the culture broth of a *Streptomyces* sp., are described. The structure of this compound was established by spectral analyses of the parent compound and its tri-*O*-acetyl derivative. PD 118,576 proved to be related to the bafilomycins and therefore is a new member of this recently discovered family of macrolide antibiotics.

During the course of our antitumor screening program a complex of antitumor macrolide antibiotics was isolated from the fermentation broth of an unidentified *Streptomyces* (WP 3913). The major component of this complex, PD 118,576, was found to exhibit *in vitro* activity against L1210 lymphocytic leukemia and HCT-8 human colon adenocarcinoma cell lines. The characteristics of the microorganism and the fermentation conditions used to produce PD 118,576 will be reported separately. This paper describes the isolation, properties, and structure determination of PD 118,576 which was found to be a novel 16-membered macrolide closely related to the bafilomycins[1,2] and the L-681,110 complex of antibiotics[3].

Experimental

HPLC Analysis of PD 118,576

All HPLC assays for PD 118,576 were performed using a μBondapak C-18 column (3.9 mm × 30 cm, Waters Associates), with MeOH - 0.05 M NH_4OAc buffer (pH 6.5) (70: 30) as the mobile phase at a flow rate of 1.5 ml/minute, and detection by UV absorption at 254 nm. The retention time of PD 118,576 in this system is approximately 5.0 minutes. The concentration of PD 118,576 in fermentation broths was assayed by extracting 25 ml of unfiltered beer at pH 6.5 with two 25 ml portions of EtOAc. The combined extracts were evaporated to dryness and the residue was dissolved in 10 ml of EtOH to afford a solution which was used for HPLC analysis after filtration through a 0.45 μm filter. At 93 hours of fermentation, the concentration of PD 118,576 in broths was approximately 18 μg/ml.

Isolation

Fermentation broth (57 liters, pH 6.3) was stirred with 30 liters of EtOAc for 30 minutes. Celite 545 (3.2 kg) was added and the mixture was filtered. The lower aqueous layer was separated and extracted with a 30-liter EtOAc wash of the filter cake. The organic layers were combined, washed with H_2O, and concentrated *in vacuo* to 300 ml. This concentrate was added, with stirring, to 4 liters of heptane - MeOH - H_2O (10: 9: 1). The resulting mixture was filtered and the aqueous MeOH layer was evaporated to dryness *in vacuo* to yield 17.5 g of residue. A $CHCl_3$ solution (75 ml) of the crude concentrate was chromatographed over 680 g of Silica gel 60 (E. Merck, 40~63 μm) using increasing amounts of MeOH in $CHCl_3$. All of the PD 118,576 was eluted in the 96: 4 ($CHCl_3$ - MeOH) fractions as determined by HPLC and TLC (E. Merck Silica gel 60 plates: Rf 0.30, using $CHCl_3$ - MeOH (96: 4); Rf 0.31, using EtOAc - heptane (7: 3)). Concentration of these fractions *in vacuo* afforded 1.45 g of an oily residue. This material was further purified by chromatography over 120 g of Silica gel 60 (E. Merck, 20~40 μm) using EtOAc - hexane (6: 4) as the eluent. The fractions con-

Table 1. NMR assignments for PD 118,576 in $CDCl_3$*.

Position	^1H NMR	^{13}C NMR
1		166.5
2		141.3[a]
2-OCH_3	3.67, s	60.1
3	6.63, s	133.1[b]
4		142.9[a]
4-CH_3	1.98, d (1.1)	14.0
5	5.75, d (9.0)	142.4
6	2.52, ddq (1.2, 7.0, 9.0)	36.8
6-CH_3	1.07, d (7.0)	17.4[c]
7	3.29, br m**	81.2
8	~1.88, m (obsc.)	39.8[d]
8-CH_3	0.94, d (6.3)	10.7
9a	1.95, m (obsc.)	41.3[d]
9b	2.11, br d	
10		132.9
10-CH_3	1.91, s	20.0
11	5.79, d (10.6)	125.3
12	6.47, dd (10.6, 15.0)	132.8[b]
13	5.15, dd (9.0, 15.0)	127.0
14	3.80, dd (8.3, 9.0)	83.3
14-OCH_3	3.21, s	55.7
15	5.03, dd (1.3, 8.3)	76.3
16	2.05, m (obsc.)	38.5
16-CH_3	0.95, d (6.9)	21.8
17	3.73, m (obsc.)	72.7
18	2.95, dq (4.2, 7.0)	46.5
18-CH_3	1.21, d (7.0)	10.3
19		203.1
20	6.26, d (1.3, 15.8)	129.1
21	6.82, dd (8.1, 15.8)	148.4
22	2.39, dq (1.3, 6.7)	44.0
22-CH_3	1.08, d (6.8)	14.6[c]
23	3.75, m (obsc.)	70.7
24	1.15, d (6.4)	20.4
OH	3.61, d (5.8)	
	1.57, br d (6.4)	

* NMR spectra were recorded at 300 MHz for ^1H and 75 MHz for ^{13}C. Chemical shifts are given in ppm downfield from TMS. J values, in Hz, are given in parentheses. Indicated assignments ([a, b, c, d]) are interchangeable. obsc.=obscured (overlapping signals).

** Doublet observed after addition of D_2O.

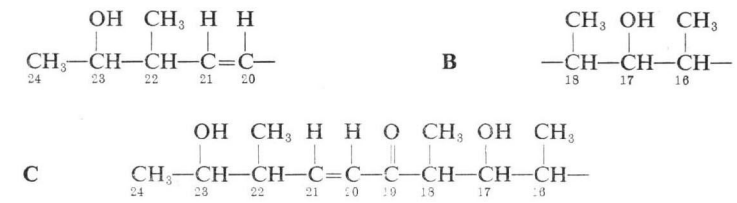
taining PD 118,576 as determined by TLC were combined and concentrated *in vacuo* to afford 736 mg of PD 118,576 as a viscous oil. This product was 98.5% pure by HPLC and gave a single spot in the two TLC systems described above: UV λ_{max}^{MeOH} nm (ε) 244 (28,230) and 284 (10,500); IR ($CHCl_3$) 3620, 3480, 1705 (sh), 1691, 1648, 1625, 1109 and 1095 cm^{-1}; $[\alpha]_D$ −6.5° (*c* 0.17, MeOH); EI-MS *m/z* 576 (M^+), 544, 420, 391, 351, 334, 250, 225, 191, 183, 181, 169, 151, 137, 125, 124, 112, 109, 93 and 83; ^1H and ^{13}C NMR data are listed in Table 1.

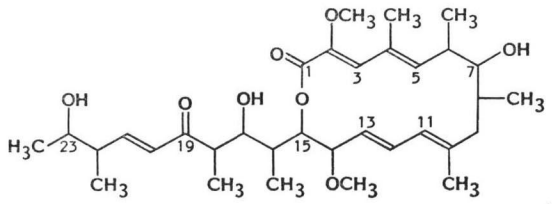
Acetylation of PD 118,576

A solution of PD 118,576 (10 mg) in 1.0 ml of pyridine - Ac_2O (1: 1) was allowed to stand at room temp for 21 hours. Volatile components were removed with a stream of N_2 and the residue was chromatographed on a 20×20 cm preparative TLC plate (E. Merck, Silica gel 60, 0.5 mm) using EtOAc - hexane (6: 4) as the mobile phase. The major TLC band, visualized under UV light, was removed and eluted with EtOAc to yield a tri-*O*-acetyl derivative (6 mg) which was homogeneous in two TLC systems: UV λ_{max}^{MeOH} nm (ε) 242 (36,800) and 281 (13,500); IR ($CHCl_3$) 2965, 2933, 1730, 1708 (sh), 1628, 1457, 1375, 1101, 1022, 975 and 912 cm^{-1}; $[\alpha]_D$ −15.1° (*c* 0.205, MeOH); EI-MS *m/z* 702 (M^+), 642, 582, 376, 347, 332, 316, 241, 217, 191, 151 and 109; ^1H NMR (200 MHz, $CDCl_3$) 2.01, 2.02, 2.15 (3×3H, OAc), 4.74 (1H, dd, J=2.4, 6.1, H-7), 4.87 (1H, dq, J=6.4, 6.4, H-23) and 5.19 (1H, obscured m, H-17).

Structure Determination

A molecular ion is observed in the electron impact mass spectrum of PD 118,576 at *m/z* 576, corresponding to a molecular formula of $C_{33}H_{52}O_8$. UV absorption maxima in methanol appear at 244 and 284 nm which indicate that PD 118,576 may be assigned to the class of macrolide antibiotics possessing an isolated conjugated diene and $\alpha,\beta,\gamma,\delta$-unsaturated lactone functionalities. This class includes both 18-membered lactones (concanamycins[4~6] and virustomycin[7]) and 16-membered lactones (bafilomycins[1,2], hygrolidin[8,9], leucanicidin[10,11], L-115,175[12] and the L-681,110 complex[3]). Comparison of the ^1H NMR spectrum of PD 118,576 (Table 1) with the corresponding signals reported for bafilomycin A_1 (**2**), L-681,110 A_1 (**3**) and related compounds revealed that PD 118,576 possesses the same 16-membered lactone moiety. This was further confirmed by analysis of fragmentation patterns in the mass





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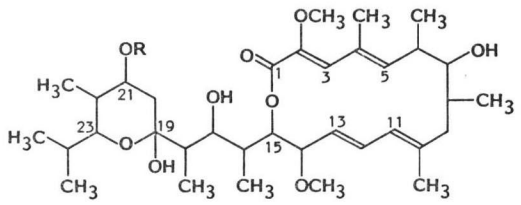

2 R = H

3 R = COCH=CHCOOH

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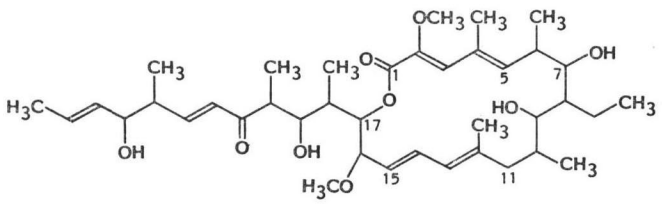

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spectrum of PD 118,576 in which the same fragment ions assigned to the macrolide portion of L-681,110[3] are also observed.

The structure of the remaining portion of PD 118,576 was readily discerned by analysis of the ^{1}H and ^{13}C NMR signals that distinguish this compound from the known antibiotics of the same class. The results of homonuclear and heteronuclear correlation NMR experiments suggested the presence of partial structures **A** and **B** which could be expanded to fragment **C** by the following arguments. A downfield signal at 203 ppm in the ^{13}C NMR spectrum could be assigned to an α,β-unsaturated ketone functionality, which was further confirmed by a shoulder in the IR spectrum at 1705 cm^{-1} and a positive reaction with 2,4-dinitrophenylhydrazine. The presence of an α,β-unsaturated aldehyde was ruled out by the absence of a downfield signal in the ^{1}H NMR spectrum. Attachment of fragments **A** and **B** to the ketone carbonyl as shown is justified on the basis of coupling constants and chemical shift data. The olefinic proton signals at 6.26 and 6.82 ppm (fragment **A**) are consistent with the α- and β-protons of the α,β-unsaturated ketone moiety, while the signal at 2.95 ppm (fragment **B**) must be assigned to the α'-proton. The proton connectivity from H-18 (H-α') to H-15 of the macrolide

is clearly revealed by the homonuclear correlation data, thereby allowing the attachment of fragment **C** to the macrolide ring. The placement of hydroxyl groups at C-17 and C-23 was verified by the sharpening of the signals at 3.73 and 3.75 ppm upon addition of D_2O, and their downfield shifts to 5.19 and 4.87 ppm, respectively, upon acetylation. Homonuclear spin decoupling experiments with the acetylated derivative confirm the indicated proton assignments, completing the assignment of structure **1** to PD 118,576. Additional verification of the side-chain structure is obtained by comparison of the ^1H NMR chemical shift and coupling data reported for similar protons in the alkaline degradation product (**4**) of concanamycin A[4].

Discussion

PD 118,576 represents the first example of a bafilomycin-type macrolide lacking a hemi-ketal ring in the side chain. The presence of a *trans* double-bond precludes the formation of the usual tetrahydropyran moiety. The possibility that PD 118,576 is an artifact produced during the isolation procedure can be excluded since the compound is readily detected in fermentation broths by HPLC analysis. PD 118,576 is only weakly active against most bacteria and fungi. However, the IC_{50} values for PD 118,576 against L1210 lymphocytic leukemia and HCT-8 human colon adenocarcinoma cell lines are 1.4×10^{-6} M and 1.1×10^{-6} M, respectively.

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