DEMETHYLATION OF ANSAMITOCINS AND RELATED COMPOUNDS

MOTOWO IZAWA, KAZUO NAKAHAMA, FUMIKO KASAHARA,
MITSUKO ASAI and TOYOKAZU KISHI

Central Research Division, Takeda Chemical Industries, Ltd.,
Yodogawa-ku, Osaka 532, Japan

(Received for publication June 22, 1981)

Ansamitocins were converted into 20-*O*-demethyl derivatives by bacteria. The structures of these products were elucidated from their physicochemical properties and from the chemical conversion to ansamitocins with diazomethane.

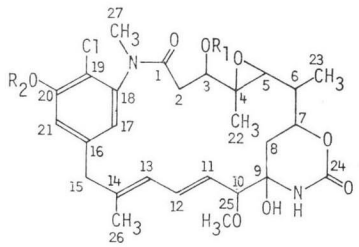
Ansamitocins (ASMs) are maytansinoid antitumor antibiotics isolated from the culture broth of *Nocardia* sp. No. C-15003 (N-1)[1~4] and a mutant strain of *Nocardia* sp. No. C-14482[5,6]. In the course of studies on microbial modification of ASMs and related compounds, 20-*O*-demethyl derivatives were obtained with cultures of *Bacillus megaterium* IFO 12108[7].

20-*O*-Demethylansamitocin P-3 (PDM-3) has better antitumor activity against P388 and L1210 than does ansamitocin P-3 (i.p.-i.v., s.c.-i.v.)[8]. This paper deals with the isolation of 20-*O*-demethylansamitocins (PDMs) and their structural elucidation from spectroscopic and chemical evidence.

Isolation and Physicochemical Properties of Products

These conversion products were isolated by the general procedure for lipophilic weakly acidic substances. The reaction mixture obtained after microbial modification of ansamitocin P-3 (P-3) was extracted with ethyl acetate at pH 5.0 and transferred into 3% aqueous sodium carbonate at 5°C. The aqueous layer was reextracted with ethyl acetate at pH 5.0 and the extract was concentrated to give crude PDM-3. This was chromatographed on a silica gel column with a mixture of chloroform and methanol to give pure PDM-3.

Fig. 1. Structures of PDMs.



	R_1	R_2
P-3	$COCH(CH_3)_2$	CH_3
PDM-4	$COCH_2CH(CH_3)_2$	H
PDM-3	$COCH(CH_3)_2$	H
PDM-2	$COCH_2CH_3$	H
PDM-1	$COCH_3$	H
PDM-0	H	H

By similar procedures, PDM-4, PDM-2, PDM-1 and PDM-0 were isolated and purified from cultures given ansamitocin P-4, propionylmaytansinol (P-2), maytanacine (P-1) and maytansinol (P-0), respectively. The Rf values observed after TLC are shown in Table 1. The

Table 1. TLC Rf values of PDMs on silica gel.

	$CHCl_3$ - MeOH (9: 1)	EtOAc saturated with H_2O
P-3	0.52	0.42
PDM-4	0.44	0.47
PDM-3	0.42	0.40
PDM-2	0.40	0.36
PDM-1	0.38	0.29
PDM-0	0.30	0.22

Table 2. Characteristic mass fragment peaks of PDMs.

	M^+-a	$-(a+b)$	$-(a+b+CH_3)$	$-(a+Cl)$	$-(a+b+Cl)$
P-3	573	485	470		450
PDM-4	573	471	456		436
PDM-3	559	471	456		436
PDM-2	545	471	456		436
PDM-1	531	471	456		436
PDM-0	489	471	456	454	436

a=HNCO+H_2O, b=R_1OH

Table 3. ^1H NMR spectral data of PDMs.

	CH_3 22	CH_3 23	CH_3 26	N-CH_3 27	O-CH_3 25	O-CH_3 28	Others
P-3	0.84	1.28	1.71	3.18	3.38	4.00	1.27 (3H, d), 1.28 (3H, d)
PDM-4	0.86	1.27	1.70	3.17	3.39	—	1.03 (6H, d)
PDM-3	0.85	1.21	1.71	3.19	3.38	—	1.27 (3H, d), 1.29 (3H, d)
PDM-2	0.82	1.12	1.62	3.04	3.27	—	1.07 (3H, t)
PDM-1	0.83	1.12	1.62	3.06	3.27	—	2.14 (3H, s)
PDM-0	0.77	1.13	1.60	3.06	3.26	—	10.31 (1H, s)

δ In ppm downfield from internal TMS. P-3, PDM-4 and PDM-3 were measured in $CDCl_3$, PDM-2, PDM-1 and PDM-0 in DMSO-d_6.

UV, NMR and mass spectra of these conversion products are similar to those of the substrates. The ^1H NMR spectra and fragmention peaks of the PDMs are summarized in Tables 2 and 3, respectively.

Structural Elucidation of PDMs

In the mass spectrum of PDM-3, the characteristic fragmention of maytansinoids were observed at m/z 471 [$M^+-(a+b)$], 456 [$M^+-(a+b+CH_3)$] and 436 [$M^+-(a+b+Cl)$], as shown in Table 2. The values were each 14 mass units less than that of the substrate, P-3, indicating that PDM-3 lacked a methyl group present in P-3. Table 4 shows that a methyl carbon at C_{25} or C_{28} was absent from the ^{13}C NMR spectrum of PDM-3. Signals in the ^{13}C NMR spectra of P-3 and PDM-3 were assigned from the multiplicities observed in off-resonance spectra, by selective proton decoupling, and by comparing chemical shift values with those of model compounds.

The ^1H NMR spectrum of P-3 showed a signal for the C_{20} methoxy group at δ 4.00 which was absent from the spectrum of PDM-3 (Table 2). From these results it was concluded that PDM-3 differed from P-3 only in having an OH instead of an OCH_3 at C_{20}. The structure of PDM-3 was confirmed by its chemical conversion into P-3 by methylation with diazomethane. The monomethyl derivative so obtained was identified by its TLC Rf values, UV, IR, NMR and mass spectra. Except for ^{13}C NMR spectroscopy, these methods were used to determine the structures of PDM-4, PDM-2, PDM-1 and PDM-0.

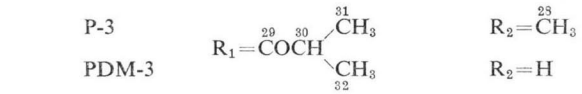
Experimental

Melting points were determined with a Mettler FP-5 instrument at 3°C/minute. UV spectra were measured with a Shimadzu UV-200 double beam spectrophotometer. IR spectra were recorded with a Hitachi 285 grating spectrophotometer. NMR spectra were obtained using Varian XL-100-12 and Varian EM-390 instruments; chemical shifts (δ) are reported in ppm downfield from TMS. Mass

Table 4. ^{13}C NMR spectral data of P-3 and PDM-3.

C-No	P-3	PDM-3	C-No	P-3	PDM-3
1	s 168.3	s 168.9	17	d 121.8	d 120.9
2	t 32.6	t 32.6	18	s 142.3*	s 141.4*
3	d 76.6	d 76.5	19	s 119.3	s 116.8
4	s 60.3	s 60.4	20	s 155.9	s 153.4
5	d 66.1	d 66.2	21	d 112.8	d 117.5
6	d 38.6	d 38.5	22	q 12.1	q 12.0
7	d 74.1	d 74.2	23	q 14.4	q 14.4
8	t 35.9	t 35.9	24	s 151.9	s 152.6
9	s 80.7	s 80.6	25	q 56.5	q 56.5
10	d 88.4	d 88.3	26	q 15.6	q 15.6
11	d 127.6	d 127.1	27	q 35.3	q 35.6
12	d 132.4	d 132.6	28	q 56.5	—
13	d 124.3	d 123.9	29	s 175.4	s 175.7
14	s 139.7*	s 140.0*	30	d 33.7	d 33.7
15	t 47.0	t 46.5	31, 32	q 17.8	q 17.8
16	s 139.9*	s 140.2*		q 19.8	q 19.8

δ In ppm downfield from internal TMS; $CDCl_3$.
Numbering of carbons is shown in Fig. 1 and below.



* Tentatively assigned.

spectra were determined with a JMS-OISC spectrometer equipped with a direct inlet system. For TLC, silica gel 60 F_{254} plates (E. Merck, A.G., Germany, 0.25 mm thick) were used.

Isolation of PDMs

B. megaterium IFO 12108 was grown for 18 hours at 28°C in a medium (pH 7.5) containing 2% dextrin, 0.5% peptone, 0.5% yeast extract and 0.5% meat extract on a rotary shaker. P-3 (2.2 g) was added to the culture broth (11 liters), and the mixture was incubated for 48 hours at 28°C with shaking. The reaction mixture was adjusted to pH 5.0 with dilute HCl and extracted twice with 5.5 liters each of EtOAc. The EtOAc layers were combined, washed with aqueous 0.005 N HCl and 0.5% $NaHCO_3$ and then stirred four times at 5°C with 1/3 volume of 3% aqueous Na_2CO_3. The aqueous layer was adjusted to pH 5.0 with 6 N HCl and PDM-3 was removed from it by two extractions with 1/3 volume of EtOAc. The EtOAc layers were combined, washed and concentrated, giving crude PDM-3. The crude material (2.05 g) was chromatographed on silica gel (50 g, E. Merck) successively with $CHCl_3$ (200 ml) and $CHCl_3$ - MeOH at 40: 1 (500 ml) and 20: 1 (200 ml). Fractions of the effluent (10 ml) were examined by TLC using as solvent system, $CHCl_3$ - MeOH (9: 1). Fractions showing absorbing zone under light of 2537 Å and having an Rf value of 0.42 were concentrated to dryness. When EtOAc (20 ml) was added to the residue and the solution was kept at 5°C, pure crystals of PDM-3 (1.5 g) were obtained.

Demethylation and purification of P-4, P-2, P-1 and P-0 were carried out in the same manner.

PDM-3: $C_{31}H_{41}ClN_2O_9$=621.14; m.p. 200~202°C (decomp.): $[\alpha]_D^{22}$ −120° (*c* 0.52, MeOH); UV (MeOH) 232 nm (δ 28900), 242 (29200), 251 (29200), 280 (6150), 288 (5970); IR (KBr) 1740, 1710, 1642, 1598 cm^{-1}.

PDM-4: $C_{32}H_{43}ClN_2O_9$=635.17; m.p. 192~194°C (decomp.); $[\alpha]_D^{22}$ −112° (*c* 0.51, MeOH); UV (MeOH) 232 nm (δ 30000), 242 (30400), 251 (30500), 280 (6360), 288 (6290); IR (KBr) 1738, 1715, 1662, 1585 cm^{-1}.

PDM-2: $C_{30}H_{39}ClN_2O_9$=607.12; m.p. 193~195°C (decomp.); $[\alpha]_D^{22}$ −112° (*c* 0.54, MeOH); UV

(MeOH) 232 nm (δ 30000), 242 (30900), 251 (31200), 280 (6380), 288 (6320); IR (KBr) 1737, 1715, 1642, 1594 cm^{-1}.

PDM-1: $C_{29}H_{37}ClN_2O_9$=593.09; m.p. 224~226°C (decomp.); $[\alpha]_D^{22}$ −98° (*c* 0.505, MeOH); UV (MeOH) 232 nm (δ 29700), 242 (30700), 251 (34000), 280 (6890), 288 (6840); IR (KBr) 1726, 1697, 1640, 1594 cm^{-1}.

PDM-0: $C_{27}H_{35}ClN_2O_8$=551.01; m.p. 194~196°C (decomp.); $[\alpha]_D^{22}$ −209° (*c* 0.5, MeOH); UV (MeOH) 232 nm (δ 31200), 242 (33400), 251 (34000), 280 (6890), 288 (6840); IR (KBr) 1726, 1697, 1640, 1587 cm^{-1}.

Chemical Conversion of PDM-3 into P-3

To a solution of PDM-3 (40 mg) in tetrahydrofuran (4 ml), 1 ml of fresh CH_2N_2 - ether solution was added. After 2 hours at room temperature the reaction mixture was concentrated to dryness and a small portion of EtOAc was added. After cooling at 5°C, the solution deposited crystals of P-3 (36 mg), which had the same Rf values on TLC with various solvent systems and the same UV, IR, NMR and mass spectra as an authentic sample.

Acknowledgements

We are grateful to Drs. E. OHMURA, M. YONEDA and E. HIGASHIDE for encouragement throughout this work. We are also indebted to Drs. T. HASEGAWA, M. KIDA, E. MIZUTA and the members of the physical analysis and biological assay groups. Thanks are also due to Mr. H. FUJIUCHI for his skillful assistance.

References

1) HIGASHIDE, E.; M. ASAI, K. OOTSU, S. TANIDA, Y. KOZAI, T. HASEGAWA, T. KISHI, Y. SUGINO & M. YONEDA: Ansamitocin, a group of novel maytansinoid antibiotics with antitumor properties from *Nocardia*. Nature 270: 721~722, 1977
2) ASAI, M.; E. MIZUTA, M. IZAWA, K. HAIBARA & T. KISHI: Isolation, chemical characterization and structure of ansamitocin, a new antitumor ansamycin antibiotic. Tetrahedron 35: 1079~1085, 1978
3) TANIDA, S.; T. HASEGAWA, K. HATANO, E. HIGASHIDE & M. YONEDA: Ansamitocins, maytansinoid antitumor antibiotics. Producing organism, fermentation and antimicrobial activities. J. Antibiotics 33: 192~198, 1980
4) IZAWA, M.; K. HAIBARA & M. ASAI: Analytical studies and preparative separation of ansamitocins and related antibiotics using high-performance liquid chromatography. Chem. Pharm. Bull. 28: 789~794, 1980
5) TANIDA, S.; M. IZAWA & T. HASEGAWA: Ansamitocin analogs from a mutant strain of *Nocardia*. I. Isolation of the mutant, fermentation and antimicrobial properties. J. Antibiotics 34: 489~495, 1981
6) IZAWA, M.; S. TANIDA & M. ASAI: Ansamitocin analogs from a mutant strain of *Nocardia*. II. Isolation and structure. J. Antibiotics 34: 496~506, 1981
7) NAKAHAMA, K.; M. IZAWA, M. ASAI, M. KIDA & T. KISHI: Microbial conversion of ansamitocin. J. Antibiotics 34: 1581~1586, 1981
8) KOZAI, Y. & K. OOTSU: Antitumor activities of C_{20}-demethyl derivatives of ansamitocins. Proceeding of the Japan Cancer Association; The 39th Annual Meeting, Tokyo, November, 1980