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ISOLATION, CHEMICAL CHARACTERIZATION AND STRUCTURE OF ANSAMITOCIN, A NEW ANTITUMOR ANSAMYCIN ANTIBIOTIC

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Abstract—In this paper we report the isolation, chemical characterization and structural elucidation of Ansamitocin, a new antitumor antibiotic obtained from *Nocardia* No. C-15003 (N-1). Ansamitocin P-3, P-3' and P-4 with molecular formulae $C_{32}H_{43}ClN_2O_9$, $C_{32}H_{43}ClN_2O_9$ and $C_{33}H_{45}ClN_2O_9$, respectively, were identified as novel antibiotics. Their UV spectra resemble that of maytansine obtained from a plant source. Analysis of the PMR spectrum and spin-decoup studies of P-3 demonstrated that its skeletal structure was the same as that of maytansine. Reductive cleavage of each antibiotic gave maytansinol (P-0). Alkali hydrolysis of P-3, P-3' and P-4 gave isobutyric, butyric and isovaleric acids, respectively. P-3, P-3' and P-4 were concluded to be the isobutyrate, butyrate and isovalerate ester of maytansinol at C-3, respectively. An antitumor plant product, maytanacine, and its semisynthetic derivative, maytansinol propionate, were also produced by the same strain.

Ansamitocin, group of novel antibiotics, are lipophilic neutral substances with antitumor activity, and obtained from the fermentation broth of *Nocardia* sp. No. C-15003 (N-1).[1] The microbial properites of the producing organism and production of the antibiotic have been described.[2,3] This paper deals with the isolation, physicochemical properties and structure elucidation of ansamitocin P-3, P-3' and P-4.

The isolation of antitumor maytansinoids from plants, *Maytenus serrata*,[4] *M. Buchananii*,[5] *Putterlikia verrucosa*[6] and Colubrina texemis,[7] have been published, but, the isolation of maytansinoids as microbial products has not been reported.

Isolation. In the course of screening for new antibiotics which are active against fungi and tumor cells, it was found that the fermentation broth of *Nocardia* contains such antibiotics.[1] They were tentatively designated as C-15003 P (group) antibiotics.

C-15003 P was isolated by the general procedure for lipophilic neutral substances, shown in Chart 1. The active ingredients were monitored by UV absorption method on tlc and detected as five components. They were designated as C-15003 P-1, P-2, P-3, P-3' and P-4 in the order of increasing R_f values, as shown in Fig. 1.

C-15003 P complex was extracted with ethyl acetate from the broth filtrate of *Nocardia* sp. No. C-15003 (N-1) or aqueous acetone extract of the mycelium. The extract was chromatographed on silica gel, or macropolar nonionic resin. The isolated each component was recrystallized from ethyl acetate and ether.

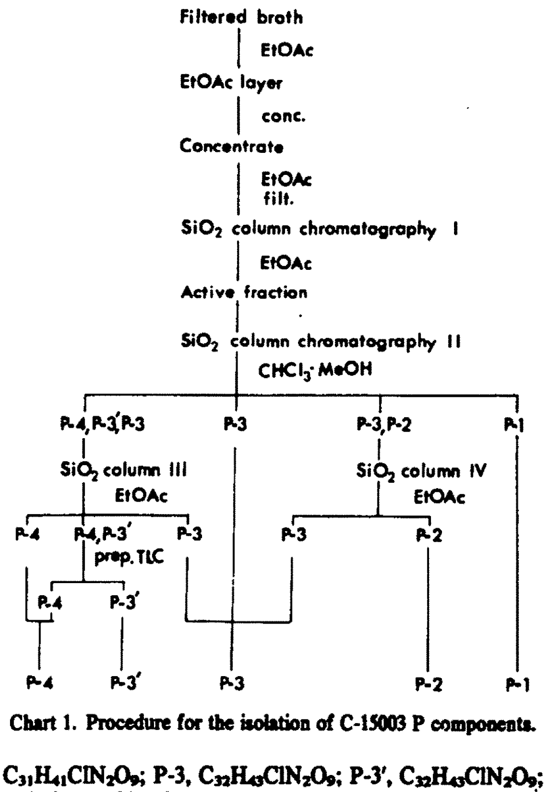


Chart 1. Procedure for the isolation of C-15003 P components.

Physicochemical properties. The physicochemical properties of each component were measured after drying at 60° under reduced pressure and are shown in Table 1. All five components of C-15003 P had very similar physicochemical properties. All were lipophilic, neutral colorless crystals. Their molecular formulas were as follows from their elemental analysis, mass spectra and ^{13}C NMR spectra; P-1, $C_{30}H_{39}ClN_2O_9$; P-2, $C_{31}H_{41}ClN_2O_9$; P-3, $C_{32}H_{43}ClN_2O_9$; P-3', $C_{32}H_{43}ClN_2O_9$; P-4, $C_{33}H_{45}ClN_2O_9$.

Biological properties. These antibiotics show strong inhibitory activity against fungi and protozoa but no activity against bacteria.[1,3] They have strong antitumor activity against leukemia P-388, melanoma B-16, sarcoma-180, Ehrlich carcinoma as reported in another paper.[1]

The acute toxicities (LD_0) of the C-15003 P mixture in mice were 0.3 mg/kg by intraperitoneal administration and 1.5 mg/kg by oral administration.†

Comparison with known antibiotics. According to these physicochemical and biological properties no related known antibiotic has been found as a microbial

†We are grateful to Dr. Murata and his colleagues for their toxicological tests.

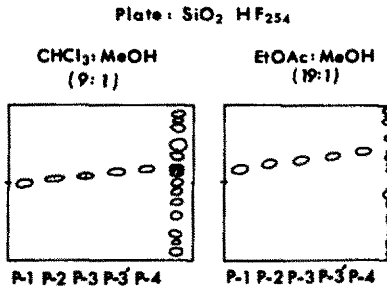


Fig. 1. Tlc of C-15003 P components.

metabolite. However, their UV spectra and physicochemical and biological properties are similar to those of maytansinoids which were obtained from plant by Kapchan *et al.*[4-6] and Wani *et al.*[7] Maytansinoids have a characteristic ansa-structure with an amino acid side chain and attracted attention because of their strong antitumor activity. These similarities suggest that C-15003 P-3, P-3' and P-4 have a maytansinoid structure.

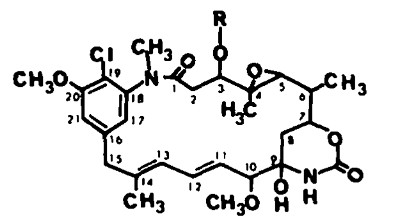
The highest mass numbers of C-15003 P-4, P-3, P-3', P-2 and P-1 were observed at *m/e* 587, 573, 573, 559, 545, respectively (Table 1). The common fragment ion peaks were found at 485, 470 and 450 for each component of C-15003 P as for maytansine and its analogues. The UV spectra of C-15003 P components resembled that of maytansine and the chromophores of both groups were identical. The mass spectra and physicochemical properties of C-15003 P-1 and P-2 were identical to those of maytanacine, isolated from *Putterlickia verrucosa* and maytansinol propionate, synthesized from maytansinol[6] (Table 2). Thus, it was concluded that antibiotic C-15003 P-1 and P-2 were identical to maytanacine and maytansinol propionate, respectively. These findings revealed that antibiotic C-15003 P-3, P-3' and P-4 were novel and they were named ansamitocin P-3, P-3' and P-3, respectively.[1]

Table 1. Physicochemical properties of C-15003 P.

	P-1	P-2	P-3	P-3'	P-4
m.p. (°C) (decomp.)	235–236	188–190	190–192	182–185	177–180
$[\alpha]_{D\,CHCl_3}$	−121° (c = 0.25)	−127° (c = 0.35)	−136° (c = 0.375)	−134° (c = 0.11)	−142° (c = 0.52)
Anal. (C H N Cl) obs.	59.62 6.93 4.28 5.74	59.93 6.82 4.32 5.57	60.06 7.04 4.33 5.37	60.06 7.04 4.33 5.37	60.65 7.05 4.25 5.23
Anal. (C H N Cl) calc.	59.35 6.48 4.61 5.84	59.94 6.65 4.51 5.91	60.51 6.82 4.41 5.58	60.51 6.82 4.41 5.58	61.05 6.99 4.32 5.46
MS m/e	545, 485, 470, 450	559, 485, 470, 450	573, 485, 470, 450	573, 485, 470, 450	587, 485, 470, 450
Mol. Form.	$C_{30}H_{39}ClN_2O_9$	$C_{31}H_{41}ClN_2O_9$	$C_{32}H_{43}ClN_2O_9$	$C_{32}H_{43}ClN_2O_9$	$C_{33}H_{45}ClN_2O_9$
UV spect λ_{max}^{MeOH} nm (ε)	233 (30330) 240sh (28240) 252 (27850) 280 (5680) 288 (5660)	233 (30240) 240sh (28400) 252 (27650) 280 (5740) 288 (5710)	233 (30250) 240sh (28450) 252 (27640) 280 (5750) 288 (5700)	233 (30155) 240sh (28250) 252 (27600) 280 (5750) 288 (5700)	233 (29900) 240sh (28240) 252 (27590) 280 (5712) 288 (5680)
IR spect. $\nu_{cm^{-1}}^{KBr}$	1740 1730 1670 1580	1740 1730 1670 1580	1740 1730 1670 1580	1740 1730 1670 1580	1740 1730 1670 1580
TLC Rf $CHCl_3$:MeOH 9 : 1	0.48	0.50	0.52	0.54	0.58
EtOAc:MeOH 19 : 1	0.58	0.62	0.63	0.65	0.68
EtOAc (H_2O sat.)	0.34	0.38	0.42	0.45	0.49
Stability	Crystal : stable Solution : stable at PH 2–9 unstable > PH 10				
Solubility	Easily soluble : methanol, ethanol, chloroform, DMSO, THF, acetone, ethyl acetate Sparingly soluble : benzene, ether Insoluble : petroleum ether, hexane, water				
Color reac.	Positive Beilstein reac., Dragendorff reac. Negative ninhydrin reac., Ehrlich reac.				

Table 2. Comparative data of P-1: maytanacine and P-2: maytansinol propionate.

	P-1	Maytanacine[6)]	P-2	Maytansinol propionate[8)]
Mol. Form.	$C_{30}H_{39}ClN_2O_9$	$C_{30}H_{39}ClN_2O_9$	$C_{31}H_{41}ClN_2O_9$	$C_{31}H_{41}ClN_2O_9$
m.p. (°c)	235—236	234—237	188—190	187.2—188.6
$[\alpha]_D$ $CHCl_3$	−121° (c=0.25)	−119° (c=0.1)	−127° (c=0.35)	−119° (c=0.133)
UV λ_{max} nm(ε)	in MeOH 233 (30330) 240sh (28240) 252 (27850) 280 (5680) 288 (5660)	in EtOH 233 (30300) 242sh (28000) 252 (27900) 281 (5360) 288 (5360)	in MeOH 233 (30240) 240sh (28400) 252 (27650) 280 (5740) 288 (5710)	in EtOH 233 (31300) 252 (28400) 280 (5610) 288 (5540)
MS m/e	545 485 470 450	545.2186 485.1969	559 485 470 450	620.2504 (M^+) 559.2330 485 470 450



	R
Maytanacine = C-15003 P-1	O-C(=O)-CH_3
Maytansinol propionate = C-15003 P-2	O-C(=O)-CH_2CH_3

Structure elucidation. The molecular formula of ansamitocin (ASM) P-3, P-3′ and P-4 are $C_{32}H_{43}ClN_2O_9$, $C_{32}H_{43}ClN_2O_9$ and $C_{33}H_{45}ClN_2O_9$, respectively, as described above. They contain two atoms of N and one atom of Cl per molecule. The same molecular formula for ASM P-3 and P-3′ suggests their isomeric relationship. The difference between the molecular formula of ASM P-3 and that of P-4 is CH_2.

Their UV maxima at 233, 240 (sh), 252, 280 and 288 nm show that they have the same chromophore of maytanacine. The IR spectra of ASM P-3, P-3′ and P-4 are very similar to that of maytanacine.[6] The intensive ester CO absorptions at 1740 cm^{-1} are observed in all of them together with other CO absorptions at 1730 and 1670 cm^{-1}.

The NMR spectra of ASM P-3 indicate the presence of one tertiary Me group, three secondary Me groups, one olefinic Me group, one N-Me group, two O-Me groups, two O-methine groups, three olefinic protons, two aromatic protons, two protons which disappeared upon addition of D_2O, four O-methine or other special methylene protons and six methylene or methine protons as shown in Figs. 2 and 4.

In the spectra of ASM P-3′ and P-4, one primary Me group at 1.05 ppm as a triplet and two secondary Me groups at 1.03 ppm as a doublet are observed, respectively, instead of the two secondary Me groups at 1.27 and 1.28 ppm in that of ASM P-3. These findings suggest that structural differences among these three components exist in the Me and methylene groups.

Upon acetylation of ASM P-3 or P-4, the starting material was recovered. This indicated the absence of primary or secondary OH groups. The two protons at 6.27 and 3.40 ppm which disappeared with addition of D_2O are assumed to be amide and tertiary OH groups.

The principal fragments in the mass spectra of ASM P-3, P-3′ and P-4 together with P-1† and P-2† are listed in Table 3.

The highest mass numbers of ASM P-3 and P-4 were *m/e* 573.2471 (calc. $C_{31}H_{40}ClNO_7 = 573.2493$) and 587.2626 (calc. $C_{32}H_{42}ClNO_7 = 587.2649$), respectively. These mass numbers are identical to the calculated mass numbers of the molecular formula substracting $CH_3NO_2(H_2O + NHCO)$. The mass spectral characteristics of maytansinoids are: (i) there is little parent peak (M^+) and (ii) the common fragments M^+–a, M^+–(a + b), M^+–(a + b)–CH_3 and M^+–(a + b)–Cl (a = H_2O + HNCO, b = ROH) are observed. As shown in Table 3, the fragmentation patterns of ASM P-3, P-3′ and P-4 are the same as those of maytanacine and maytansinol propionate.[6] The difference of their mass numbers exists in their highest fragments (M^+–a). Thus the structural difference should be present in part b which indicates the presence of a fatty acid moiety of maytansinoid. In the case of P-1 and P-2, the substraction of the fragment (M^+–(a + b) = 485) from the highest mass number (M^+–a = 545, 559) gave 60 and 74 which corresponded to

†P-1 = maytanacine; P-2 = maytansinol propionate.

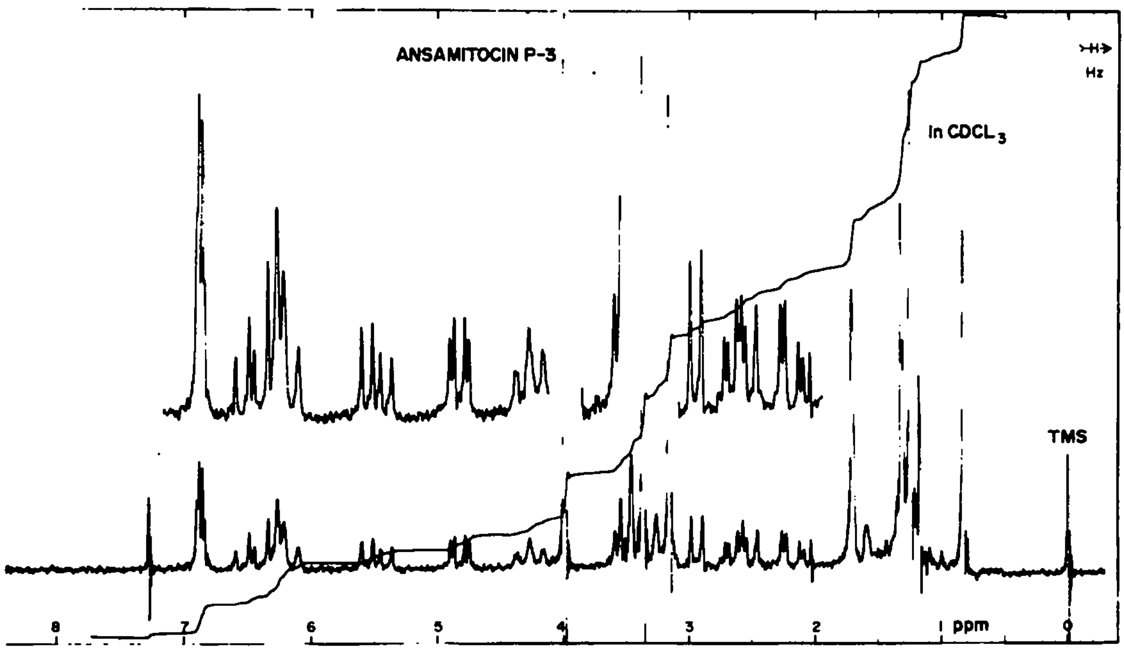


Fig. 2. PMR spectrum of ASM P-3.

Table 3. Principal fragments in the mass spectra of ASM P-3, P-3' and P-4.

	M^+ - a	M^+-(a+b)	485-15(CH_3)	485-35(Cl)
P-3	m/e 573.2471	485	470	450
P-3'	573	485	470	450
P-4	587.2626	485	470	450
P-1	545	485	470	450
P-2	559	485	470	450

a = H_2O + HNCO

b = ROH

acetic and propionic acid, respectively. Those of ASM P-3, P-3' and P-4 gave 88, 88 and 102 which corresponded to butyric, butyric and valeric acid, respectively.

To confirm the fatty acid moiety of ASM, alkaline hydrolysis of these components was carried out. After the hydrolysis of ASM P-3, P-3' or P-4 with sodium hydroxide, the fatty acids formed were separated by gas chromatography on chromosorb 101 and identified with authentic samples from the retention time as isobutyric, butyric and isovaleric acid, respectively.

NMR spin-decoupling studies of ASM P-3 were carried out to confirm the maytansinoid structure as shown in Figs. 2 and 3. When the methine proton at 4.83 ppm (dd, J = 12, 3 Hz), which was assumed to combine with the fatty acid ester group at C-3, was irradiated, two methylene protons at 2.19 (dd, J = 3, 15 Hz) and 2.61 ppm (dd, J = 12, 15 Hz) collapsed to each doublet (J = 15 Hz). Irradiation of the proton at 2.61 ppm made the double doublet methine at 4.83 ppm into a doublet (J = 3 Hz) together with sharpening of the two Me groups of the fatty acid moiety at 1.27 and 1.28 (d) into a singlet. This indicates the presence of a methine proton of a fatty acid moiety at about 2.61 ppm. Irradiation of Me protons at 1.28 sharpened the multiplet methine at 2.65 ppm into a singlet. When the methine proton at 1.51 ppm of C-6 was irradiated, the methine protons at 2.95 (d) of C-5 and 4.27 ppm (m) of C-7 collapsed into a singlet and a double doublet (J = 3, 11 Hz), respectively.

When the methine proton (d) at 3.48 ppm (J = 9 Hz) was irradiated, the olefinic proton (dd) at 5.49 ppm collapsed into a doublet (J = 15 Hz). On the contrary, irradiation of this olefinic proton at 5.49 (dd, J = 15, 9 Hz) sharpened the methine proton at 3.48 ppm (d) into a singlet and the double doublet olefinic proton at 6.48 ppm into a doublet (J = 11 Hz). When the Me proton at 1.71 ppm [s (b)] was irradiated, the olefinic proton at 6.15 ppm [d (b)] collapsed into a doublet (J = 11 Hz).

From the spin-decoupling study of ASM P-3 and the chemical shifts and coupling constants of maytansine,[4] the partial structure of ASM P-3 can be explained well as shown in Fig. 4. There is almost no difference between these two maytansinoids except for the chemical shifts of H_{13}. The difference is thought to result from the

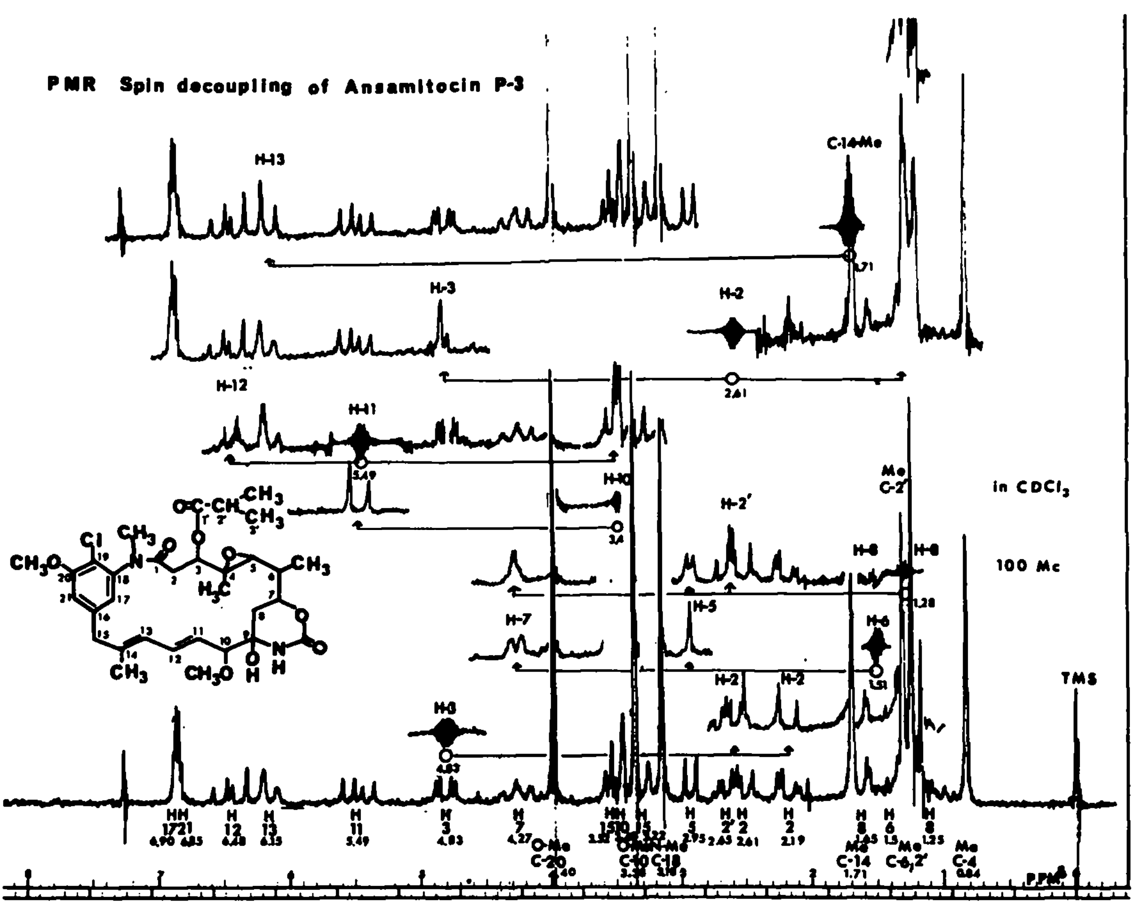


Fig. 3. PMR spin-decoupling of ASM P-3.

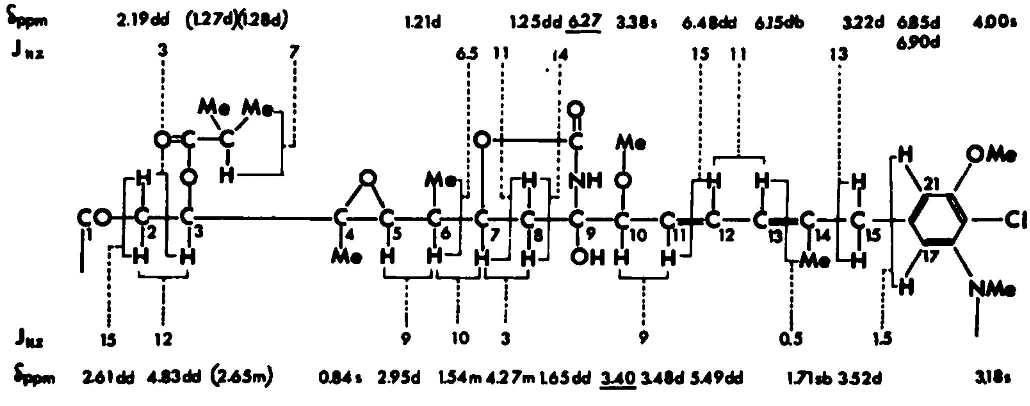
N-acetyl-N-methylalanine moiety at C-3 because it is near the double bond at C-13 according to X-ray analysis of maytansine 3-bromopropyl ether.[4] Their coupling constants were identical: $J_{2,2} = 15$, $J_{2,3} = 3$, 12 Hz, $J_{5,6} = 9$ Hz, $J_{10,11} = 9$ Hz, $J_{11,12} = 15$ Hz, $J_{12,13} = 11$ Hz, $J_{15,15} = 13$ Hz and $J_{17,21} = 1.5$ Hz.

The mutual stereochemical relations between H_2 and H_3, H_5 and H_6, H_{10} and H_{11}, and H_{12} and H_{13} in ASM P-3 were the same as those of maytansine.

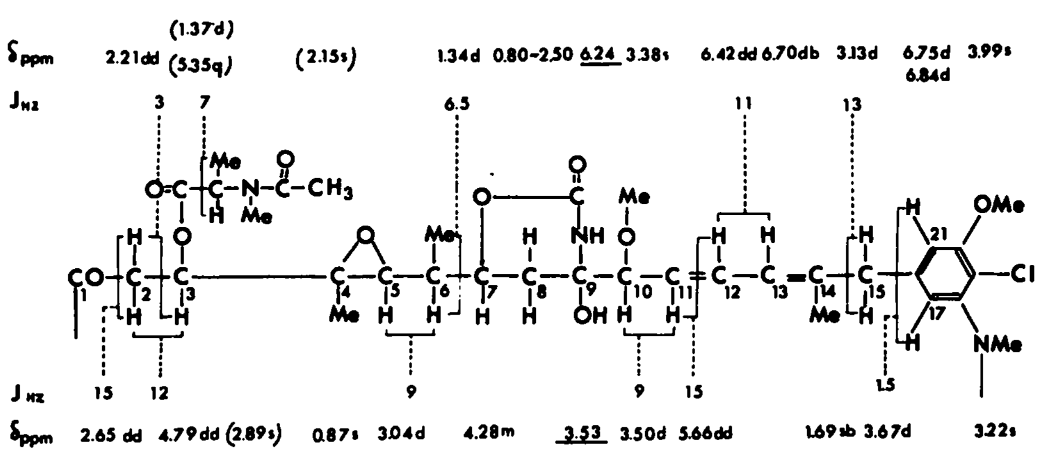
According to the method of maytansinol preparation,[6] ASM P-3, P-3', P-4, P-2 and P-1 were reductively cleaved into the same product, P-0, independently. The physicochemical properties of P-0 are: m.p. 198°;† molecular formula $C_{28}H_{37}ClN_2O_8$; $[\alpha]_D$ −198°† ($c = 0.5$, $CDCl_3$); UV spectrum λ_{max}^{MeOH} nm (ϵ): 232 (32750), 244 sh (30850), 252 (31650), 281 (5750), 288 (5700); IR spectrum, an absorption at 1740 cm^{-1} (ester carbonyl group found in P-1, P-2, ASM P-3, P-3', P-4) disappeared; mass spectrum, m/e 503, 485, 468 (503–Cl), 451 (468–17). In the NMR spectrum of P-0, six Me protons are observed at δ 0.86 ppm (3H, s, $-\overset{|}{\underset{|}{C}}-\underline{CH_3}$), 1.30 (3H, d, J = 6 Hz, CH–$\underline{CH_3}$), 1.70 (3H, bs, $=CH_3$), 3.22 (3H, s, N–$\underline{CH_3}$), 3.38 (3H, s, O–$\underline{CH_3}$), 4.00 (3H, s, O–$\underline{CH_3}$), but the six gem-Me protons [–CH($\underline{CH_3}$)$_2$] found in ASM P-3 and P-4 do not appear. Three olefinic protons at 5.55 (1H, dd, J = 9, 15 Hz), 6.16 (1H, d, J = 11 Hz) and 6.46 (1H, dd, J = 11, 15 Hz), and two aromatic protons at 6.82 (1H, d, J = 1.5 Hz) and 7.10 (1H, d, J = 1.5 Hz) exist in ASM P-0, but a methine proton shifts to 3.48 (1H, dd, J = 3, 12 Hz) from 4.83 (1H, dd, J = 3, 12 Hz, $-C_{(3)}\underline{H}$–OR) in ASM P-3. This means that the acyl ester group at C-3 is reductively cleaved. Three protons were observed at 1.15–1.55 ($C\underline{H}_2$ or $-\overset{|}{\underset{|}{C}}-\underline{H}$), two methylene protons at 2.04 (1H, dd, J = 3, 15 Hz) and 2.28 (1H, dd, J = 12, 15 Hz), 3.1 (1H, d, J = 13 Hz) and 3.51 (1H, d, J = 13 Hz), one methine proton (–O–$\overset{|}{\underset{|}{C}}\underline{H}$) at 4.38 (1H, m), and three replaced protons with D_2O at 2.79 (1H, s), 3.45 (1H, s) and 6.50 (1H, s).

The physicochemical properties of P-0 described above indicate that it is identical to maytansinol. Clearly the skeleton structure of P-3, P-3' or P-4 is maytansinol, like those of P-1 and P-2. P-0 treated with acetic anhydride in pyridine yielded a monoacetate: m.p. 231°, $C_{30}H_{39}ClN_2O_9$. The mass spectrum (m/e 545, 485, 470, 450), specific rotation ($[\alpha]_D^{23}$ −117.4°), NMR spectrum (2.18 ppm, 3H, s, $C\underline{H}_3CO$–), (4.90, 1H, dd, J = 3, 12 Hz, RO–$\overset{|}{\underset{|}{C}}-\underline{H}$), R_f values on silica gel tlc and IR spectrum of P-0 monoacetate were identical to those of P-1, which is maytanacine according to Kupchan.[6]

†The difference of melting point and specific rotation of P-0 from that of maytansinol (174°, −304°) might depend on their purity.



Ansamitocin P-3



Maytansine[4]

Fig. 4. PMR data of ASM P-3 and maytansine.

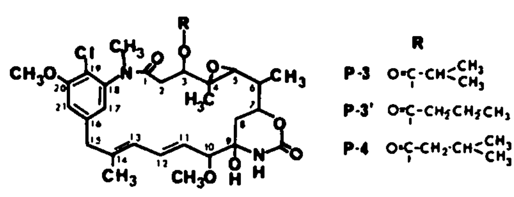


Fig. 5. Structure of ansamitocin.

All of this evidence shows that ASM P-3, P-3' and P-4 are new maytansinoids and the structural difference from maytansine exists only in the ester side chain at C-3 (Fig. 5).

EXPERIMENTAL

M.ps were determined by Metler FP-5 3°/min. UV spectra were measured on a Shimadzu UV-200 double beam spectrophotometer. IR spectra were recorded with Hitachi 285 grating IR spectrophotometer. NMR spectra were obtained using a Varian XL-100-12 instrument: chemical shifts (δ) are reported in ppm downfield from internal TMS. Mass spectra were determined on a JEOL JMS-OISC spectrometer equipped with a direct inlet system. For tlc silica gel GF_{254} (E. Merck, A.G., Germany) were used: thickness employed was 0.25 mm.

I. *Separation of* C-15003 P *mixture*

The filtrate (900 l) of cultured broth of *Nocardia* C-15003 (N-1) was adjusted to pH 7, and extracted with one third volume of EtOAc. The EtOAc extract was washed with water and concentrated *in vacuo* to obtain about 100 ml of concentrate. One liter of petroleum ether was added into the concentrate giving an oily material. A soln of the material in EtOAc (500 ml) was concentrated to 150 ml, kept under cooling and filtered after 24 hr to obtain a soln.

The soln was chromatographed on silica gel column (250 g, E. Merck 0.05–0.2 mm) with petroleum ether (500 ml), hexane (500 ml), hexane-EtOAc (4:1) (300 ml), (3:1) (300 ml), (2:1) (300 ml), (1:1) (500 ml), EtOAc (200 ml) and EtOAc-MeOH (19:1) 1000 ml successively. Each fraction of effluent (100 ml) was tested by tlc using the solvent system of EtOAc saturated with water. The fractions (No. 24 to No. 38) which were detected as absorption spots of 2537 Å, having the R_f values of 0.40–0.50, were combined, concentrated to 100 ml and filtrated after leaving at 5° for 24 hr. The filtrate was rechromatographed on silica gel column (150 g) successively with petroleum ether (300 ml), hexane (200 ml), hexane–$CHCl_3$ (1:1) (200 ml), $CHCl_3$ (400 ml), $CHCl_3$–MeOH (100:1) (1000 ml) and $CHCl_3$–MeOH (9:1) (400 ml) successively. Each fraction (50 ml) of effluent was tested by the above described tlc method. Fraction (a) (Nos. 40–46) of R_f 0.50–0.42, fraction (b) (Nos. 50–54) of R_f 0.42–0.38 and fraction (c) (Nos. 57–58) of R_f 0.34 were respectively concentrated. Each residue was crystallized from EtOAc. The mixture (800 mg) of P-4, P-3' and P-3 from (a), the mixture (1050 mg) of

P-3 and P-2 from (b) and 47 mg of P-1 from (c) were obtained as colorless crystals.

II. *Isolation of* C-15003 P-4, P-3', P-3, P-2 *and* P-1

1. The mixed crystals (600 mg) of P-4, P-3' and P-3 obtained from fraction (a) were dissolved in $CHCl_3$ and chromatographed on silica gel (400 g) successively with a mixture (500 ml) of hexane–EtOAc (1:4) saturated with water, a mixture (600 ml) of hexane–EtOAc (1:5) saturated with water and a mixture (500 ml) of hexane–EtOAc (1:7) saturated with water. Each fraction (10 ml) of effluent was tested by the tlc method. The fractions giving the same single spot were combined and evaporated to obtain crystals. Thus 210 mg of pure C-15003 P-4 and 175 mg of P-3 were isolated as colorless crystals. The fraction of the mixture of P-4 and P-3' was chromatographed on a preparative thin layer (E. Merck 0.25 mm, 20 × 20) using EtOAc saturated with water as the developing solvent. The bands of R_f 0.49 (P-4) and R_f 0.45 (P-3') were extracted with the mixture of EtOAc and water. Each EtOAc layer was separated, washed with water and concentrated to obtain the crystals of P-4 (51 mg) and P-3' (18 mg).

2. The mixed crystals (500 mg) of P-3 and P-2, obtained from fraction (b), were dissolved with $CHCl_3$ and chromatographed on silica gel (100 g) with the above described solvent systems. Each fraction (10 ml) of effluent was tested by the tlc method and the fractions giving the same single spot were combined and concentrated to dryness. Crystallization of each residue from EtOAc gave crystals of C-15003 P-3 (220 mg) and P-2 (68 mg), respectively.

3. The crude crystals were recrystallized from EtOAc to give 35 mg of pure P-1.

4. The mixture (150 mg) of C-15003 P-3, P-3' and P-4, obtained from fraction (a), was dissolved in a mixture of MeOH (15 ml), water (15 ml) and NaCl (300 mg), and was chromatographed on a Dia ion HP-10 (Mitsubishi Kasei) (200 ml) column, which had been prepared by the treating with a soln (600 ml) of 50% aqueous MeOH containing 5% NaCl, with 60% aqueous MeOH containing 5% NaCl, and followed by a gradient elution using solvent system from 60% aqueous MeOH (containing 5% NaCl) 1.5 l. to 95% aqueous MeOH 1.5 l. Each fraction (15 ml) of effluent was tested by the above described tlc method. Thus P-3, a mixture of P-3' and P-4 were detected in the fractions Nos. 145–153, 167–180 and 185–190, respectively. Each fraction containing the same component was combined and concentrated to dryness, dissolved with 80 ml of water and extracted with 100 ml of EtOAc. The EtOAc layer was separated, washed with water and concentrated to give 47 mg of P-3, 17 mg of mixture of P-3' and P-4 and 52 mg of P-4 as crystals.

III. *Alkali hydrolysis of ansamitocin* P-3, P-3' *and* P-4

Crystals (10 mg) of P-3, P-3' and P-4 were dissolved in MeOH (5 ml), mixed with 0.5N–NaOH 5 ml, then hydrolyzed at 60° for 5 hr. The hydrolyzate was diluted with 5 ml of water yielding a ppt. The resulting filtrate was concentrated to 0.5 ml and passed through a column of Amberlite IR-120 (H) (1 ml). The first acidic effluent was analyzed by gas chromatography on chromosorb 101 (80–100 mesh) (120 cm). The resulting data were: isobutyric acid was detected from the hydrolysate of P-3; butyric acid was detected from the hydrolysate of P-3'; isovaleric acid was detected from the hydrolysate of P-4. Acetic acid and propionic acid were observed from the hydrolysate of P-1 and P-2 by a similar method, respectively.

IV. *Reductive cleavage of ansamitocin* P-3, P-3' *and* P-4

Crystals (120 mg) of ansamitocin P-3, P-3' and P-4 were cleaved reductively in THF (8 ml) at −20° with LAH (100 mg) for 2 hr. After the reaction the mixture was acidified with N–HCl (6.5 ml), diluted with cold water (24 ml) and extracted with 24 ml portions of EtOAc three times. The extracts were combined, washed with H_2O and concentrated to obtain a crude material. The crude material was chromatographed on silica gel (90 g), with EtOAc saturated with water. Each fraction (15 ml) of effluent was tested by the tlc method using EtOAc saturated with water as the developing solvent, and detected at 2537 Å as an absorption spot. The fractions giving a single spot of R_f 0.27 were combined and concentrated to 0.5 ml. The concentrate was added 20 ml of Et_2O and a ppt was obtained (36 mg of P-0). M.p. 198° (dec). (Found: C, 59.35; H, 6.88; N, 4.85; Cl, 6.08. Calc. for $C_{28}H_{37}N_2ClO_8$: C, 59.52; H, 6.60; N, 4.96; Cl, 6.27%. MS *m/e* 503, 485, 468, 451).

V. P-0-*Monoacetate* (P-1, *synthesis of maytanacine*)

P-0 (100 mg) in pyridine (0.7 ml) was acetylated with Ac_2O (0.35 ml) for 18 hr at room temp. and the mixture was poured into ice water to give a crude acetate. The crude acetate (93 mg) was dissolved in $CHCl_3$ (0.5 ml) then passed through silica gel (40 g) and developed with EtOAc saturated with water. Each fraction of effluent was tested by the tlc method. The fractions (Nos. 37–44) having the R_f value 0.34, were combined and concentrated to dryness. The residue was crystallized with a small portion of EtOAc to give pure crystals (78 mg) of P-0-monoacetate, m.p. 231–233°. (Found: C, 59.58; H, 6.55; N, 4.45; Cl, 5.64. Calc. for $C_{30}H_{39}ClN_2O_9$: C, 59.35; H, 6.48; N, 4.61; Cl, 5.84%); $[\alpha]_D$ −117.4° (c = 0.54). MS *m/e* 545, 485, 470, 450. The P-0-monoacetate was identified as C-15003 P-1 (Maytanacine), described in above, due to its same R_f value, IR spectrum, UV spectrum and all physicochemical properties.

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REFERENCES

[1]E. Higashide, M. Asai, K. Ootsu, Y. Kozai, T. Hasegawa, T. Kishi, Y. Sugino and M. Yoneda, *Nature* **270**, 721 (1977).

[2]T. Hasegawa, S. Tanida, K. Hatano, E. Higashide and M. Yoneda, in preparation.

[3]S. Tanida, T. Hasegawa and E. Higashide, in preparation.

[4]S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger and R. F. Bryan, *J. Am. Chem. Soc.* **94**, 1354 (1972).

[5]S. M. Kupchan, Y. Komoda, G. J. Thomas and H. P. J. Hintz, *J. Chem. Soc.* Chem. Comm. 1065 (1972); S. M. Kupchan, Y. Komoda, A. R. Branfman, R. G. Dailey, Jr. and V. A. Zimmerly, *J. Am. Chem. Soc.* **96**, 3706 (1974).

[6]S. M. Kupchan, A. R. Branfman, A. T. Sneden, A. K. Verma, R. G. Dailey, Jr., Y. Komoda and Y. Nagao, *Ibid.* **97**, 5294 (1975); S. M. Kupchan, Y. Komoda, A. R. Branfman, A. T. Sneden, W. A. Court, G. J. Thomas, H. P. J. Hintz, R. M. Smith, A. Karim, G. A. Howie, A. K. Verma, Y. Nagao, R. G. Dailey, Jr., V. A. Zimmerly and W. C. Sumner, Jr. *J. Org. Chem.* **42**, 2349 (1977).

[7]M. C. Wani, H. L. Taylor and M. E. Wall, *J. Chem. Soc.* Chem. Comm. 390 (1973).

[8]S. M. Kupchan, A. T. Sneden, A. R. Branfman, G. A. Howie, L. I. Rebhun, W. E. McIvor, R. W. Wang and T. C. Schnaitman, *J. Med. Chem.* 21, 31 (1978).