STRUCTURE OF CERVINOMYCIN, A NOVEL XANTONE ANTIBIOTIC ACTIVE AGAINST ANAEROBE AND MYCOPLASMA

AKIRA NAKAGAWA and SATOSHI ŌMURA*

The Kitasato Institute and School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan

KATSUHIKO KUSHIDA

Varian Instrument Ltd., Shinjuku-ku, Tokyo 160, Japan

HIDEKI SHIMIZU

Asahi Chemical Industries Co., Ltd., Miyazaki 882, Japan

GABOR LUKACS

CNRS-Institute de Chimie des Substances Naturelles, 91190 Gif-Sur-Yvette, France

(Received for publication August 30, 1986)

The structures of cervinomycins A_1 (**1**) and A_2 (**2**), a potent anti-anaerobic and anti-mycoplasmal antibiotic were investigated by means of recent NMR techniques of *O*-methyl ether (**3**) and *C,O*-dimethyl ether (**4**) obtained by methylation of **2** with CH_3I in the presence of Ag_2O. The antibiotic **2** posesses a polycyclic structure involving a xanthone skeleton. The structure of **1** was confirmed to be a hydroquinone of **2** from the evidences that oxidation of **1** with Ag_2O and acetylation of **1** with $(CH_3CO)_2O$ in pyridine afforded quantitatively **2** and triacetylcervinomycin A_1 (**7**), respectively.

Cervinomycin is an anti-anaerobic and anti-mycoplasmal antibiotic produced by *Streptomyces cervinus* sp. nov.[1] The antibiotic posesses a novel xanthone skeleton[2] and consists of two components, A_1 (**1**), mp (dec) >240°C, $C_{29}H_{23}NO_9$ and A_2 (**2**), mp (dec) >290°C, $C_{29}H_{21}NO_9$ which are barely soluble in most solvents. Both components and their acyl derivatives afforded a monocrystal in appropriate solvents, however the X-ray crystallographic analyses were unsuccessful because of extreme instability of the crystals on exposing to air. Therefore, NMR spectroscopic analysis was carried out for cervinomycin methyl derivatives. In this paper we wish to report the structure elucidation of cervinomycin by means of NMR spectroscopy.

Methylation of **2** with CH_3I in the presence of Ag_2O in $CHCl_3$ - MeOH afforded two methyl derivatives, *O*-methylcervinomycin A_2 (**3**), $C_{30}H_{23}NO_9$ and *C,O*-dimethylcervinomycin A_2 (**4**), $C_{31}H_{25}NO_9$. As shown in Fig. 1, the ^{13}C NMR spectrum (in $CDCl_3$) of **3** indicated the presence of a methyl (δ 23.3), two methylenes (δ 42.2 and 43.5), three methoxyls (δ 56.4, 56.7 and 63.2), and an oxymethylene (δ 64.4) and a quaternary carbon (δ 92.1) bonded to an oxygen and a nitrogen atom. Compound **3** contains further thirteen olefinic carbons in the region at 100~140 ppm, an amide group and five olefinic oxycarbons in 149~160 ppm, a doubly α,β-unsaturated carbonyl (δ 172.4) and two quinone carbonyls (δ 178.2 and 183.2). These data suggest that cervinomycin has a conjugated poly-

Fig. 1. ^{13}C NMR spectrum of *O*-methylcervinomycin A_2 (**3**) ($CDCl_3$).

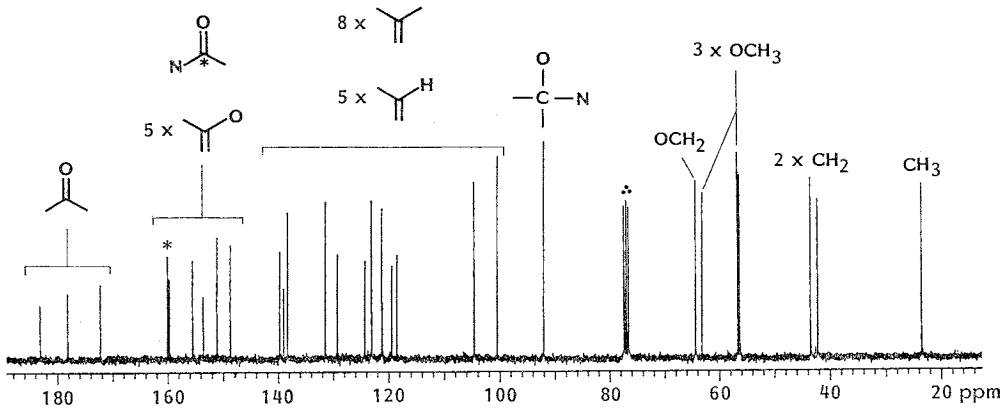
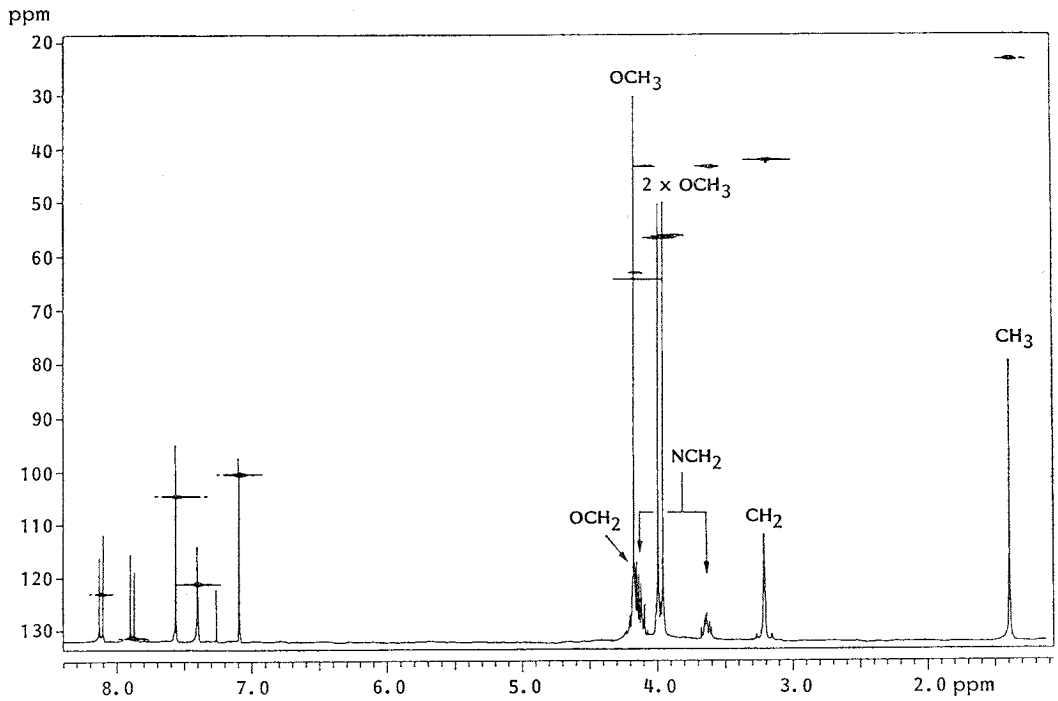


Fig. 2. C-H Shift correlated 2D NMR spectrum of *O*-methylcervinomycin A_2 (**3**) ($CDCl_3$).



cyclic skeleton containing quinone nucleus. As shown in Fig. 2, the C-H shift correlated 2D NMR spectrum (in $CDCl_3$) of **3** indicated the presence of a tertiary methyl (δ 1.43), three methylenes (*C*-, *N*- and *O*-methylenes), three methoxyls (δ 4.01, 4.03 and 4.11) in which the latter has been introduced by *O*-methylation of **2**. In the low field region, AB type protons (δ 7.93 d and 8.18 d) and three isolated olefinic protons (δ 7.64, 7.44 and 7.13) were observed. The location of two isolated olefinic protons (δ 7.64 and 7.13) and two methoxyl groups was assigned from the structure of alkaline degradation product (**5**), $C_9H_{10}O_5$ of **2** (or **1**). The NMR data [δ 7.22 (1H, s), 6.43 (1H, s), 3.80 and 3.85 (2× OCH_3), δ_C 172.1 (COOH)] confirmed the structure of 3,4-dimethoxy-6-hydroxybenzoic acid for **5**, indicating the presence of a partial structure **I** in cervinomycin. The presence of structure **I** was con-

firmed from a long range selective decoupling (LSPD) experimental data [C-H couplings of H-16 (δ 7.13) with C-8 ($^3J_{CH}$=4.1 Hz) and C-20 ($^3J_{CH}$=3.7 Hz), and H-19 (δ 7.64) with C-15 ($^3J_{CH}$=7.4 Hz), C-17 ($^3J_{CH}$=4.4 Hz) and C-21 ($^3J_{CH}$=4.0 Hz)].

For the connectivity of the remaining each functional group, a long range ^{1}H and ^{13}C shift correlated 2D NMR[3] and LSPD experiment[4] were carried out for **3**. The existence of neighboring two *N*- and *O*-methylene groups (C-1; δ 43.5, H-1a,1b; δ 3.67 (1H, m), 4.18 (1H, m) and C-2; δ 64.4, H-2a,2b; δ 4.19 (2H, t)) placed between a nitrogen and an oxygen atom was confirmed from the proton decoupling experiment. The observation of the ^{1}H and ^{13}C long range couplings between H-1 and amide carbon (C-28, δ 160.1) and between H-2 and a quaternary carbon (C-4; δ 92.1) indicated the existence of a five-membered ring including a nitrogen atom of an amide group and an oxygen atom. Long range couplings were observed between a methyl proton (δ 1.43) and C-4 and between methylenic protons (H-5a,5b; δ 3.21, 3.29) and two sp^2 carbons (C-7; δ 121.3 and C-27; δ 118.5) and C-4. In the A, B type protons H-9, δ 7.93 and H-10, δ 8.18 the former couples with C-7 ($^3J_{CH}$=4.0 Hz), C-11 ($^3J_{CH}$=6.9 Hz) and C-25 ($^3J_{CH}$=4.9 Hz) and the latter, with C-8 ($^3J_{CH}$=7.7 Hz), C-12 ($^3J_{CH}$=3.6 Hz) and C-24 ($^3J_{CH}$=5.3 Hz). The location of a phenolic hydroxyl group on the C-26 of **2** (or **1**) was assigned from the β-shift of C-25 (Δ 3.6 ppm) and C-27 (Δ 11.2 ppm) by methylation of the hydroxyl. These NMR evidences deduced the existence of a partial structure **II** consisting of conjugated five rings including a 1,4-benzoquinone moiety for **3**. The validity of structure **II** was also confirmed from the observation of the nuclear Overhauser effect (NOE) of H-7 in ring C with H-5 in ring B and H-9 in ring D. There are two possibilities (A) and (B) for the connectivity of **I** to **II** as shown in Fig. 3. In general, the chemical shift value of a quinone carbonyl carbon (or hydroquinone carbonyl) neighbor to the carbonyl of γ-pyrone system, as indicated in bikaverin,[5] a fungal metabolite, appears to be deshieled compared with the value when a quinone carbonyl locates to the ether oxygen of a γ-pyrone. The observation of $^4J_{CH}$=0.7 Hz between H-10 and C-13 (δ 153.6) attached to an

Fig. 3. Partial structures **I** and **II** and C-H three bond coupling patterns.

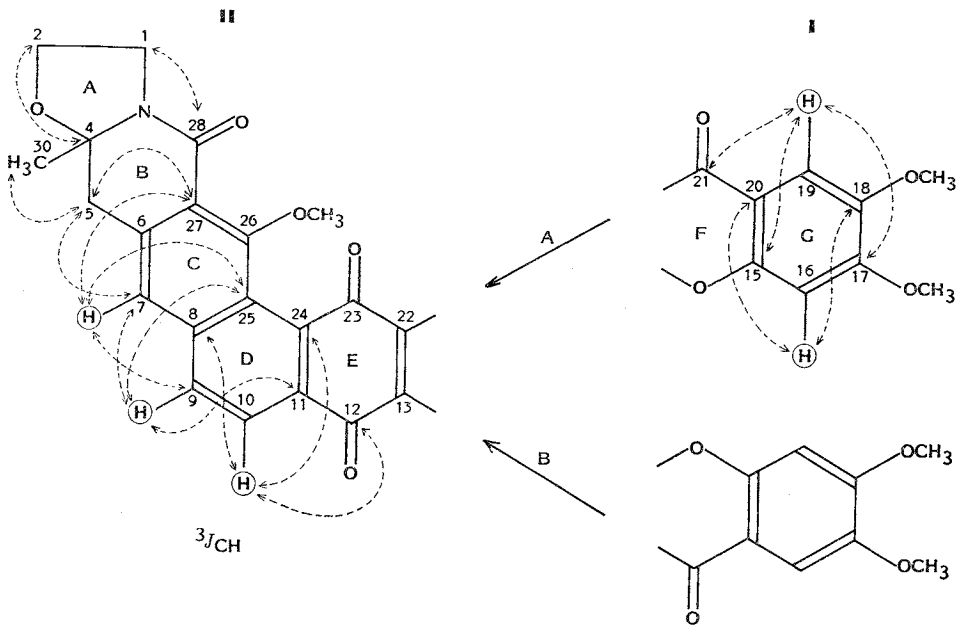
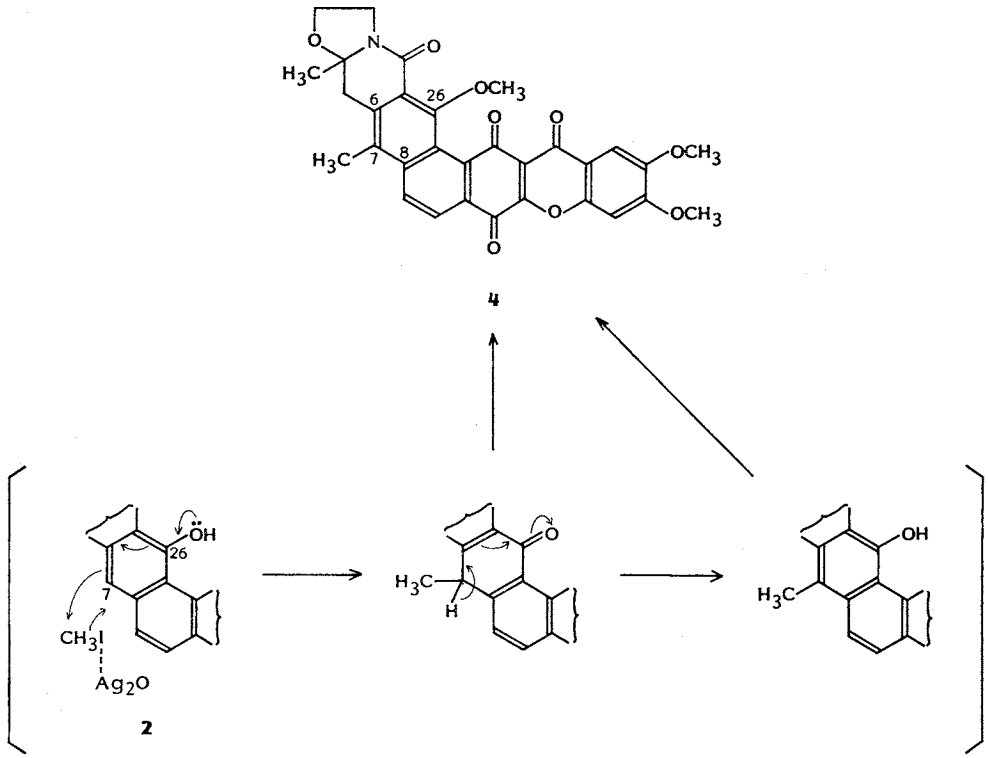
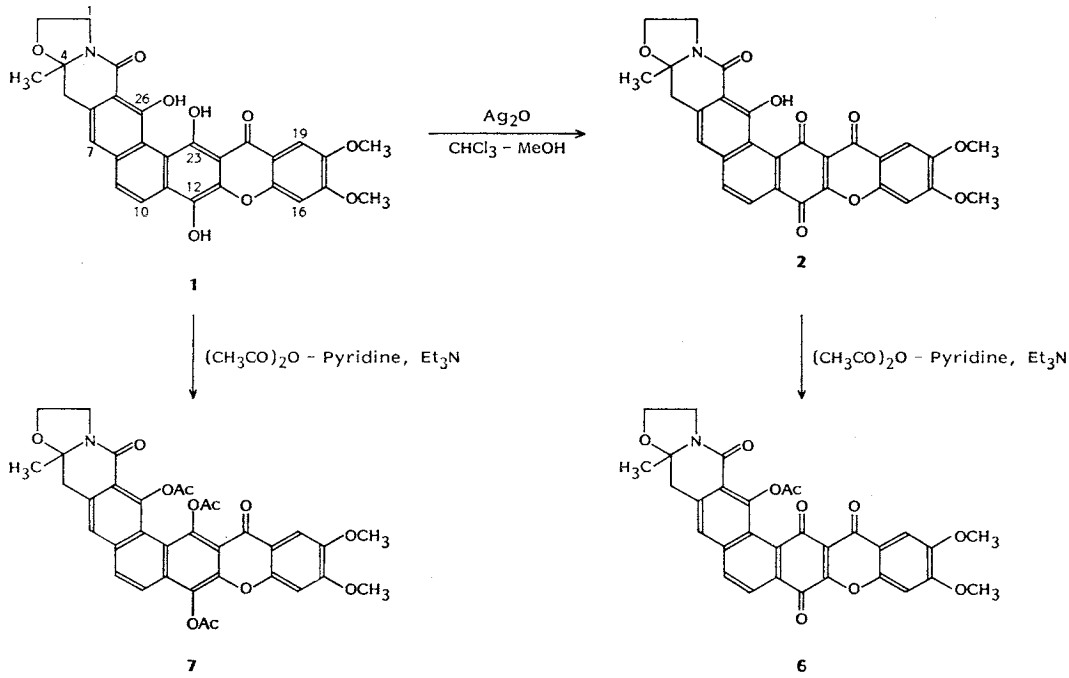


Fig. 4. Structure of *C,O*-dimethylcervinomycin A_2 (**4**) and its reaction mechanism.



Scheme 1.



ether oxygen in addition to the above chemical shift values (C-12; δ 178.2 and C-23; δ 183.2) validated the connectivity (A). The structures of **2** and **3** were further confirmed from the detailed NMR data of monoacetylcervinomycin A_2 (**6**), $C_{31}H_{23}NO_{16}$ which was obtained by acetylation of **2** with $(CH_3CO)_2O$ in pyridine in the presence of Et_3N. Thus, a novel polycyclic structure containing a xanthone skeleton was assigned for **2**.

The structure of the second methylation product **4** was assigned as the C-7 methylation product from a comparison with the chemical shift values of **3** and the observation of $^3J_{CH}$ of the methyl proton (δ 2.47) at C-7 with C-6 (δ 135.6) and C-8 (δ 138.9). It seems to be quite rare that the *C*-methylation occurs at *para*-position of a doubly α,β-substituted phenol derivative with CH_3I in the presence of Ag_2O. The formation of **4** seems to proceed the mechanism through *C*-methylation and then *O*-

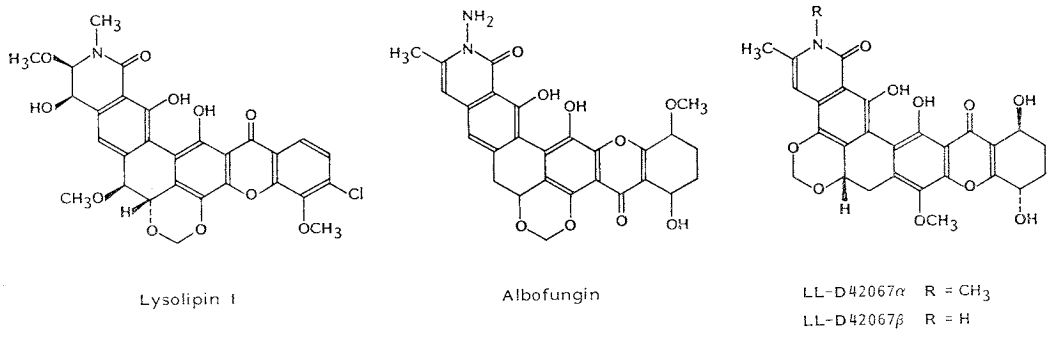
Table 1. ^1H and ^{13}C chemical shift values* of cervinomycin A_2 (**2**) and its derivatives **3**, **4** and **6**.

Carbon No.	Cervinomycin A_2 (**2**)		*O*-Methyl-cervinomycin A_2 (**3**)		*C*,*O*-Dimethyl-cervinomycin A_2 (**4**)		Monoacetyl-cervinomycin A_2 (**6**)	
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	3.64 m, 3.92 m	41.9	3.67 m, 4.18 m	43.5	3.62 m, 4.02 m	43.4	3.68 m, 4.01 m	43.0
2	4.18 t	64.1	4.19 t	64.4	4.13 t	64.2	4.20 t	64.3
4		92.1		92.1		91.9		92.0
5	3.13 d, 3.22 d	40.8	3.21 d, 3.29 d	42.2	2.95 d, 3.34 d	40.1	3.28 d, 3.34 d	41.7
6		136.5		138.3		135.6		136.3
7	7.13 s	117.5	7.44 s	121.3		125.9	7.68 s	124.6
8		140.6		139.8		138.9		139.3
9	7.86 d	132.2	7.93 d	131.4	8.08 d	128.8	8.05 d	132.4
10	8.12 d	123.6	8.18 d	123.1	8.12 d	122.7	8.26 d	123.1
11		129.6		129.3		128.6		130.5
12		178.3		178.2		178.0		178.2
13		153.7		153.6		153.6		153.2
15		151.0		151.1		151.1		151.0
16	7.11 s	100.3	7.13 s	100.5	7.07 s	100.5	7.13 s	100.4
17		155.6		155.5		155.5		155.6
18		148.7		148.7		148.6		148.7
19	7.55 s	104.8	7.64 s	104.7	7.49 s	104.4	7.62 s	104.6
20		119.5		119.5		119.2		119.4
21		172.7		172.4		172.5		172.4
22		120.6		121.1		120.8		120.9
23		181.7		183.2		183.3		182.8
24		137.7		139.0		139.1		137.2
25		120.6		124.3		124.2		124.6
26		160.8		159.7		157.7		159.3
27		107.3		118.5		118.2		123.0
28		164.4		160.1		160.6		159.4
30	1.32 s	22.6	1.43 s	23.3	1.34 s	23.8	1.42 s	23.1
17-OCH_3	3.95 s	56.6	4.01 s	56.7	3.94 s	56.6	4.02 s	56.7
18-OCH_3	3.92 s	56.3	4.03 s	56.4	3.90 s	56.2	4.00 s	56.4
26-OCH_3			4.11 s	63.2	4.08 s	62.9		
7-CH_3					2.47 s	14.2		
26-$OCOCH_3$								169.4
26-$OCOCH_3$							2.67 s	21.5

Compound **2** was measured in $CDCl_3$ - CD_3OD and **3**, **4**, **6** in $CDCl_3$.

* ppm.

Fig. 5. Structures of lysolipin I, albofungin and LL-D42067.



methylation *via* enolization, as shown in Fig. 4. The hydroquinone structure of **1** was determined from the fact that acetylation of **1** with $(CH_3CO)_2O$ in pyridine in the presence of Et_3N and oxidation of **1** with Ag_2O afforded triacetylcervinomycin A_1 (**7**), $C_{35}H_{29}NO_{12}$ and **2**, respectively, as shown in Scheme 1. The complete assignment of ^1H and ^{13}C chemical shift values for compounds **2**, **3**, **4** and **6** is shown in Table 1.

Three xanthone antibiotics, lysolipin I, a glycopeptide synthesis inhibitor,[6,7] albofungin (BA-180265, kanchanomycin) and chloroalbofungin, a DNA and RNA synthesis inhibitor[8,9] have been reported as cervinomycin related antibiotics. The structures of lysolipin I and albofungin have been established by X-ray analysis and chemical degradation, respectively, as shown in Fig. 5. Recently, LL-D42067α and β were reported as an antibacterial and anti-protozoal antibiotic in which the structure and stereochemistry were assigned by X-ray analysis.[10] The location of the carbonyl group in the xanthone ring of cervinomycin is identical with that of lysolipin I and LL-D42067. However, it is a noteworthly that the carbonyl group in albofungin is oppositely located compared with that of other three antibiotics. We are now investigating the biosynthetic correlation among cervinomycin, lysolipin I, and albofungin by feeding experiment using ^{13}C-labeled precursors.

Triacetate **7** showed enhanced anti-anaerobic activity against *Clostridium difficile*, *Peptococcus variabilis* and *Streptococcus mutans* and anti-mycoplasmal activity.[11] It is being developed as a medicinal drug because of its high solubility in most solvents and low toxicity in addition to a potent antimicrobial activity.

Experimental

General Methods

Melting points were determined with a Yanagimoto MP-S3 apparatus and are uncorrected. Optical rotations were measured with a polarimeter Jasco DIP-181. ^1H and ^{13}C NMR spectra were recorded with TMS as an internal standard in $CDCl_3$ (or $CDCl_3$ - CD_3OD) with a Varian XL-400. Mass spectra were recorded with a JMS-D100 mass spectrometer. IR spectra were recorded with Shimadzu UV-210 A infrared spectrometer. Silica gel TLC was performed on pre-coated TLC plates Silica gel 60 F_{254} (Merck).

O-Methylcervinomycin A_2 (**3**) and *C*,*O*-Dimethylcervinomycin A_2 (**4**)

Excess CH_3I (2.5 ml) was added to a suspension of **2** (200 mg) in $CHCl_3$ (5 ml) and MeOH (2 ml) in the presence of Ag_2O (70 mg). The reaction mixture was stirred at 30°C for 72 hours and then filtered. The filtrate was concentrated to dryness. The reaction products were purified on silica gel TLC (0.5 mm) using a solvent system of $CHCl_3$ - MeOH - NH_4OH (15 : 1 : 0.01) to afford **3** (56 mg)

as a brown crystals and **4** (62 mg) as a yellow crystals. Compound **3**: MP >300°C (dec); $[\alpha]_D^{27}$ −499° (*c* 0.5, $CHCl_3$); MS (electron impact (EI)-MS) *m/z* 541 (M^+); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 248.0 (42,740), 317.3 (27,320); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2990, 1690, 1650, 1615, 1505, 1450, 1420, 1270; TLC (silica gel) Rf 0.59 (solvent system: $CHCl_3$ - MeOH - NH_4OH, 15 : 1 : 0.01). Compound **4**: MP >255°C (dec 9; $[\alpha]_D^{27}$ −459° (*c* 0.2, $CHCl_3$); MS (EI-MS) *m/z* 555 (M^+); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 252.2 (35,070), 327.2 (21,540); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2990, 1690, 1645, 1620, 1500, 1455, 1421, 1270; TLC (silica gel) Rf 0.47 (solvent system: $CHCl_3$ - MeOH - NH_4OH, 15 : 1 : 0.01).

Alkaline Degradation Product (**5**) from **2**

A solution of **2** (80 mg) (or **1**) in 0.5 N KOH (3 ml) and dioxane (10 ml) was allowed to stand at 100°C for 48 hours. After acidification of the reaction mixture with 1 N HCl, the solution was extracted with EtOAc (20 ml). The extract was concentrated to dryness. The residue was purified on silica gel TLC (0.5 mm) using a solvent system of $CHCl_3$ - MeOH - NH_4OH (20 : 1 : 0.01) to obtain colorless needles **5** assigned as 3,4-dimethyl-6-hydroxybenzoic acid. MP 242~245°C; MS (EI-MS) *m/z* 198 (M^+); ^1H NMR ($CDCl_3$) δ 3.80 (OCH_3), 3.84 (OCH_3), 6.41 (1H, s), 7.21 (1H, s), ^{13}C NMR ($CDCl_3$ - CD_3OD) δ 55.8 (OCH_3), 56.0 (OCH_3), 99.7, 103.0, 110.8, 141.8, 155.5, 158.2, 172.0 (COOH).

Monoacetylcervinomycin A_2 (**6**)

A suspension of **2** (100 mg) in pyridine (3 ml) and $(CH_3CO)_2O$ (0.3 ml) in the presence of Et_3N as a catalyst was stirred at room temp for 24 hours. The reaction mixture was poured on ice-water (70 ml) and then extracted with EtOAc (25 ml). The extract was washed with 1% $NaHCO_3$ and then evaporated to dryness. The residue was purified on silica gel TLC (0.5 mm) using a solvent system of $CHCl_3$ - MeOH - NH_4OH (15 : 1 : 0.01) to obtain monoacetate **6** as an orange needles. MP 283°C; $[\alpha]_D^{27}$ −297.5° (*c* 0.3, $CHCl_3$); MS (EI-MS) *m/z* 569 (M^+); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 244.1 (30,950), 274.5 (21,390), 307.4 (25,490), 374.6 (91,040); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3000, 1775, 1690, 1650, 1620, 1501, 1430, 1270, 1170; TLC (silica gel) Rf 0.40 (solvent system: $CHCl_3$ - MeOH - NH_4OH, 15 : 1 : 0.01).

Triacetylcervinomycin A_1 (**7**)

Acetylation of **1** was carried out in a similar manner with **2** to afford triacetate **7** as a yellow needles. MP 283~285°C; $[\alpha]_D^{26}$ −115° (*c* 0.3, $CHCl_3$); MS (EI-MS) *m/z* 655 (M^+); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 258.8 (21,220), 299.4 (31,180), 336.2 (7,600), 354.7 (10,480), 371.2 (16,500), 414.0 (5,760); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3000, 1770, 1650, 1610, 1179; TLC (silica gel) Rf 0.59 (solvent system: $CHCl_3$ - MeOH - NH_4OH, 15 : 1 : 0.01).

Oxidative Transformation of **1** to **2**

A suspension of **1** (50 mg) in $CHCl_3$ (4 ml) and MeOH (3.5 ml) in the presence of Ag_2O (30 mg) was stirred at 65°C for 24 hours. After filtration of reaction mixture, the solution was evaporated to dryness. The residue was purified on silica gel TLC (0.5 mm) using a solvent system of $CHCl_3$ - MeOH - NH_4OH (15 : 1 : 0.01) to afford an orange crystalline powder, which was identified with **2** from the IR, MS, UV and NMR spectral data.

Acknowledgment

The authors are indebted to Miss M. NAKAJIMA for excellent technical assistance and Mrs. A. HATANO for the measurement of 400 MHz NMR.

References

1) ŌMURA, S.; Y. IWAI, K. HINOTOZAWA, Y. TAKAHASHI, J. KATO, A. NAKAGAWA, A. HIRANO, H. SHIMIZU & K. HANEDA: Cervinomycin A_1 and A_2, new antibiotics active against anaerobes, produced by *Streptomyces cervinus* sp. nov. J. Antibiotics 35: 645~652, 1982
2) ŌMURA, S.; A. NAKAGAWA, K. KUSHIDA & G. LUKACS: Structure of cervinomycin, a novel antianaerobic antibiotic. J. Am. Chem. Soc. 108: 6088~6089, 1986
3) BENN, R. & H. GUNTHER: Modern pulse methods in high-resolution NMR spectroscopy. Angew. Chem. Int. Ed. Engl. 22: 350~380, 1983
4) SETO, H.; T. SASAKI, H. YONEHARA & J. UZAWA: Studies on the biosynthesis of pentalenolactone.

Part I. Application of long range selective proton decoupling (LSPD) and selective ^{13}C-{^{1}H} NOE in the structural elucidation of pentalenolactone G. Tetrahedron Lett. 1978: 923~926, 1978

5) McILNNESS, A. G.; D. G. SMITH, J. A. WALTER, L. C. VINING & J. L. C. WRIGHT: Homonuclear ^{13}C decoupling in ^{13}C nuclear magnetic resonance studies of biosynthesis using doubly labelled precursors. Assembly pattern of the acetate units in bikaverin. J. Chem. Soc. Chem. Commun. 1975: 66~68, 1975

6) DOBLER, M. & W. KELLER-SCHIERLEIN: Metabolites of microorganisms, 162nd Communication. The crystal and molecular structure of lysolipin I. Helv. Chim. Acta 60: 178~185, 1977

7) DRAUTZ, H.; W. KELLER-SCHIERLEIN & H. ZÄHNER: Stoffwechselprodukte von Mikroorganismen 149. Mitteilung, Lysolipin I ein neues Antibioticum aus Streptomyces violaceoniger. Arch. Microbiol. 106: 175~190, 1975

8) GUREVICH, A. I.; M. G. KARAPETYAN, M. N. KOLOSOV, V. N. OMELCHENKO, V. V. ONOPRIENKO, G. I. PETRENKO & S. A. POPRAVKO: The structure of albofungin. Tetrahedron Lett. 1972: 1751~1754, 1972

9) LIU, W.-C.; W. P. CULLEN & K. V. RAO: BA-180265: A new cytotoxic antibiotic. Antimicrob. Agents Chemother. —1962: 767~771, 1963

10) LEE, T. M.; D. B. BORDERS, G. T. CARTER, M. HERTZ & J. P. KIRBY: LL-D42067α and β, novel antibacterial and antiprotozoal agents: Isolation, characterization and structure determination. Program and Abstracts of the 26th Intersci. Conf. on Antimicrob. Agents Chemother., No. 222, p. 136, New Orleans, Sept. 28~Oct. 1, 1986

11) NAKAGAWA, A.; Y. IWAI, H. SHIMIZU & S. ŌMURA: Enhanced antimicrobial activities of acetyl derivatives of cervinomycin. J. Antibiotics 39: 1636~1638, 1986