STUDIES ON T-2636 ANTIBIOTICS. II

ISOLATION AND CHEMICAL PROPERTIES OF T-2636 ANTIBIOTICS

SETSUO HARADA, TOYOKAZU KISHI and KOMEI MIZUNO

Microbiological Research Laboratories, Research and Development Division,
Takeda Chemical Industries, Ltd., Osaka, Japan

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Five anti-Gram-positive bacterial components were isolated from the fermented broth of *Streptomyces rochei* var. *volubilis* and named as T-2636 A, B, C, D and E. T-2636 A, C, D and E are N-containing neutral lipophilic antibiotics and have the molecular formulae of $C_{27}H_{35}NO_8$, $C_{25}H_{33}NO_7$, $C_{27}H_{37}NO_8$ and $C_{26}H_{37}NO_6$, respectively. T-2636 B, $C_{43}H_{72}O_{17}$, is a neutral macrolide. T-2636 M obtained from the mycelium was assumed to be a polyene macrolide. The physico-chemical characterization of these components reveals that T-2636 B, D and E are new antibiotics and that T-2636 A, C, D and E belong to the lankacidin group. T-2636 A and C are identical with bundlin B and lankacidin, respectively.

Antibiotics T-2636, active against Gram-positive bacteria, were isolated from the fermented broth of *Streptomyces rochei* var. *volubilis*[1].

The antibiotic mixture was extracted with organic solvents from the filtered broth and separated by silica gel chromatography. These components were named T-2636 A, B, C, D and E in accordance with their Rf values on thin-layer chromatogram. T-2636 M, obtained from mycelium, seems to be a polyene macrolide from its ultraviolet spectrum. T-2636 A, C, D and E belong to lankacidin group while T-2636 B is a neutral macrolide.

In this paper, we describe the isolation and chemical properties of antibiotics T-2636 A, B, C, D, E and M[2].

The filtered broth of *Streptomyces rochei* var. *volubilis* was extracted at pH 5~7 with ethyl acetate counter-currently. After washing with 0.1 N hydrochloric acid and 2% sodium bicarbonate the ethyl acetate extract was concentrated under reduced pressure and precipitated with *n*-hexane. The individual components, T-2636 A, B, C, D and E, in the crude precipitate were isolated by column chromatography on silica gel using the solvent system of benzene - ethyl acetate - acetone-methanol or chloroform - methanol, and crystallized from suitable solvents (Chart 1, Table 1).

Aqueous acetone extract of mycelium was concentrated *in vacuo*, treated with ethyl acetate and re-extracted with *n*-butanol. After washing with water, the *n*-butanol extract was concentrated and crystallized from methanol to obtain T-2636 M (Chart 1).

T-2636 A (I) was obtained as neutral lipophilic substance, colorless plates, recrystallized from ether and ethyl acetate; m.p. 207~210°C (decomp.), $[\alpha]_D^{25}$ −235° (*c* 1.0 in EtOH).

Chart 1. Isolation of T-2636 A, B, C, D, E and M

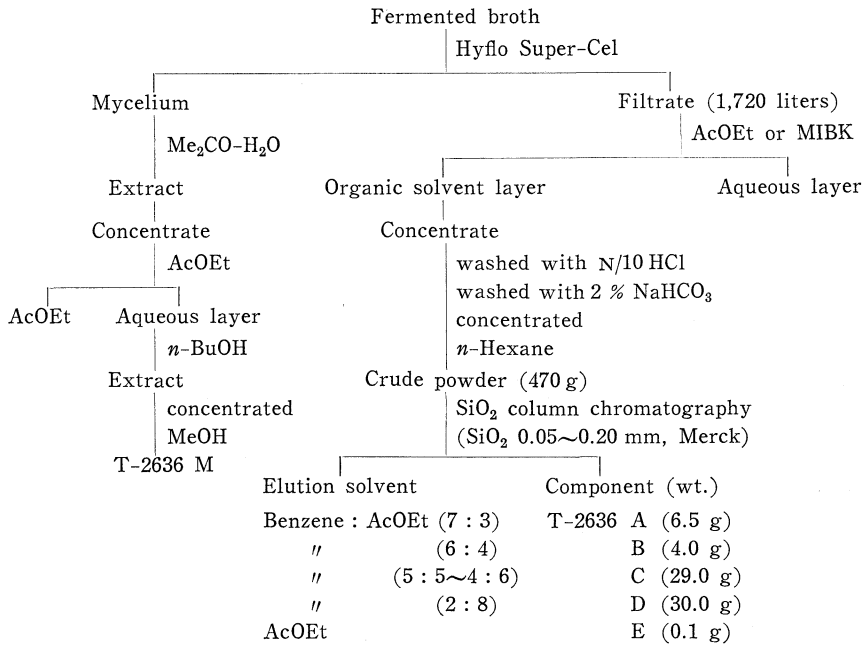


Fig. 1. Ultraviolet absorption spectra of T-2636 A, C, D and E (EtOH).

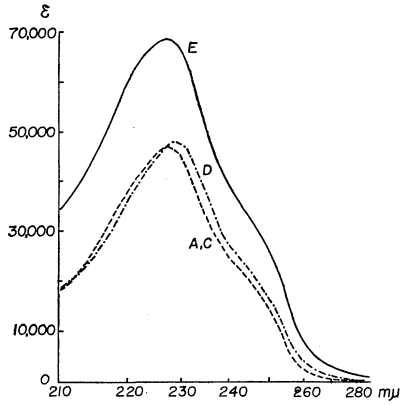


Table 1. Rf values of thin-layer chromatograms of T-2636 A, B, C, D, E and M

Compound	Solvent system			
	$CHCl_3$ – MeOH (93 : 7)	AcOEt – Me_2CO (95 : 5)	MeEtCO – Et_2O (1 : 3)	Benzene – Me_2CO (1 : 1)
T-2636 A	0.87	0.77	0.85	0.82
B	0.85	0.67	0.78	0.81
C	0.51	0.51	0.57	0.69
D	0.41	0.47	0.53	0.56
E	0.33	0.35	0.35	0.49
M	0.0	0.0	0.0	0.0

Absorbent : Kiesel gel F_{254} Merck.
Detected with conc. H_2SO_4 or I_2.

It is slightly soluble in *n*-hexane, petroleum ether and soluble in ethyl acetate, acetone, chloroform and methanol. This antibiotic shows a blue-violet color with conc. H_2SO_4 and blue color with FISCHBACH and LEVINE's carbomycin test[3], but is negative to erythromycin test[3]. The molecular weight is assumed to be 501 from V.P.O. method*, 490 in ethyl acetate, and MS* spectrum, m/e 441 ($M^+ - 60$ (AcOH)). The elemental analysis and the molecular weight indicate that the molecular formula of T-2636 A is $C_{27}H_{35}NO_8$. The above-mentioned molecular formula was ascertained from the fact that T-2636 A gave a monoacetate (II), NMR* spectrum, $\delta_{ppm}^{CDCl_3}$ 2.00 (3H, s, -OAc), MS

* The abbreviations used are as follows; V.P.O. : vapor pressure osmometry, MS : mass, NMR : nuclear magnetic resonance, UV : ultraviolet absorption, IR : infrared absorption.

Fig. 2. Infrared absorption spectrum of T-2636 A (KBr)

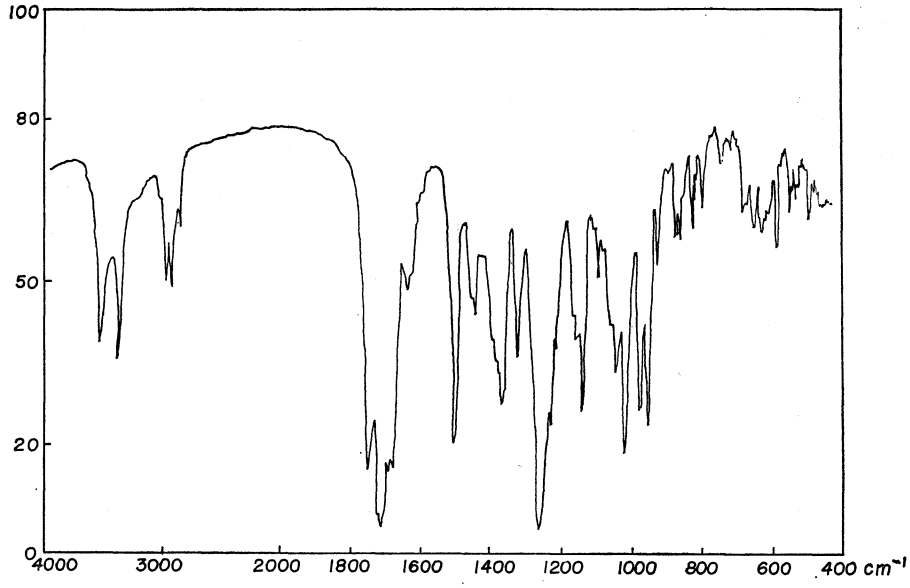
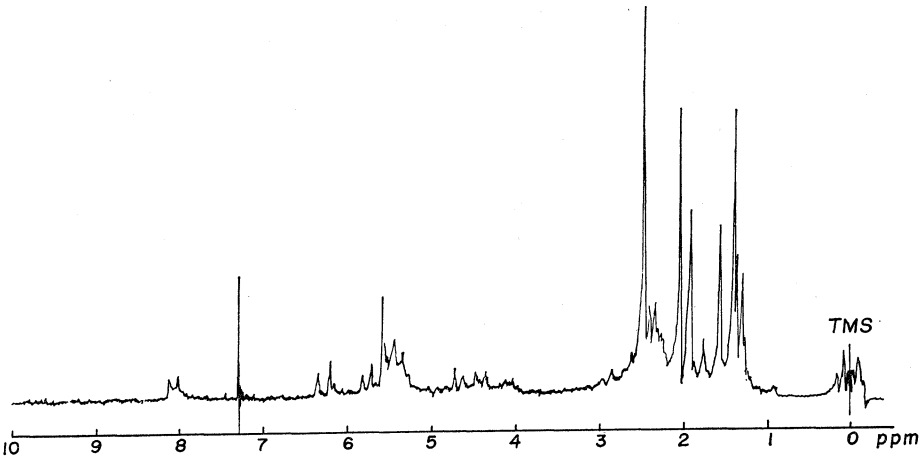


Fig. 3. NMR spectrum of T-2636 A (100 Mc, $CDCl_3$)

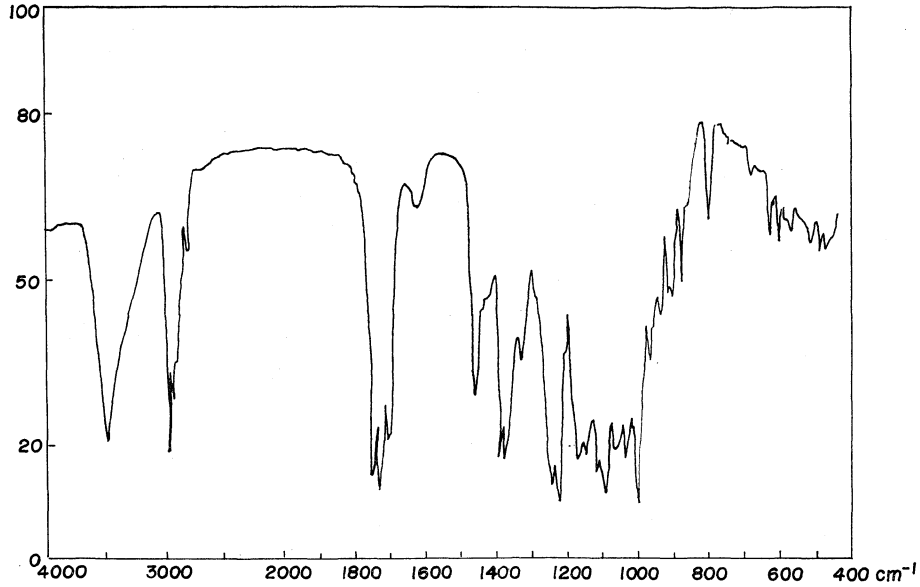


spectrum, m/e 543 (M^+). The UV* spectrum of T-2636 A in ethanol exhibits a maximum at 228 mμ (ε 46,900) (Fig. 1). The IR* spectrum indicates the presence of -OAc (ν_{max}^{KBr} 1735, 1260 cm^{-1}) and lactone (1755 cm^{-1}) groups (Fig. 2). The NMR spectroscopy indicates the existence of -CONH-, $\delta_{ppm}^{CDCl_3}$ 8.00 (1H, d, J=9 cps, disappeared on addition of D_2O), -COMe, 2.40 (3H, s) and -OAc, 2.00 (3H, s), groups (Fig. 3).

T-2636 B (III) was obtained as colorless hexagonals from diethyl ether; m.p. 205 ~207°C, $[\alpha]_D^{24}$ −92.4° (*c* 1.0 in EtOH). It is insoluble in petroleum ether and water, and soluble in diethyl ether, ethyl acetate, acetone, chloroform and methanol.

This antibiotic shows red-violet color with MOLISCH reagent and erythromycin test[3] and is negative to carbomycin test[3]. The molecular weight is assumed to be 860 from V.P.O. method, 851 in ethyl acetate and MS spectrum, m/e 860 (M^+). The elemental analysis and the molecular weight indicate that the molecular formula of

Fig. 4. Infrared absorption spectrum of T-2636 B (KBr)



T-2636 B is $C_{43}H_{72}O_{17}$. The UV spectrum of T-2636 B in methanol exhibits a maximum at 287 mμ (ε 54). In the IR spectrum the presence of -OAc (ν_{max}^{KBr} 1730, 1250 cm^{-1}) and lactone (1750 cm^{-1}) groups (Fig. 4) are observed.

T-2636 C (IV) was obtained as neutral colorless needles from ethyl acetate, m.p. 201~203°C (decomp.), $[\alpha]_D^{25}$ −240° (*c* 1.0 in EtOH). It is slightly soluble in diethyl ether, moderately soluble in ethyl acetate and soluble in acetone, chloroform, methanol and ethanol. This antibiotic shows light blue color with carbomycin test and blue color with conc. H_2SO_4 and is negative to erythromycin test. It gives the diacetate (II').

Fig. 5. Infrared absorption spectrum of T-2636 C (KBr)

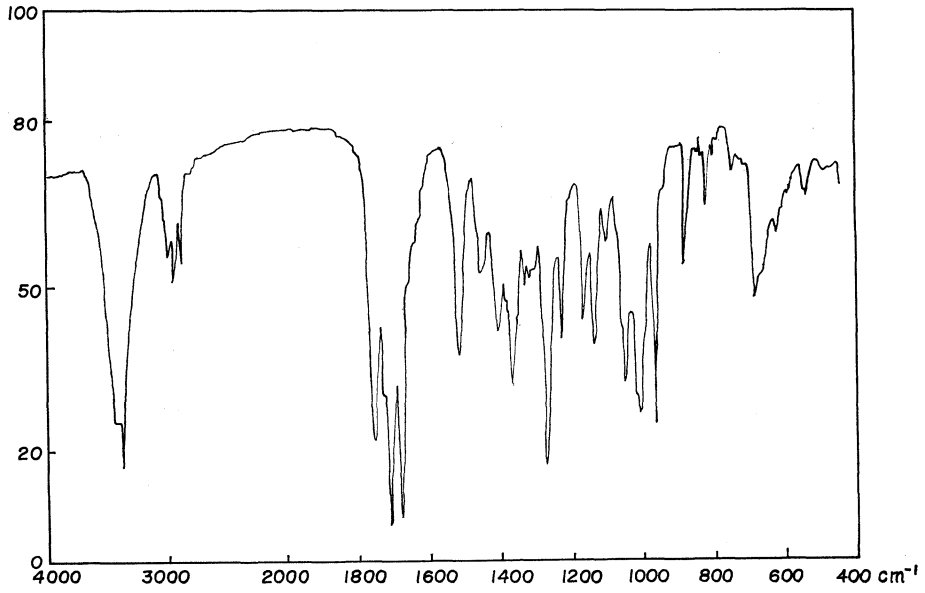
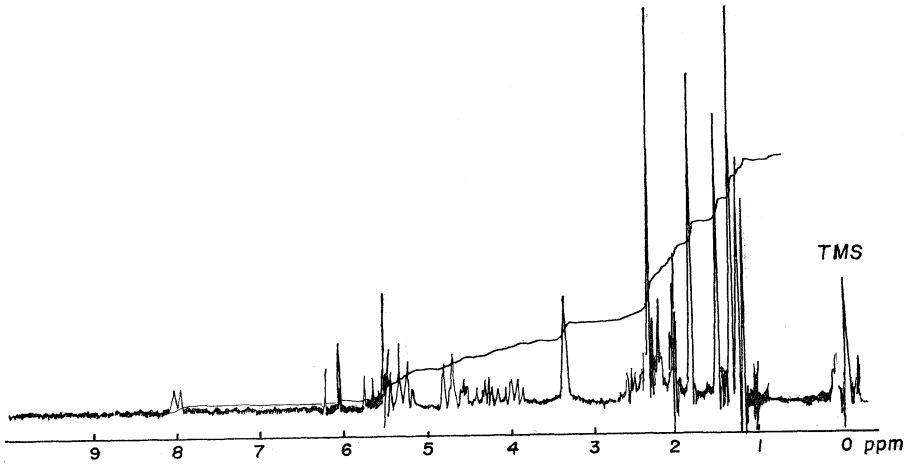


Fig. 6. NMR spectrum of T-2636 C (100 Mc, d_6-Me_2CO)



The molecular weight is assumed to be 459 from V.P.O. method, 439 in ethyl acetate, and MS spectrum, m/e 459 (M^+), 441 ($M^+ -18$ (H_2O)), and m/e 543 (M^+) of T-2636 C diacetate (II'). T-2636 A monoacetate (II) and T-2636 C diacetate (II') show the same melting point, elemental analysis, IR, NMR, MS spectra and antimicrobial activities. The elemental analysis and the molecular weight of T-2636 C indicate that the molecular formula of **IV** is $C_{25}H_{33}NO_7$. The UV spectrum of **IV** in ethanol shows a maximum at 227 mμ (ε 46,700) (Fig. 1). The IR spectrum exhibits four characteristic peaks (ν_{max}^{KBr} 1755, 1725, 1710, 1680 cm^{-1}) in the carbonyl region (Fig. 5). The NMR spectrum is similar to that of **I**, however, it is lacking in the acetyl group which observed in the spectrum of **I** (Fig. 6).

T-2636 D (**V**) was obtained as neutral colorless needles from ethyl acetate and methanol, m.p. 190~191°C (decomp.), $[\alpha]_D^{24}$ −226° (*c* 1.0 in EtOH). It is slightly soluble in ethyl acetate and chloroform, moderately soluble in acetone and soluble in methanol, ethanol, dimethyl formamide and pyridine. This antibiotic shows violet-blue color with carbomycin test and conc. H_2SO_4 and gives a negative to erythromycin test, ninhydrin and benzidine reactions. The molecular weight is assumed to be 503 from V.P.O. method, 509 in acetone, and MS spectrum, m/e 458 ($M^+ -45$ (-$CHOHCH_3$)), 443 ($M^+ -60$ (AcOH)). The elemental analysis and molecular weight indicate that the molecular formula is $C_{27}H_{37}NO_8$. T-2636 D afforded T-2636 D diacetate (**VI**) NMR spectrum, $\delta_{ppm}^{CDCl_3}$=2.00 (3H, s, -OAc), 2.16 (3H, s, -OAc), MS spectrum, m/e 587 (M^+). The UV spectrum of **V** in ethanol exhibits a maximum at 229 mμ (ε 48,000) (Fig. 1). The IR spectrum suggests the presence of lactone (ν_{max}^{KBr} 1740 cm^{-1}) and -OAc (1720 cm^{-1}) groups (Fig. 7). The NMR spectrum is lack-in -COMe group which observed in T-2636 A but has a new signal appearing at δ_{ppm}^{DMSO}=1.3 (3H, d, J=6 cps) (Fig. 8).

T-2636 E (**VII**) was obtained as neutral colorless needles from acetone, m. p. 228~230°C (decomp.), $[\alpha]_D^{25}$ −320° (*c* 0.25 in DMSO - EtOH (1 : 1)). This antibiotic is slightly soluble in ethyl acetate and chloroform, moderately soluble in acetone, methanol and ethanol and soluble in dimethyl sulfoxide and pyridine. The molecular weight is

Fig. 7. Infrared absorption spectrum of T-2636 D (KBr)

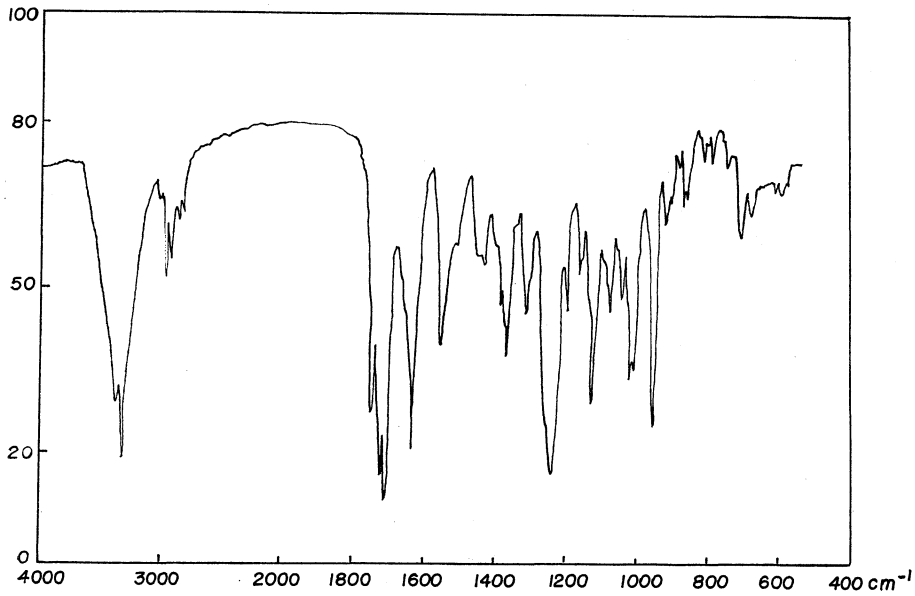
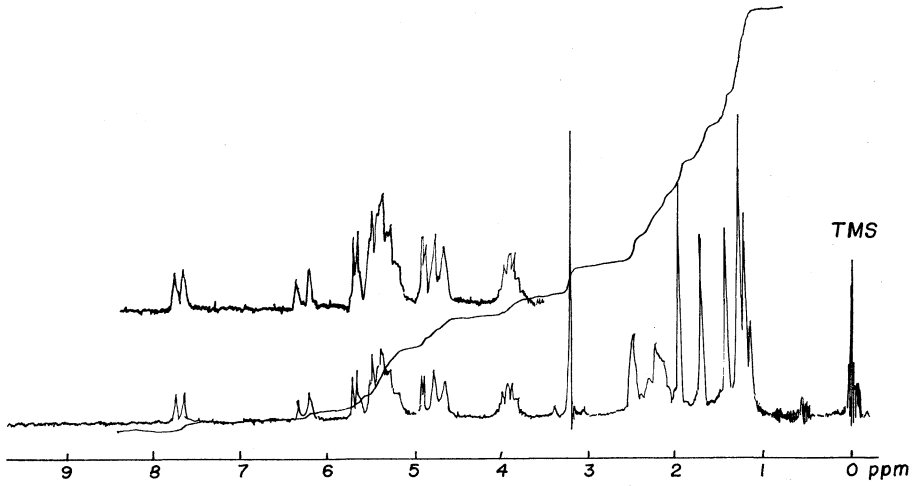


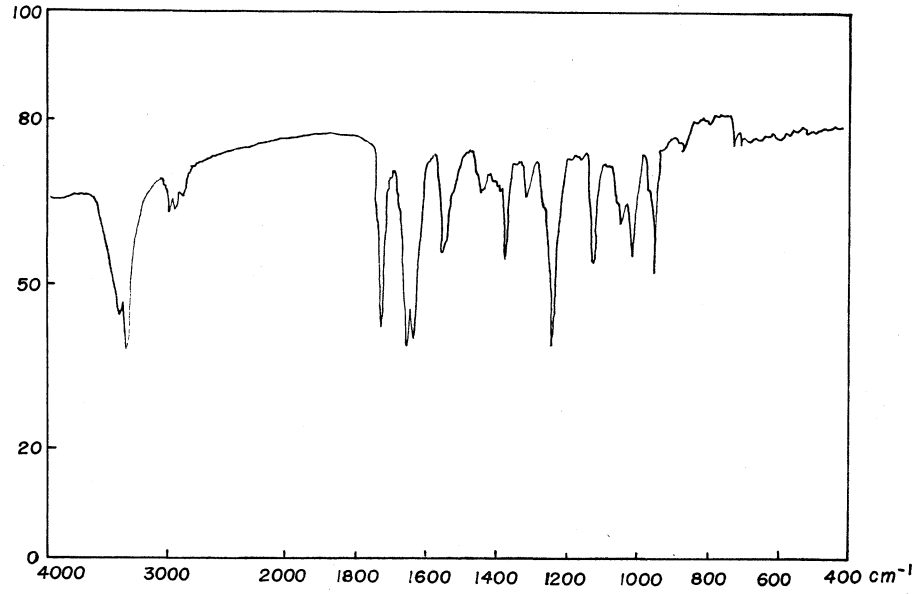
Fig. 8. NMR spectrum of T-2636 D (100 Mc, d_6-DMSO)



assumed to be 459 from MS spectrum, m/e 459 (M^+), 441 ($M^+ - 18$ (H_2O)). The elemental analysis and the molecular weight indicate that the molecular formula is $C_{26}H_{37}NO_6$. The UV spectrum shows a maximum at 227 mμ (ε 68,200) in ethanal (Fig. 1). The IR spectrum is shown in Fig. 9.

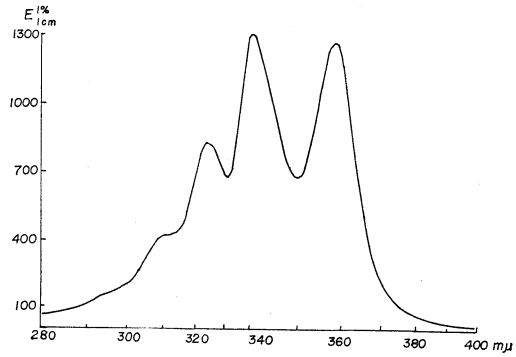
T-2636 M (**IX**) was obtained as a pale yellow crystalline powder, $[\alpha]_D^{24}$ −210° (*c* 0.5 in EtOH). It is moderately soluble in methanol and ethanol, and soluble in aqueous methanol or acetone and dimethyl sulfoxide. The elemental analysis is found; C, 61.23, 61.32; H, 8.56, 8.43; N, 0 (%). The UV spectrum in ethanol shows maxima at 310 mμ (shoulder), 324 mμ ($E_{1cm}^{1\%}$ 816), 341 mμ ($E_{1cm}^{1\%}$ 1294), 358 mμ ($E_{1cm}^{1\%}$ 1251) (Fig. 10). The IR spectrum is shown in Fig. 11.

Fig. 9. Infrared absorption spectrum of T-2636 E (KBr)



T-2636 C shows strong *in vitro* activities against Gram-positive bacteria such as *Staphylococcus aureus*, including oleandomycin and erythromycin resistant strain and *Sarcina lutea*[1], and Gram-negative bacteria such as *Neisseria gonorrhoeae* and *Vibrio cholerae*[4]. T-2636 M is active against molds[1]. T-2636 C is also active against staphylococci isolated from patients[4]. T-2636 A and C show curative effect to the *S. aureus* and *Streptococcus pyogenes* infected mice, when administered via oral or intraperitoneal routes[4].

Fig. 10. Ultraviolet absorption spectrum of T-2636 M (EtOH)



The LD_{50} of T-2636 A, C and D for mice via oral route are up to 10 g/kg. When administered intraperitoneally, those of T-2636 A, B, C, D and M are 8~10, *ca.* 0.4, 4.5, 10 and 0.02 g/kg, respectively.

Comparison of the components of T-2636 antibiotics with other known antibiotics is shown in Table 2. T-2636 A is similar to bundlin B (Meiji Seika Co., Ltd.)[5], but different from it in elemental analysis, molecular weight and IR spectrum[11]. Thereafter M. URAMOTO *et al.*[9] found bundlin B as anti-*Xanthomonas oryzae* antibiotic and isolated as pure crystal which was in accord with T-2636 A[2] in melting point, specific rotation, molecular formula and chemical structure[10].

T-2636 B is shown to be different from lankamycin[6,7] donated from Ciba Ltd. in the Rf values of thin-layer chromatograms, molecular formula and NMR spectrum. T-2636 C had been recognized to be different from lankacidin[6] or bundlin A (Meiji

Fig. 11. Infrared absorption spectrum of T-2636 M (KBr)

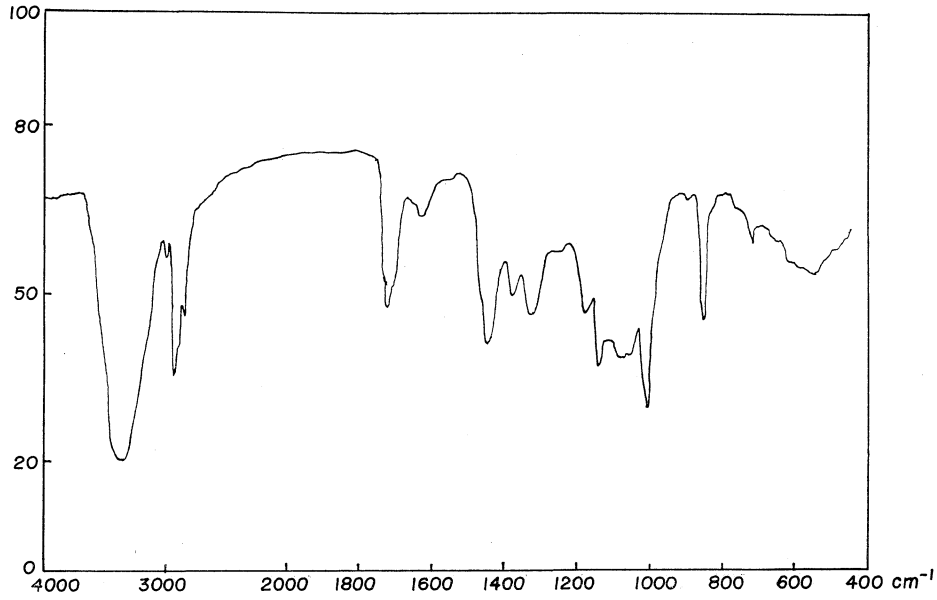
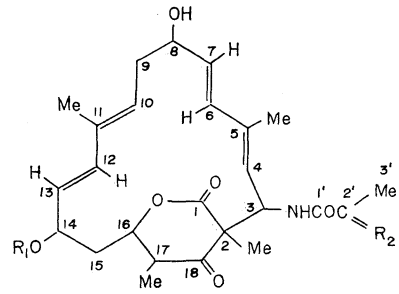


Table 2. Comparison of antibiotics T-2636 components with other antibiotics

	Melting point (°C)	$[\alpha]_D$	UV	Analysis			Melecular weight	Molecular formula	Reference
				C	H	N			
T-2636 A	207~210 (decomp.)	−235° (EtOH) −221° (MeOH)	227 mμ (ε 48,900) (EtOH)	64.62	7.10	2.79	501	$C_{27}H_{35}NO_8$	2)
Bundlin B (Meiji Seika)	188 (decomp.)	−208° (MeOH)	227 mμ ($E^{1\%}_{1cm}$ 1,020) (MeOH)	63.24	7.20	2.30	600		5)
Bundlin B (Univ. Tokyo)	213~215 (decomp.)	−212.1°(MeOH)	227 mμ (ε 80,000) (MeOH)				501	$C_{27}H_{35}NO_8$	9)
T-2636 B	205~207	−92.4° (EtOH)	287 mμ (ε 54) (EtOH)	59.87	8.59	0	860	$C_{43}H_{72}O_{17}$	2)
Lankamycin (Ciba)	146~148	−94° (EtOH)	289 mμ (log ε 1.50) (EtOH)	60.45	8.78	0	832	$C_{42}H_{72}O_{16}$	6), 7)
T-2636 C	201~203 (decomp.)	−237° (EtOH) −227° (MeOH)	226 mμ (ε 46,800) (EtOH)	65.30	7.20	3.13	459	$C_{25}H_{33}NO_7$	2)
Lankacidin (Ciba)	165~168 (decomp.)	−161° (EtOH)	227 mμ (log ε 2.95) (EtOH)	62.71	7.11	2.87	898	$C_{49}H_{66}O_{16}N_2$	6)
Bundlin A (Meiji Seika)	157~161 (decomp.)	−177° (MeOH)	227 mμ ($E^{1\%}_{1cm}$ 982) (MeOH)	64.35	7.00	2.99	420		5)
Lankacidin**	194~196 (decomp.)			64.20	6.96	3.13	459		
Bundlin A (Univ. Tokyo)	205~206 (decomp.)	−222.0°(MeOH)	227 mμ (ε 80,000) (MeOH)				459	$C_{25}H_{33}NO_7$	9)

** A purified sample from Ciba Ltd.

Seika Co., Ltd.)[5] in the melting point, specific rotation, elemental analysis, IR spectrum and other data[11]. However, recently reexamination of the identity with the purified sample of lankacidin given from Ciba Ltd. revealed that our sample was identical with lankacidin in the physico-chemical coefficients of melting point, IR, UV and MS spectra and Rf values of thin-layer chromatograms. The melting point, specific rotation, molecular formula and chemical structure of T-2636 C[2] was in good accord with those of bundlin A (Univerity of Tokyo)[9,10] which is identical with lankacidin. Thus T-2636 C is identical with purified lankacidin and bundlin A. On the other hand, T-2636 B, D and E are apparently new antibiotics. T-2636 F indicated in the above structural formula will be reported in the next paper[12].



	R_1	R_2
T-2636 A	-COMe	=O
T-2636 C	-H	=O
T-2636 D	-COMe	-H,-OH
T-2636 F	-H	-H,-OH

The studies on the isolation, chemical structure and X-ray analysis of T-2636 have been reported[2,8].

Experimental

T-2636 A, B, C, D and E: Fermented broth was filtered with 2% Hyflo Super-Cel and 1,720 liters of the filtrate (*S. aureus*, 50~75, *Bacillus subtilis* 15 du/ml) was extracted at pH 6 with 1/3 volume of ethyl acetate in a counter-current extractor. After concentration of the organic solvent layer (*S. aureus* 350, *B. subtilis* 75 du/ml), the concentrate (28 liters, *S. aureus* 7,500, *B. subtilis* 2,000 du/ml) was washed with 1/4 volume of N/10 hydrochloric acid, 1/4 volume of 2% sodium bicarbonate (2 times) and 1/4 volume of water (2 times), concentrated *in vacuo* and added into the 10 liters of *n*-hexane to give 470 g of crude powder (*S. aureus* 350, *B. subtilis* 75 du/mg). The crude powder was separated by a column chromatography on 3.5 kg of silica gel (0.05~0.20 mm) and eluted with 15 liters of benzene, benzene-ethyl acetate (9 : 1), (8 : 2), (7 : 3), (6 : 4), (5 : 5), (4 : 6), (3 : 7), (2 : 8), (1 : 9), ethyl acetate, ethyl acetate-acetone (9 : 1), acetone, and methanol. Each fraction was checked by the paper disc method (test organism: *S. aureus* and *B. subtilis*) and thin-layer chromatography of silica gel HF_{254} (color reagent: conc. H_2SO_4, solvent system: diethyl ether-ethyl acetate (3 : 1)). Pure fractions were concentrated under reduced pressure and crystallized from the suitable solvents; T-2636 A 6.5 g (diethyl ether-ethyl acetate), T-2636 B 4 g (diethyl ether), T-2636 C 29 g (ethyl acetate), T-2636 D 30 g (ethyl acetate-methanol) and T-2636 E 0.1 g (acetone).

Compound	m.p. (°C) (decomp.)	$[\alpha]_D$	Anal. (%) Found C	H	N	O	Calcd. C	H	N	O
T-2636 A	207~210	−235°	64.62	7.10	2.83		64.67	6.99	2.79	
B	205~207	−92.4°	59.87	8.59	0	30.81	59.98	8.43	0	31.59
C	201~203	−240°	65.30	7.20	3.13		65.34	7.24	3.05	
D	190~191	−226°	64.22	7.67	2.83		64.40	7.41	2.78	
E	228~230	−320°	67.42	8.29	3.14		67.95	8.11	3.05	
M		−210°	61.23	8.56	0					

T-2636 A monoacetate (II) was prepared by dissolving 300 mg of T-2636 A in 3 ml of pyridine and 1.5 ml of acetic anhydride. After standing at room temperature for 10 hours, the reaction mixture was poured into iced water. The precipitate was separated by filtra-

tion and recrystallized from diethyl ether-*n*-hexane to yield 170 mg of colorless prisms, m. p. 136~140°C (decomp.), $[\alpha]_{24}^{D}$ −212° (*c* 0.5 in EtOH). Elemental analysis is calcd. for $C_{29}H_{37}NO_9$; C 64.07, H 6.86, N 2.58 (%), found, C 63.75, H 6.81, N 2.52 (%). The UV spectrum in ethanol solution shows a peak at 226.5 mμ (ε 47,000).

T-2636 C diacetate was prepared by dissolving 5 g of T-2636 C in 20 ml of pyridine and 10 ml of acetic anhydride. After treatmeat of the reaction mixture as mentioned above 3 g of pure crystal was obtained; m. p. 136~140°C (decomp.), $[\alpha]_{D}^{24}$ −211° (*c* 0.5 in EtOH). The elemental analysis is found; C 63.77, H 6.89, N 2.74 (%). The IR, UV, MS and NMR spectra were in accordance with those of T-2636 A monoacetate (II).

T-2636 D diacetate (VI). A solution of 225 mg of T-2636 D in 20 ml of pyridine and 10 ml of acetic anhydride was held at 5°C for 15 hours, then, poured into ice-water. The crude crystal was filtered and recrystallized from diethyl ether-ethyl acetate mixtures; colorless prisms, 125 mg, m. p. 211~215°C (decomp.), $[\alpha]_{D}^{24}$ −216° (*c* 0.5 in EtOH). The elemental analysis is calcd. for $C_{31}H_{41}NO_{10}$; C 63.36, H 7.03, N 2.38 (%), found, C 63.13, H 6.84, N 2.52 (%). The UV spectrum is determined in ethanol: λ_{max} 229 mμ (ε 50,900).

Acknowledgement

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