DELTAMYCINS, NEW MACROLIDE ANTIBIOTICS. II

ISOLATION AND PHYSICOCHEMICAL PROPERTIES*

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The new macrolide antibiotics were isolated by silica gel chromatography. They showed a UV absorption maximum at 240 nm. Deltamycins A_1, A_2 and A_3 were demonstrated to be new macrolide antibiotics on the basis of their physicochemical properties whereas deltamycin A_4 was identified as carbomycin A.

Streptomyces halstedii subsp. *deltae* was found to produce a group of basic macrolide called deltamycins. The group of antibiotics was separated into four components by silica gel TLC and designated as deltamycins A_1, A_2, A_3 and A_4 based on the order of migration from the origin of TLC. This paper deals with the isolation and physicochemical properties of deltamycins A_1, A_2, A_3 and A_4.

1. Isolation

The isolation and purification of the deltamycin complex was accomplished by the established procedure for basic macrolide antibiotics. Culture broth of *Streptomyces halstedii* subsp. *deltae* was adjusted to pH 3.8 and the mycelium was removed by filtration using Perlite (Tōkō Perlite Co.) as a filter aid. The filtered broth was extracted twice with benzene after adjustment to pH 8.0. After concentrating the benzene extract under reduced pressure, the activity was transferred into hydrochloric acid - acetate buffer solution at pH 2.5. The acidic aqueous solution was adjusted to pH 8.0 and the activity was extracted twice with benzene. The benzene layer was concentrated to dryness. The crude powder was dissolved in a small amount of methanol and passed through a Sephadex LH-20 column using methanol as an eluant to remove colored impurities. The crude complex thus obtained was chromatographed on a silica gel column and eluted with a mixed solvent of benzene-acetone, giving four active fractions. The fractionation was checked by coloration with H_2SO_4 on a silica gel TLC plate. By repeated crystallization from benzene - *n*-hexane, deltamycins A_1, A_2, A_3 and A_4 were isolated as colorless needles from the four active fractions, respectively. The extraction and purification procedure is shown in Chart 1.

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Chart 1. Extraction and purification of deltamycin components

Culture broth (9.5 liters, 120 μg/ml)
↓ filtered at pH 3.8
Filtrate (8.1 liters)
↓ C_6H_6 2.0 liters×2, at pH 8.0
C_6H_6 layer
↓ concd.
C_6H_6 soln. (400 ml)
↓ 0.1 M HCl - acetate buffer soln. (pH 2.5) 100 ml×2
Aqueous layer (200 ml)
↓ C_6H_6 100 ml×2, at pH 8.0
C_6H_6 layer (200 ml)
↓ concd.
Crude powder 630 mg
↓ Sephadex LH-20 column 100 g (400 ml), eluted with MeOH
Active fraction
↓ concd.
Deltamycin complex 590 mg
↓ Silica gel column (Wakō gel) 100 g, eluted with C_6H_6 - Me_2CO

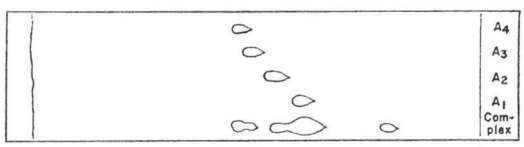
Component	A_4	A_3	A_2	A_1	minor
Elution solvent: C_6H_6 - Me_2CO	5:1	5:1	5:1	3:1	1:1
Yield (mg)	136	15	47	330	11

2. Physicochemical Properties

The physicochemical properties of each deltamycin component are listed in Table 1. The components had similar physicochemical properties. They were soluble in methanol, ethanol, butanol, acetone, ethyl acetate, chloroform, diethylether, benzene and acidic aqueous solution and slightly soluble in petroleum ether (b. p. 30~60°C), *n*-hexane and water. The antibiotics gave positive anthrone, SELIWANOFF, MOLISCH and sulfuric acid reactions and also the carbomycin test[2] and gave negative ninhydrin, biuret, MILLON, xanthoprotein and EHRLICH reactions. The components were weakly basic, had pKa values of 7.12~7.25 in 66.7% aqueous methanol and molecular weights of 800~900 by the titration equivalent method. They showed a strong UV absorption maximum at 239~240 nm (log ε=4.17~4.20).

Deltamycins A_1, A_2, A_3 and A_4 were compared on silica gel TLC plate with known basic macrolides showing absorption maxima near 240 nm.[3]. The Rf values of deltamycin components and known basic macrolides are shown in Table 2 and Fig. 1 together with their sulfuric acid coloration characteristics. From these data, they were found to differ from angolamycin[4], cirramycins A_1[5] and B_1[5] and rosamicin[6]. However deltamycin A_4 was confirmed to be identical to carbomycin A.

Fig. 1. Thin-layer chromatogram of deltamycin



Solvent a, H_2SO_4 coloration

The IR spectra of these components shown in Figs. 2, 3, 4 and 5 are very close to each other and

Table 1. Physicochemical properties of deltamycin components

Deltamycin	A_1	A_2	A_3	A_4
m.p. (°C, dec.)*	201~204	189~194	197~201	211~214
$[\alpha]_D^{24}$ (*c*, $CHCl_3$)	−49.3 (0.23)	−55.3 (0.25)	−56.7 (0.26)	−60.7 (0.25)
pKa (66.7% MeOH)	7.23	7.25	7.12	7.16
UV λ_{max}^{MeOH} (nm) (log ϵ)	240 (4.17)	239 (4.17)	239 (4.17)	240 (4.20)
Mol. Wt.				
Titration eq. (in 66.7% MeOH)	796	813	793	900
M^+ ion peak				
intact (*m/e*)	799	813	827	841
monoacetate (*m/e*)	841	855	869	883
Elem. Analysis (%) Calcd. as	$C_{39}H_{61}NO_{16}$	$C_{40}H_{63}NO_{16}$	$C_{41}H_{65}NO_{16}\cdot\frac{1}{2}H_2O$	$C_{42}H_{67}NO_{16}\cdot\frac{1}{3}C_6H_6$
	Found Calcd.	Found Calcd.	Found Calcd.	Found Calcd.
C	58.45 58.56	58.89 59.02	58.65 58.84	60.99 60.88
H	7.51 7.68	7.78 7.80	7.77 7.95	7.91 8.01
N	1.80 1.75	1.70 1.72	1.75 1.67	1.76 1.61
Formula	$C_{39}H_{61}NO_{16}$	$C_{40}H_{63}NO_{16}$	$C_{41}H_{65}NO_{16}$	$C_{42}H_{67}NO_{16}$
Mol. Wt. (Calcd.)	799.92	813.94	827.97	841.99

*: uncorr. (KOFLER)

Table 2. Rf values of deltamycin and other macrolides

Antibiotic	Solvent system			H_2SO_4 coloration
	a	b	c	
Deltamycin A_1	0.41	0.55	0.46	purplish blue
〃 A_2	0.47	0.59	0.50	〃
〃 A_3	0.51	0.61	0.55	〃
〃 A_4	0.54	0.62	0.58	〃
Angolamycin	0.23	0.42	0.44	brown
Cirramycin A_1	0.04	0.07	0.49	brownish yellow
〃 B_1	0.49	0.57	0.68	〃
Rosamicin	0.04	0.13	0.49	〃
Carbomycin A	0.54	0.63	0.58	purplish blue

Solvent a: C_6H_6 - Me_2CO (2: 3), (Merck TLC plate)*
〃 b: $CHCl_3$ - MeOH (10: 1), (Merck TLC plate)
〃 c: *n*-BuOH - AcOH - H_2O (3: 1: 1), (Merck TLC plate)
*: Merck TLC plate Silica gel 60 F_{254} pre-coated.

support the idea that the main portion of the structures is common. Each component gave strong bands at 3450 cm^{-1} (hydroxyl), 1740 cm^{-1} (carbonyl), 1695 cm^{-1} (α, β-unsaturated carbonyl) and 1620 cm^{-1} (double bond). If the IR spectrum of deltamycin A_1 is compared with those of other deltamycins, the band at 1240 cm^{-1} which was attributed to the acetyl group had twice or greater intensity. The IR spectrum of deltamycin A_4 was coincident with that of carbomycin A[7].

It is obvious that deltamycins are basic macrolide antibiotics from the above-mentioned chromatographic, physicochemical properties and solvent extraction behavior. The strong UV maximum at 240 nm of four components indicated a chromophore having γ,δ-epoxy-α,β-unsaturated

Fig. 2. Infrared spectrum of deltamycin A_1

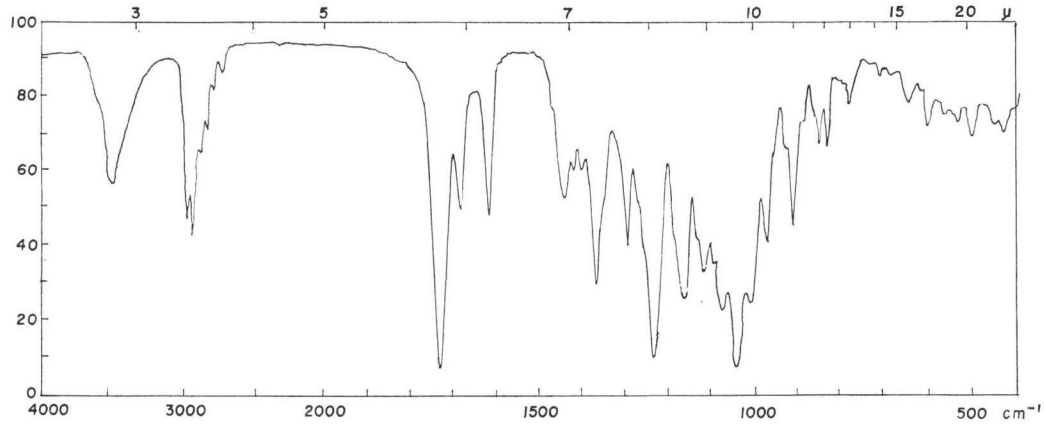


Fig. 3. Infrared spectrum of deltamycin A_2

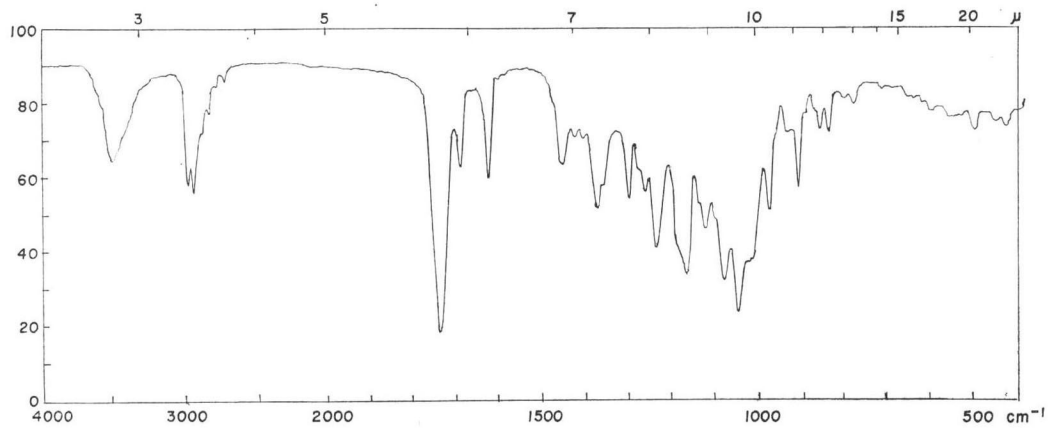


Fig. 4. Infrared spectrum of deltamycin A_3

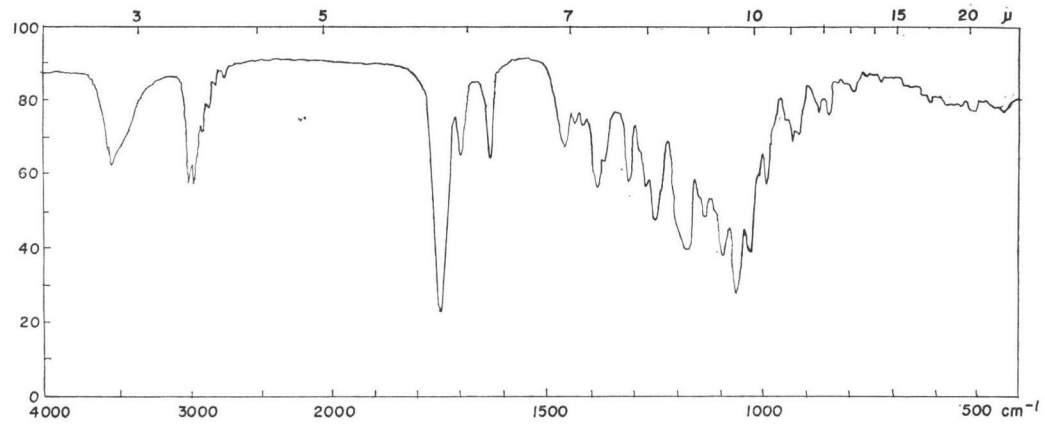
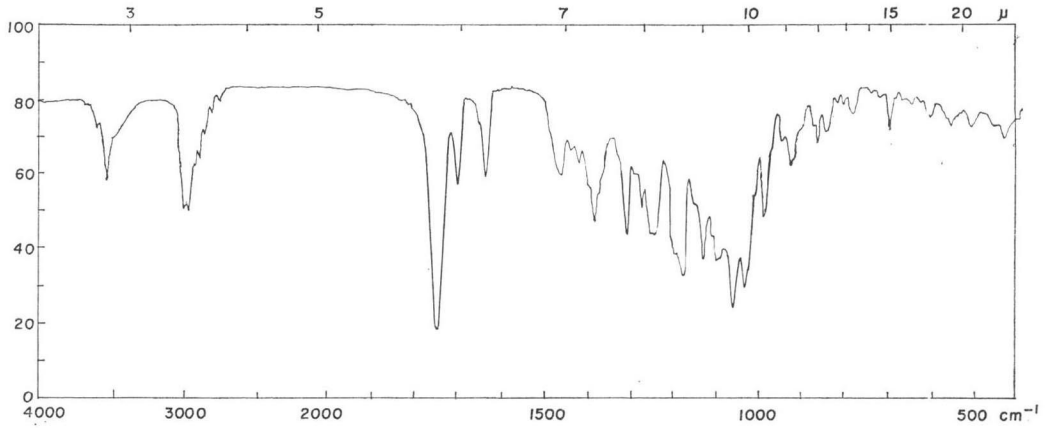


Fig. 5. Infrared spectrum of deltamycin A_4



ketone which occurs in the structure of carbomycin A, cirramycins, rosamicin and angolamycin. The IR spectra also supported the presence of the α,β-unsaturated ketone group. From the fact that deltamycin A_4 was coincident with carbomycin A in the physicochemical properties, the deltamycins were thought to relate to the carbomycin A group.

Experimental

General method: UV absorption spectra were measured with a Hitachi EPS-3T spectrophotometer, IR absorption spectra with a Hitachi EPI-G_2 spectrophotometer, mass spectra with Hitachi RMU-7L and RMU-6M mass spectrometers, optical rotation values with a JASCO DIP-180 automatic polarimeter and pKa values with a Metrohm E 436 potentiograph.

Deltamycin A_1 Monoacetate:

To dried benzene solution (1 ml) containing 100 mg of deltamycin A_1 was added acetic anhydride (0.06 ml) under stirring and ice-cooling. The solution was stirred at room temperature for 17 hours. After reaction, the solution was diluted with benzene (9 ml), washed with 5% $NaHCO_3$ aqueous solution (5 ml × 3), dried and evaporated under reduced pressure. The residue was crystallized from benzene - *n*-hexane. Colorless needles, m.p.: 134~137°C (dec.), mass spectrum *m/e*: 841 (M^+).

Deltamycin A_2, A_3 or A_4 Monoacetate:

Synthetic procedure of each monoacetate was same as that of the deltamycin A_1 derivative. Deltamycin A_2 monoacetate; colorless needles, m.p.: 134~138°C (dec.), mass spectrum *m/e*: 855 (M^+). Deltamycin A_3 monoacetate; colorless needles, m.p.: 133~137°C (dec.), mass spectrum *m/e*: 869 (M^+). Deltamycin A_4 monoacetate; colorless needles, m.p.: 138~142°C (dec.), mass spectrum *m/e*; 883 (M^+).

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References

1) SHIMAUCHI, Y.; K. OKAMURA, A. KOKI, M. HASEGAWA, M. DATE, Y. FUKAGAWA, K. KOUNO, T. ISHIKURA

& J. LEIN: Deltamycins, new macrolide antibiotics. I. Producing organism and fermentation. J. Antibiotics 31: 261~269, 1978

2) FISCHBACH, H. & J. LEVINE: The identification of the antibiotics. Antibiot. & Chemoth. 3: 1159~1169, 1953

3) CELMER, W. D.: Stereochemical problems in macrolide antibiotics. Pure Appl. Chem. 28: 413~453, 1971

4) CORBAZ, R.; L. ETTLIGER, E. GÄUMANN, W. KELLER-SCHIERLEIN, L. NEIPP, V. PRELOG, P. REUSSER & H. ZÄHNER: Stoffwechselprodukte von Actinomyceten. Angolamycin. Helv. Chim. Acta 38: 1202~1209, 1955

5) a) KOSHIYAMA, H.; M. OKANISHI, T. OHMORI, T. MIYAKI, H. TSUKIURA, M. MATSUZAKI & H. KAWAGUCHI: Cirramycin, a new antibiotic. J. Antibiotics, Ser. A 16: 59~66, 1963
b) KOSHIYAMA, H.; H. TSUKIURA, K. FUJISAWA, M. KONISHI, M. HATORI, K. TOMITA & H. KAWAGUCHI: Studies on cirramycin A_1. I. Isolation and characterization of cirramycin A_1. J. Antibiotics 22: 61~64, 1969

6) REIMANN, H. & R. S. JARET: Structure of rosamicin, a new macrolide from *Micromonospora rosaria*. J. Chem. Soc., Chem. Comm. 1972: 1270, 1972

7) WAGNER, R. L.; F. A. HOCHSTEIN, K. MURAI, N. MESSINA & P. P. REGNA: Magnamycin, new antibiotic. J. Am. Chem. Soc. 75: 4684~4687, 1953