STUDIES ON THE ANTIBIOTICS FROM *STREPTOMYCES SPINICHROMOGENES* VAR. *KUJIMYCETICUS*. III

THE STRUCTURE OF KUJIMYCIN A AND KUJIMYCIN B

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Kujimycins A and B obtained from the fermentation broth of *Streptomyces spinichromogenes* var. *kujimyceticus* have already been reported as neutral macrolides exhibiting activities against Gram-positive bacteria[1]. Recently, in the course of structural investigation, kujimycin B has been found to be identical with an authentic specimen of lankamycin. The structure of kujimycin A has been elucidated by some chemical degradation studies as lankamycin deacetylated at C-4 of arcanose.

The authors reported previously that kujimycin A (I) and kujimycin B (II) were different from lankamycin in respect to crystal form, melting point, infrared spectrum, colors of the carbomycin test and some antimicrobial activities[1]. Recently, II was found to be identical with an authentic specimen of lankamycin, which was a gift of CIBA Pharmaceutical Products Inc., by comparison of some of their physicochemical and microbiological properties. On the other hand, the structure of I resembled that of II and has been investigated by some chemical degradation studies. The present paper reports the structural elucidation of I.

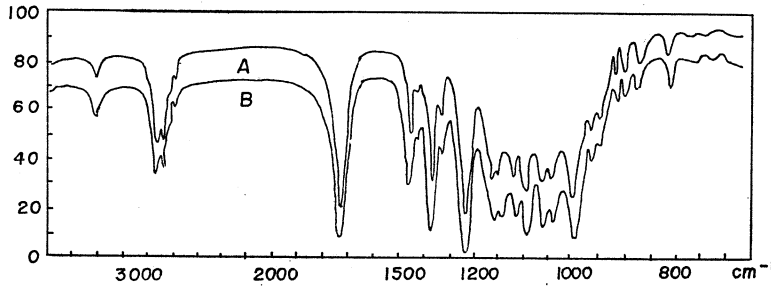
Structure of kujimycin A (I)

Kujimycin A (I) has been proven to differ from II in some physicochemical properties. Compound I can be separated from II by chromatography on a column of silica gel eluted by benzene-acetone or ethyl acetate, and obtained from carbon tetrachloride-*n*-hexane as a colorless amorphous powder, m. p. 114~115°C. The possible formula, $C_{40}H_{70}O_{15}$ (mol. wt. 791) is compatible with the elemental analysis and the molecular weight determination by vapor pressure method (mol. wt. 780±20 in acetone). The antimicrobial spectrum of I closely resembles that of II.

The infrared spectrum of I shows strong peaks at 3470 cm^{-1} (hydroxyl), 1760, 1735 cm^{-1} (carbonyl) and 1230 cm^{-1} (acetyl). Compund I has also a similar ultraviolet absorption spectrum as II. The saturated character of I is suggested by negative reactions of the decolorization tests with permanganate and bromine. In addition to the above facts, the molecular formula of I, $C_{40}H_{70}O_{15}$ suggests that I is a new neutral macrolide resembling II, $C_{42}H_{72}O_{16}$, and has only one acetyl group.

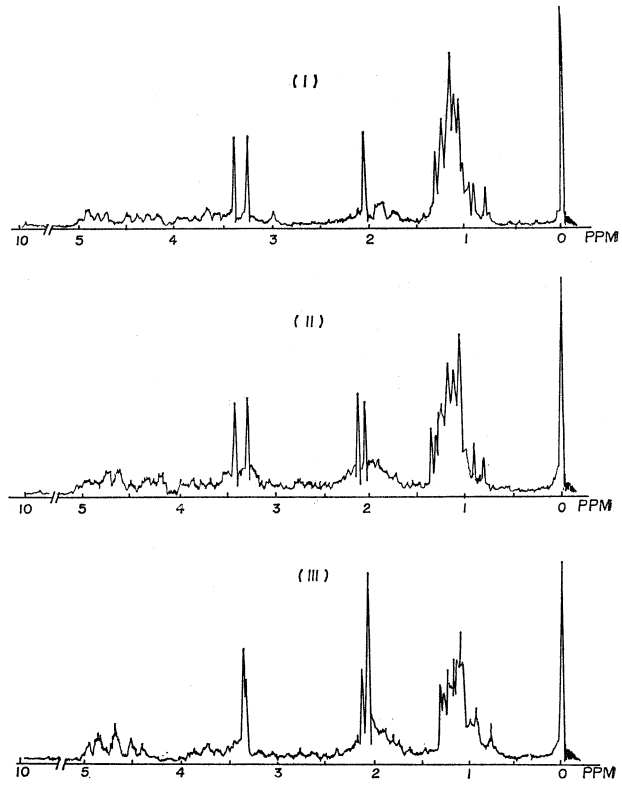
Acetyl derivatives of I and II are formed on acetylation in acetic anhydride-

Fig. 1. Infrared absorption spectra of triacetyl kujimycin A (A) and diacetyl kujimycin B (B) in KBr



pyridine. These two acetyl derivatives have been shown to be identical by the comparisons of their melting points, the infrared spectra and the behavior on thin-layer chromatography. The remaining hydroxyl group is observed in the acetate (III) by the infrared spectrum, indicating a presence of a tertiary hydroxyl group in I as shown in Fig. 1. The NMR spectra of I, II and III in deuterochloroform are shown in Fig. 2. The spectrum of I suggests the presence of two methoxyl groups (s, 3H, 3.26 ppm, s, 3H, 3.41 ppm), one acetyl group (s, 3H, 2.05 ppm) and a number of C-methyl groups at around 1 ppm. The spectrum of II supported the presence of two acetyl groups (s, 3H, 2.05 ppm, s, 3H, 2.11 ppm). In the spectrum of III were observed two acetyl signals (s, 9H, 2.05 ppm, s, 3H, 2.11 ppm) corresponding to four acetyl groups. Absence of an acetyl signal at 2.11 ppm in I indicates that I has only one acetyl group in the carbon skelton of II in which there are four possible positions to be acetylated, hydroxyl groups at C-11 and C-15 of lankolide, at C-4 of arcanose and at C-2 of lankavose.

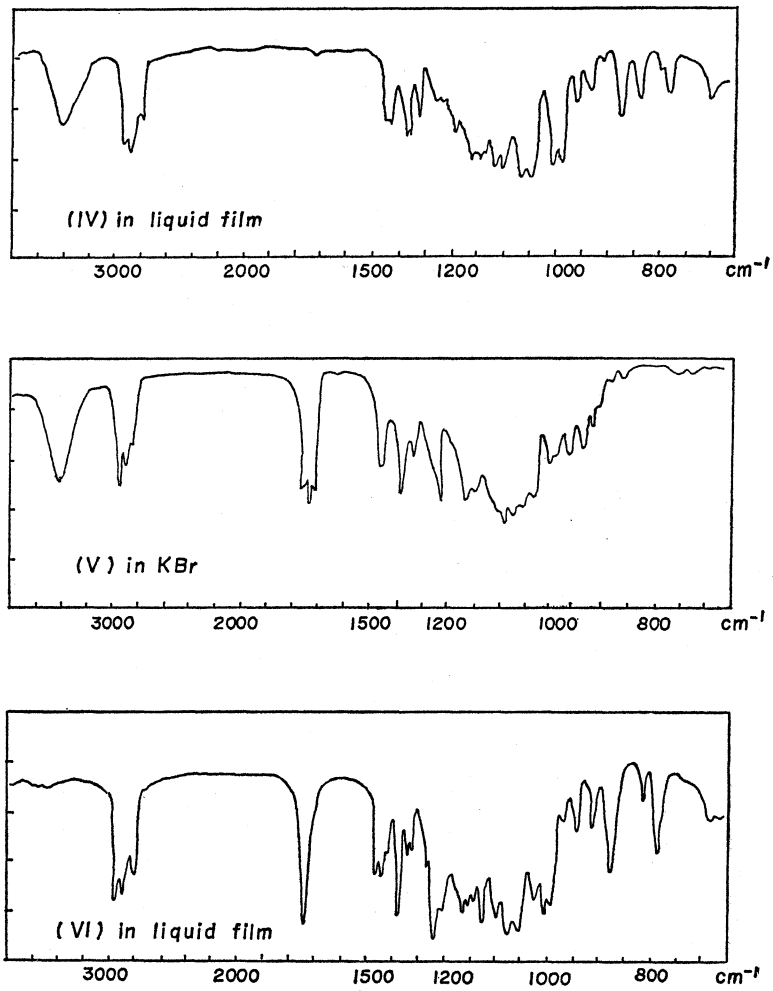
Fig. 2. NMR spectra of kujimycin A (I), kujimycin B (II) and their acetate (III) (60 Hz, in deuterochloroform)

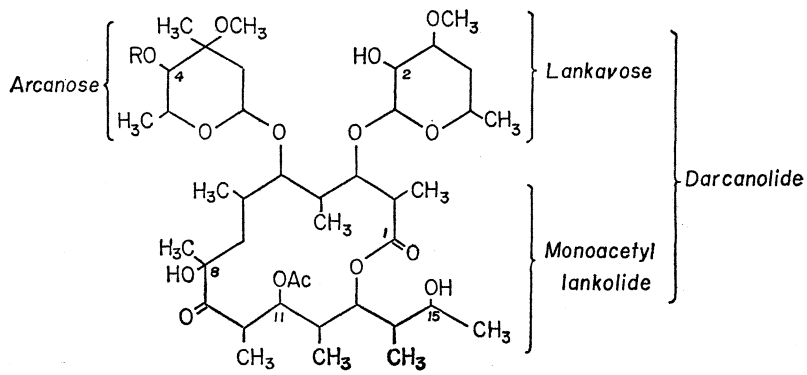


On the alkaline hydrolysis of I, one mole of acetic acid was eliminated. On the other hand, two moles of acetic acid were eliminated from II.

In order to find the position of an acetyl group in I, some degradation studies were carried out. The methanolysis of I afforded a neutral sugar (IV), which was a colorless oil and analysed as $C_9H_{18}O_4$, and compound V, which was a colorless needle

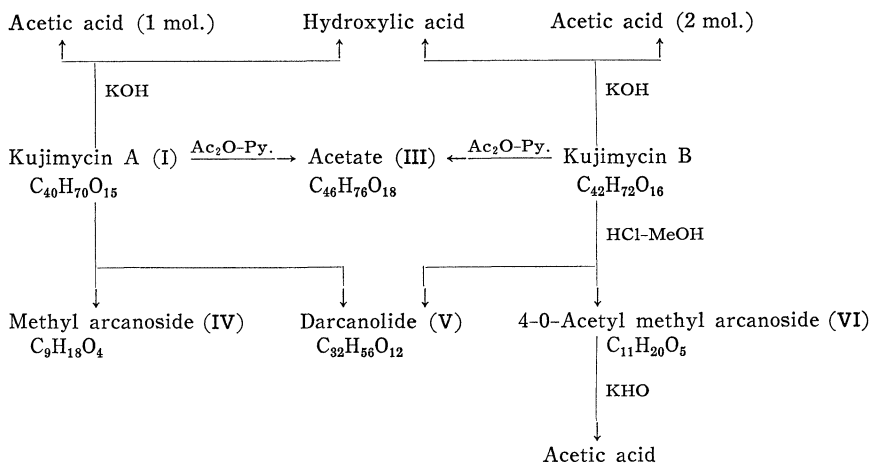
Fig. 3. Infrared absorption spectra of methyl arcanoside (IV), darcanolide (V) and 4-0-acetyl methyl arcanoside (VI)





I R=H : Kujimycin A II R=Ac : Kujimycin B

Chart 1.



and anaylsed as $C_{32}H_{56}O_{12}$, melting at 104~105°C. Compound **IV** was identified as methyl arcanoside obtained from the alkaline hydrolysate of 4-0-acetyl methyl arcanoside (**VI**) when compared by the infrared spectrum, the NMR spectrum and the thin-layer chromatography. The infrared spectrum of **IV** shows the absorption band at 3470 cm^{-1} attributed to a hydroxyl group and no absorption bands at 1740 and 1230 cm^{-1} attributed to an acetyl group. In the NMR spectrum of **IV**, two methoxyl signals (s, 3H, 3.21 ppm, s, 3H, 3.48 ppm), one hydroxyl signal (broad, 1H, 2.10 ppm), and no acetyl signal were recognized, which suggested that hydroxyl group at C-4 of arcanose was not acetylated in the intact molecule of **I**.

On the other hand, **V** was identified through comparisons of the elemental analysis, the melting point, the infrared spectrum, the NMR spectrum and the behavior on thin-layer chromatography with an authentic specimen of darcanolide which was obtained by methanolysis of **II**. The infrared spectrum of **V** shows the strong peaks at 1755, 1735, 1720 and 1230 cm^{-1} which suggest the presence of a ketone, an acetyl and a lactone group as shown by the structure of lankamycin[2,3].

Also in the NMR spectrum of **V** a singlet acetyl signal is observed at 2.05 ppm (3H). On alkaline hydrolysis of **V** one mole of volatile acid was liberated. Therefore, the results of these studies support the above structure for kujimycin A (**I**).

Experimental

Comparative studies of II and lankamycin Compound **II** was obtained as a colorless prism, m. p. 181~182°C (authentic sample of lankamycin, m. p. 147~150°C, 181~182°C). The infrared spectrum and the analytical data of **II** were also identical with those of the authentic sample of lankamycin and **II** was shown not to have such absorption bands at 1634 (6.12 μ), 1497 (6.68 μ), 752 (13.30 μ) and 692 (14.45 μ) cm^{-1} as previously reported[4,5]. Also both gave a yellow color in hydrochloric acid and pale yellowish green color in butanol extract in the carbomycin test. Compound **II** had the same antimicrobial activities as lankamycin against some test organisms by the two-fold agar dilution method. Molecular weight was estimated by vapor pressure method with Hitachi-Perkin Elmer-115 Molecular Weight Apparatus. NMR spectra at 60 Hz were measured with a Hitachi-Perkin Elmer R-20 spectrometer.

Triacetyl-kujimycin A (III) (=Diacetyl-kujimycin B) Two-hundred mg of I (or II) were acetylated with 1 ml of pyridine containing 0.2 ml of acetic anhydride. After standing for 20 hours at room temperature the reaction mixture was concentrated *in vacuo* at 40°C. The residue was dissolved in benzene and chromatographed on a silica gel column using the solvent system, benzene - acetone (5 : 1). Compound III in the eluate was detected by thin-layer chromatography, and the fractions giving one spot were collected. The solution was concentrated *in vacuo*, and the amorphous powder was obtained from CCl_4-*n*-hexane (yield 120 mg). m. p. 98~100°C, Anal. Calcd. for $C_{46}H_{76}O_{18}$: C 60.24, H 8.35. Found: C 60.30, H 8.09.

The infrared spectra in KBr are shown in Fig. 1. The NMR spectrum in $CDCl_3$ is shown in Fig. 2 (III): 3.29 ppm (s, 3H, $-OCH_3$), 3.31 ppm (s, 3H, $-OCH_3$), 2.05 ppm (s, 9H, 3 $-OCOCH_3$), 2.11 ppm (s, 3H, 1 $-OCOCH_3$). Rf values of the thin-layer chromatography on silica gel G plate: 0.92 (benzene - acetone 1 : 1), 0.90 (ethyl acetate). The spot on the plate heated at 100°C for 5 minutes after spraying conc. H_2SO_4 gives a dark gray or dark brown color.

Alkaline hydrolysis of I Compound I (100 mg) was dissolved in 6 ml of EtOH to which was added 0.5 ml of 1.00 N KOH. After refluxing for 30 minutes the solution was titrated with 0.10 N HCl (1.95 molar equivalents of alkali were consumed). After evaporation of the EtOH, the remaining aqueous solution was acidified with 5 ml of 30 % phosphoric acid and 0.85 molar equivalent of acetic acid was obtained by steam distillation[6].

Methanolysis of I Compound I (300 mg) was dissolved in 2 ml of MeOH and 0.2 ml of conc. HCl. The solution was allowed to stand for 18 hours at 4°C, then neutralized to pH 4.0 with dil. NaOH. The neutralized mixture was concentrated under reduced pressure to remove MeOH and the residue was extracted with ethyl acetate. The extract was concentrated to a small volume and chromatographed on silica gel with benzene - acetone (5 : 2). The first eluate was shown to contain the sugar moiety which was identified as methyl arcanoside (**IV**) (yield 30 mg), a colorless oil, Anal. Calcd. for $C_9H_{18}O_4$: C 56.82, H 9.54. Found: C 57.02, H 9.39.

The infrared spectrum in liquid film is shown in Fig. 3 (**IV**). The NMR spectrum in $CDCl_3$: 1.24 ppm (s, 3H, $-CCH_3$); 1.24 ppm (d, J=7 Hz, 3H, $-CCH_3$); 3.21 ppm (s, 3H-OCH_3); 3.48 ppm (s, 3H, $-OCH_3$); 4.50 ppm (d, d, J=3 Hz, J=9.5 Hz, 1H at C-1); 1.37 ppm (d, d, J=9.5 Hz, J=15 Hz, 1H at C-2); 1.90 ppm (d, d, J=3 Hz, J=15 Hz, 1H at C-2); 4.07 ppm (d, q, J=2 Hz, J=7 Hz, 1H at C-5); 3.06 ppm (d, J=2 Hz, 1H at C-4); 2.10 ppm (broad, 1H at C-4 OH). The hydroxyl signal of C-4 at 2.10 ppm shifted to 2.05 ppm at 50°C and disappeared by addition of D_2O. Rf value on silica gel G plate developed with benzene - acetone (2 : 1) was 0.69 and the color of the spot was violet by heating at 100°C for 5 minutes after a spray of conc. H_2SO_4.

The second eluate contained the aglycone moiety which was identified as darcanolide (**V**) (yield 100 mg), a colorless needle, m. p. 104~105°C. Anal. Calcd. for $C_{32}H_{56}O_{12}$: C 60.74, H 8.92. Found: C 60.67, H 8.92. The infrared spectrum in KBr is shown in Fig. 3 (**V**). NMR spectrum in $CDCl_3$: a number of C-methyl groups at around 1 ppm: 2.05 ppm (s, 3H, $-OCOCH_3$); 3.34 ppm (s, 3H, $-OCH_3$). Rf value of silica gel thin-layer chromatography developed with benzene - acetone (2 : 1) was 0.44 and the color of the spot was yelowish brown by heating at 100°C for 5 minutes after spraying with conc. H_2SO_4. The yield of volatile acid was 0.8 mole.

Methanolysis of II The methanolysis and the purification of the products were carried out by the same procedure employed in I. 4-0-Acetyl methyl arcanose (**VI**), a colorless oil. Anal. Calcd. for $C_{11}H_{20}O_5$: C 56.88, H 8.68. Found: C 56.92, H 8.75. The infrared spectrum in liquid film is shown in Fig. 3 (**VI**). NMR spectrum in $CDCl_3$: 1.10 ppm (s, 3H, $-CCH_3$); 1.12 ppm (d, J=6Hz, 3H, $-CCH_3$); 2.12 ppm (s, 3H, $-OCOCH_3$); 3.23 ppm (s, 3H, $-OCH_3$); 3.48 ppm (s, 3H, $-OCH_3$); 4.55 ppm (d, d, J=3 Hz, J=9.5 Hz, 1H at C-1); 1.49 ppm (d, d, J=9.5 Hz, J=15 Hz, 1H at C-2); 1.90 ppm (d, d, J=3 Hz, J=15 Hz, 1H at C-2); 4.07 ppm

(d, d, J=2 Hz, J=7 Hz, 1H at C-5); 4.73 ppm (d, J=2 Hz, 1H at C-4). The Rf value on silica gel thin-layer chromatography developed with benzene - acetone (2 : 1) was 0.88, and the color of the spot was dark gray by heating at 100°C for 5 minutes after spraying with conc. H_2SO_4. The aglycone moiety was identified as **V** obtained from **I**.

Alkaline hydrolysis **VI** One hundred milligrams of **VI** were dissolved in 5 ml of EtOH to which was added 0.5 ml of 1 N KOH. After refluxing for 30 minutes, the solution was neutralized with 0.1 N HCl. The solution was extracted with ethyl acetate and the extract was concentrated under reduced pressure. Sixty milligrams of **IV** were obtained as a colorless oil. Anal. Calcd. for $C_9H_{18}O_4$: C 56.82, H 9.54. Found: C 57.02, H 9.40.

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