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Studies on an Antibiotic, Albocycline. III.[1] Partial Structure of Albocycline

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Albocycline, $C_{18}H_{28}O_4$, is a neutral antibiotic produced by *Streptomyces* sp. The physico-chemical properties indicated that it was one of the macrolide antibiotic consisting of only macrocyclic lactone without any carbohydrate moiety. Analytical and spectral data on albocycline, its acetate and ozonolysis products made it possible to assume that albocycline possessed a hydroxy, a methoxy, a lactone, three olefinic linkages and also four branched-methyl groups. From these data the partial structure of albocycline was suggested as illustrated in Chart 1 and 3.

Albocycline is an antibiotic isolated by the present authors from the culture broth of *Streptomyces brunneogriseus*, *S. roseocinereus* and *S. roseochromogenes* var. *albocyclini* in 1967. It was also isolated from the culture products of *S. maizeus* in 1969.[3] The antibiotic was mainly active against *Staphylococci*.[4] The physico-chemical properties and the preliminary investigations suggested that the antibiotic might be a kind of the neutral macrolide. However, different from the known neutral-macrolide antibiotics such as lankamycin,[5] chalcomycin[6] and neutramycin,[7] it indicated no evidence of the presence of a carbohydrate moiety. Therefore, it seemed to consist of only an aglycone part (namely a macrocyclic lactone ring) of the usual macrolide antibiotics. Such substances were often found in metabolites of fungal origin.[8,9] However, in *Streptomyces* sp. borrelidin[10] was reported as an only illustrative antibiotic produced.

In this paper, authors wish to report on the partial structure of albocycline elucidated from the physico-chemical properties of albocycline, its acetate and its ozonolysis products.

1) Part II: T. Furumai, N. Nagahama and T. Okuda, *J. Antibiotics* (Tokyo), Ser. A, **21**, 85 (1968).
2) Location: *50-2-2 Kawagishi, Toda, Saitama.*
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Albocycline is colorless plates with the molecular formula of $C_{18}H_{28}O_4$. In the infrared (IR) spectrum (Fig. 1), it exhibited the absorptions at 3430, 1720 and 1640 cm^{-1}, which probably due to hydroxy group, lactonic carbonyl moiety, and ethylene linkage, respectively.

In the ultraviolet (UV) spectrum the characteristic absorption was unobserved, but in the optical rotatory dispersion (first minimum: $[\phi]_{250}$-18500°) and circular dichroism (negative maximum: $[\theta]_{232}$-44900), an optical active absorption band at 232 mμ was observed which seemed to be ascribed to α,β-unsaturated lactone structure as in the case of brefeldin[8] and phomin.[11]

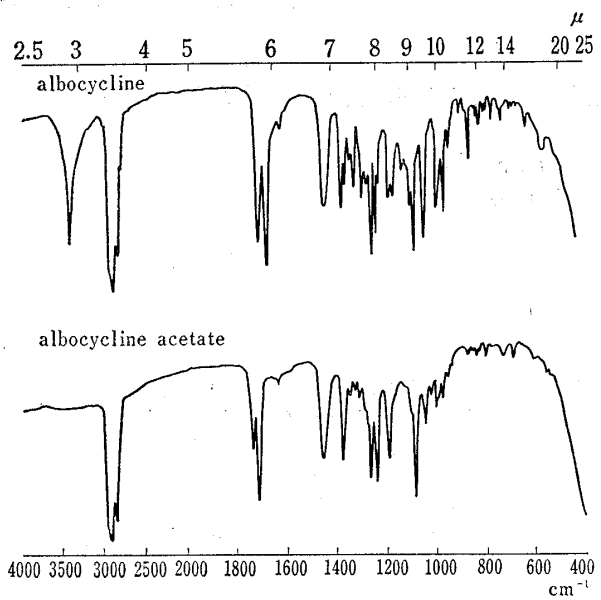

Fig. 1. IR Spectra of Albocycline and Albocycline Acetate (Nujol)

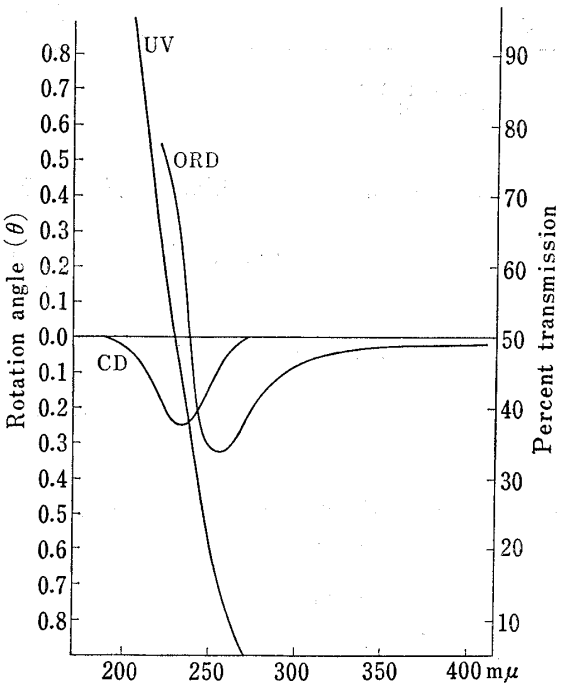

Fig. 2. UV, ORD and CD Curves of Albocycline (MeOH)

Albocycline was positive to color-reactions of iodoform and ferric hydroxamate, and decolorized potassium permanganate and bromine solutions, but was negative to all of the detective reaction of a carbohydrate. When albocycline, neutral in nature, was potentiometrically titrated with dilute potassium hydroxide solution, it was confirmed that the equimolecular alkali was consumed. This result, together with IR at 1720 cm^{-1}, suggested the presence of a lactone moiety in an albocycline molecule. Albocycline acetate, $C_{20}H_{30}O_5$, was obtained in low yield by treating albocycline with acetic anhydride and pyridine. Nuclear magnetic resonance (NMR) and IR (Fig. 1) spectral data suggested that it was a monoacetate without any more hydroxy group. Therefore, albocycline must contain only one hydroxy function in the molecule. The fourth oxygen atom in albocycline was present in a methoxy group, which was found in NMR spectrum at 6.71 τ (3H, singlet) as shown in Fig. 3.

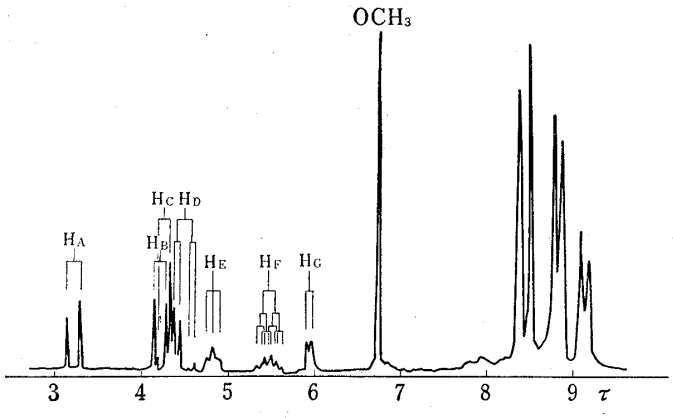

Fig. 3. NMR Spectrum of Albocycline (100 MHz, $CDCl_3$)

11) W. Rothweiler and Ch. Tamm, *Experientia*, **22**, 750 (1966).

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Thus, four oxygens in albocycline were defined as one hydroxy, one methoxy and one lactone groups.

NMR spectrum of albocycline (Fig. 3) was very informative to determine the partial structure of albocycline. In the higher field, two doublet methyl protons centered at 9.13τ (J= 6.5 Hz) and 8.83τ (J=6.5 Hz) were observed. The latter was decoupled to singlet by irradiation on the signal at 5.46 τ.

The broad peak at 8.36 τ (3H, singlet) corresponded to methyl protons connecting to olefinic carbon, because it moved to higher field by hydrogenation and changed to a doublet signal.

The sharp signal at 8.48 τ (3H, singlet) moved to lower field by 25 Hz on acetylation. It meant that hydroxy and methyl functions were connected to the same carbon, namely, a hydroxy function was tertiary. This fact made us realize that acetylation of albocycline was achieved only with a very low yield.

The olefinic protons were observed in the low field of NMR spectrum of albocycline; two protons H_A (3.13 τ, J=15 Hz) and H_B (4.14 τ, J=15 Hz), which showed AB system, were considered to be olefinic protons conjugating with a carbonyl function. They indicated the partial structure as shown in Chart 1.

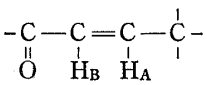

Chart 1

Other olefinic protons H_C centered at 4.19 τ (doublet, J=16 Hz) and H_D centered at 4.40 τ (doublet of doublet, J=16 Hz and J=5 Hz), which showed AB components of ABX system, were observed. H_D was coupled to the methine proton at 5.94 τ (doublet, J=6 Hz). The remaining olefinic proton H_E at 4.72 τ (triplet, J=6 Hz) seemed to be adjacent to methylene group on olefinic linkage, because it showd a clear triplet signal and coupled to methylene protons at 7.9—8.2 τ.

Two signals at 5.94 τ, H_G (1H, doublet), and 5.46 τ, H_F (1H, doublet of quartet), were ascribed to methines shifted to lower field by an oxygen function. It was assigned that H_G proton was located on a carbon bearing a methoxy group and H_F was located on a carbon bonded to lactonic oxygen. The doublet of quartet in the splitting pattern of H_F seemed to be arised from both methyl protons and a methine proton on the adjacent carbon. By irradiation at H_F signal the doublet signals of methyl proton at 8.83 τ was decoupled to reduce to a singlet.

Albocycline was oxidized with ozone, followed by oxidative degradation with hydrogen peroxide in an alkaline solution. The reaction products were then esterified with diazomethane and purified by silicagel chromatography.

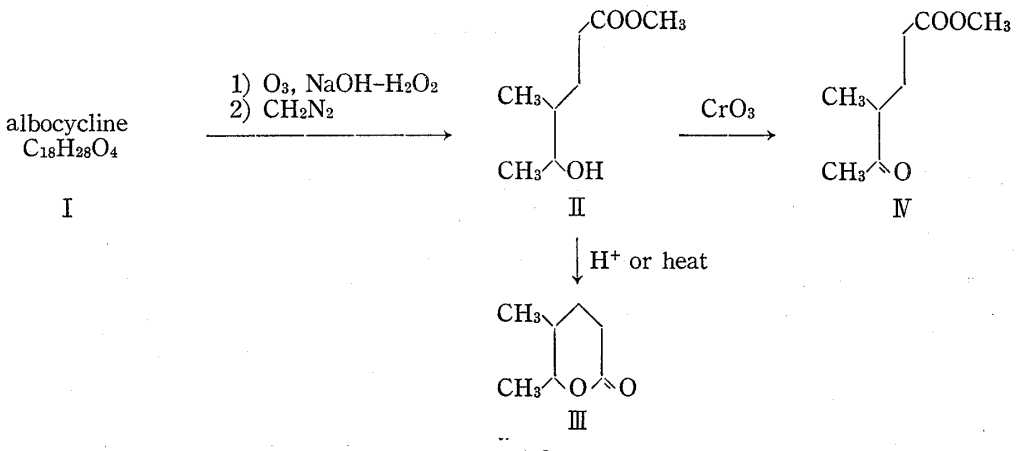

Chart 2

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The main product thus obtained was defined to be methyl 5-hydroxy-4-methylcaproate (II) by the analytical and spectral data. By treating with acid or by heating, II was immediately converted to III. Elemental analysis gave the values consistent with a molecular formula $C_7H_{12}O_2$, and IR and NMR spectra suggested the presence of a δ-lactone (1735 cm^{-1}) and two C-methyl groups (9.00 τ, 6H, doublet).

Mass spectrometric pattern of III (Fig. 4) exhibited the molecular ion peak at *m/e* 128 and the predominant peaks at 113 (M-15), *m/e* 84(M-CO_2), *m/e* 69 (M-15-CO_2), and *m/e* 56.

On the basis of these results, the structure of III was determined as 4-methyl-δ-caprolactone. The liquid IV was obtained by oxidation of II using Jones reagent in acetone.[12] IR spectrum of IV showed two carbonyl absorption at 1737 and 1712 cm^{-1} and mass spectrum (Fig. 5) gave the fragment ions originated from a ketone and a methyl ester at *m/e* 115 and *m/e* 99 respectively.

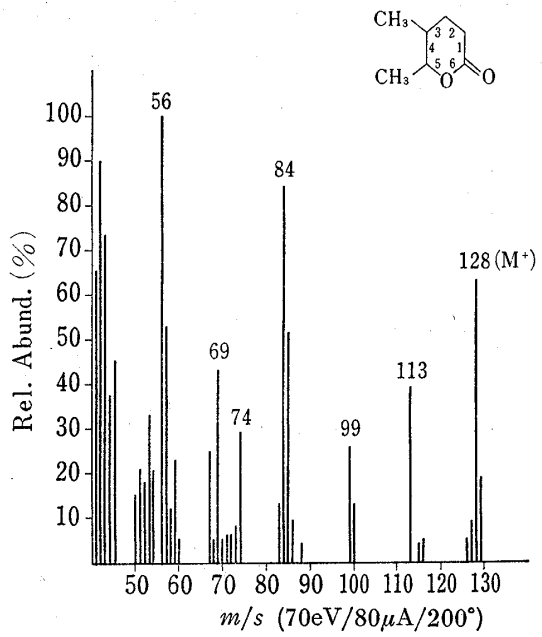

Fig. 4. Mass Spectrum of III

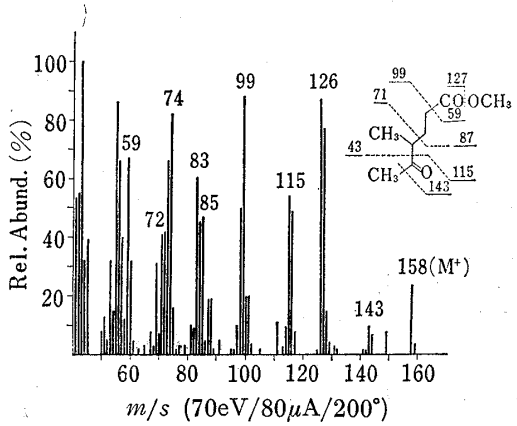

Fig. 5. Mass Spectrum of IV

Judging from the structure of the ozonolysis products, the partial structure shown in Chart 3 was proposed to albocycline. Another ozonolysis product could not be obtained in a pure form.

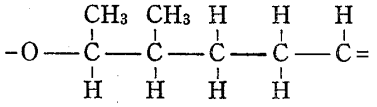

Chart 3

Cineromycin B reported by Miyairi, *et al.*[13] had the formula, $C_{17}H_{26}O_4$, and was very similar to albocycline in biological and some chemical properties. In mass spectrum, it gave the molecular ion peak at *m/e* 294 which was less than albocycline by 14 mass units corresponding to CH_2, and it gave the similar fragmentation patterns to albocycline. In NMR spectrum cineromycin B gave almost the same signals to albocycline in all field with the exception of the signal at 6.8 originated from methoxy protons (Fig. 6). Thus, cineromycin B was different

12) K. Bowden, I.M. Heilbron, E.R.H. Jones and B.C.L. Weedon, *J. Chem. Soc.*, **1946**, 39.
13) N. Miyairi, M. Takashima, K. Shimizu and H. Sakai, *J. Antibiotics* (Tokyo), Ser. A, **19**, 56 (1966).

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from albocycline in point of possession of a hydroxy instead of a methoxy moiety in albocycline molecule; namely cineromycin B was des-O-methyl albocycline.

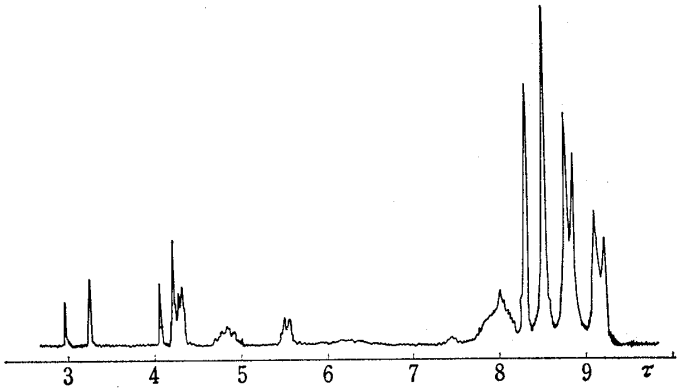

Fig. 6. NMR Spectrum of Cineromycin B (60 MHz, $CDCl_3$)

Periodate oxidation was performed for these two antibiotics. When the consumed volume of periodate in each antibiotic was compared, cineromycin B consumed about one mole of periodate, while albocycline did about a quarter moles. From these results it was estimated that there were vicinal hydroxy group in cineromycin B, and consequently, hydroxy and methoxy functions were adjacent in albocycline. The periodate oxidation data will be discussed again in following reports.

Experimental

Albocycline Acetate——500 mg of albocycline was dissolved in a solution of 10 ml of Ac_2O and 2 ml of dry pyridine, and kept at room temperature for 3 days. The reaction mixture was poured into ice water, and extracted with $CHCl_3$, which was washed with 5% $NaHCO_3$ solution and water. The extract was evaporated *in vacuo* to obtain an oily residue, which was separated by means of the preparative TLC using ether–isopropyl ether (1:1). The ethereal extract of silica gel containing acetate was concentrated *in vacuo* and stored at 0° overnight to give crystals, which were recrystallized from *n*-hexane as colorless needles. Yield, 80 mg, mp 84°. *Anal.* Calcd. for $C_{20}H_{30}O_5$: C, 68.45; H, 8.57. Found: C, 68.24; H, 8.40. Mol. wt. 350 (Mass Spectrum). IR ν_{max}^{Nujol} (cm^{-1}): 1740, 1720, 1640, 1270, 1190, 1090, 1050. NMR τ ($CDCl_3$): 7.88 (3H, s, CH_3 of acetate), 8.22 (3H, s, shifted from 8.54 of albocycline).

Methyl 5-Hydroxy-4-methylcaproate (II)——1.0 g of albocycline was dissolved in 75 ml of AcOEt and ozonized at −70°. The reaction mixture was bubbled by N_2 to remove the excess ozone and then, added drop by drop into the mixed solution of 30% H_2O_2 (45 ml) and 10% NaOH (150 ml) under stirring at below 0°. It was set aside at room temperature for 2 hours and then refluxed at 85° in a water bath for 1 hour. The reaction mixture was concentrated *in vacuo* to about 130 ml, and continuously extracted with ether at pH 4.0 (adjusted with 10% H_2SO_4) for 30 hours. The extract was dried with Na_2SO_4 and concentrated to about 30 ml *in vacuo*, and then esterified with diazomethane. After standing for several hours, the reaction mixture was evaporated to dryness to furnish an oily residue (300 mg), which gave many spots on TLC. It was passed through the column of Silica gel H (Merck, 30 g) using the mixed solvent of ether and petroleum ether. The elution with ether–pet. ether (4:6) was evaporated *in vacuo* and 25 mg of the hydroxy ester (II) was obtained as oily state, which was easily converted to III by heating or addition of HCl. *Anal.* Calcd. for $C_8H_{16}O_3$: C, 60.00; H, 10.00. Found: C, 60.65; H, 10.48. IR ν_{max}^{liquid} (cm^{-1}): 34.60 (OH), 1735 (C=O).

4-Methyl-δ-carpolactone (III)——III was obtained in its pure form by collecting the portion, which was eluted at 8.2 min in gas chromatography (1.5 m, 5% SE-30, N_2, 90 ml/min, 180°) of II, bp 145—147°. *Anal.* Calcd. for $C_7H_{12}O_2$: C, 65.68; H, 9.45. Found: C, 64.99; H, 9.40. IR ν_{max}^{Nujol} (cm^{-1}): 1735, 1250 and 1225. Mass Spectrum *m/e*: 128 (M^+), 113 (M-CH_3), 99 (M-HCO), 84 (M-CO_2). NMR τ ($CDCl_3$): 9.00 (3H, d, J=6.0 Hz, $C\underline{H}_3$-CH), 8.65 (3H, d, J=6.0 Hz, $C\underline{H}_3$-CH$\langle_{O}^{_}\rangle$), 8.30 (3H, m), 7.45 (2H, t).

Methyl 5-Keto-4-methylcaproate (IV)——126 mg of II was dissolved in 10 ml of acetone. Into it 0.5 ml of Jones 8N chromic acid solution was added and set aside for 10 min at room temperature. The reaction solution was poured into ice–water and was extracted with ether. The extract was washed with water and dried with Na_2SO_4, and evaporated to dryness. The residue was further purified by preparative gas chromatography (1.5 m, 5% SE-30, He, 50 ml/min). Finally, 10 mg of pure IV was obtained in oily state. Colorless liquid. *Anal.* Calcd. for $C_8H_{14}O_3$: C, 60.76; H, 8.22. Found: C, 60.10; H, 8.20. IR ν_{max}^{liquid} (cm^{-1}): 1737, 1712. Mass Spectrum *m/e*: 158 (M^+), 143 (M-CH_3), 127 (M-CH_3O), 126 (M-CH_3OH), 115 (M-CH_3CO), 99 (M-CO_2CH_3).

Periodic Acid Oxidation of Albocycline and Cineromycin B——Albocycline (1.54 mg, 0.05 mmole) and cineromycin B (14.7 mg, 0.05 mmole) were respectively dissolved in 5 ml of 70% MeOH solution containing H_5IO_6 (45.6 mg, 0.2 mmole). Each reaction mixture and the blank were allowed to stand at 17° or 25°.

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At regular intervals during the course of the reaction, 1 ml aliquot was withdrawn. To each sample solution, 3 ml of saturated $NaHCO_3$ solution, 2 ml of 0.100 N $NaAsO_2$ solution and 1 ml of 15% KI solution were added. After standing for 10 min in a dark room, each reaction mixture was respectively titrated with iodine solution using 1 drop of starch solution as an indicator. The consumed moles of periodic acid were calculated.

	Consumed mole of periodic acid (at 25°)				
	6	24	48	72	96 (hours)
Albocycline	0.08	0.30	0.48	0.55	0.58
Cineromycin B	0.46	0.92	1.00	1.02	1.08

	Consumed mole of periodic acid (at 17°)			
	16	40	64	88 (hours)
Albocycline	0.03	0.099	0.16	0.208
Cineromycin B	0.11	0.46	0.69	0.87

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