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Studies on an Antibiotic, Albocycline. IV.[1] Catalytic Hydrogenation and Structure Elucidation of Albocycline

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By the catalytic hydrogenation of albocycline at room temperature four hydrogenation products were obtained. Two of them, II and III, were confirmed to have 14-membered lactone and 5-membered lactone structure respectively from infrared (IR) and nuclear magnetic resonance (NMR) spectral data. They were the isomer and correlated to each other. These correlations suggested the position of a hydroxy group in albocycline. II was derived to the corresponding hydrocarbons (IV) by complete hydrogenation, of which mass spectra suggested the carbon skeleton and branching pattern of methyl group. From these results and the 100 MHz NMR spectral data of albocycline and its acetate, the structure of albocycline was finally proposed as Ia.

In the previous paper, the partial structure and functional groups of albocycline, a macrocyclic lactone antibiotic without any carbohydrate moiety, were presented on the basis of the spectrometric assignment of the antibiotic and its ozonolysis product.

The present paper deals with catalytic hydrogenation products of albocycline and the hydrocarbon derived from them by catalytic hydrogenation. The later products played an important role in determining the carbon skeleton of albocycline and the branching position of four methyl groups in albocycline molecule. From the structural elucidation of the hydrocarbon, albocycline was assigned to be 14-membered lactone similar to the aglycones of erythromycin,[3] oleandomycin,[4] narbomycin[5] and lankamycin.[6] On the basis of the present experimental data, and by detailed analysis of nuclear magnetic resonance (NMR) of albocycline, the structure Ia shown in Chart 1 was proposed for albocycline.

When albocycline was hydrogenated with palladium–charcoal or Adam's platinum as the catalysts, rapid uptake of 2.5—3.0 moles of hydrogen was observed. In the reaction mixture, many hydrogenation products were detected on thin-layer chromatography (TLC). By means of silica gel column chromatography, four main hydrogenation products (named H-2, H-3, H-4 and H-5) were successfully isolated and their physico-chemical properties were shown in Table I. Among these products H-4 (II) and H-5 (III) were of great use for the determination of the structure of albocycline.

According to the Cope's method applied successfully to determine the structure of polyene macrolide antibiotics,[7,8] II was further converted to the corresponding hydrocarbon as follows.

II was first reduced with lithium aluminium hydride in tetrahydrofuran to a polyol, which was then treated with red phosphorus in hydriodic acid. The deoxy compound thus obtained

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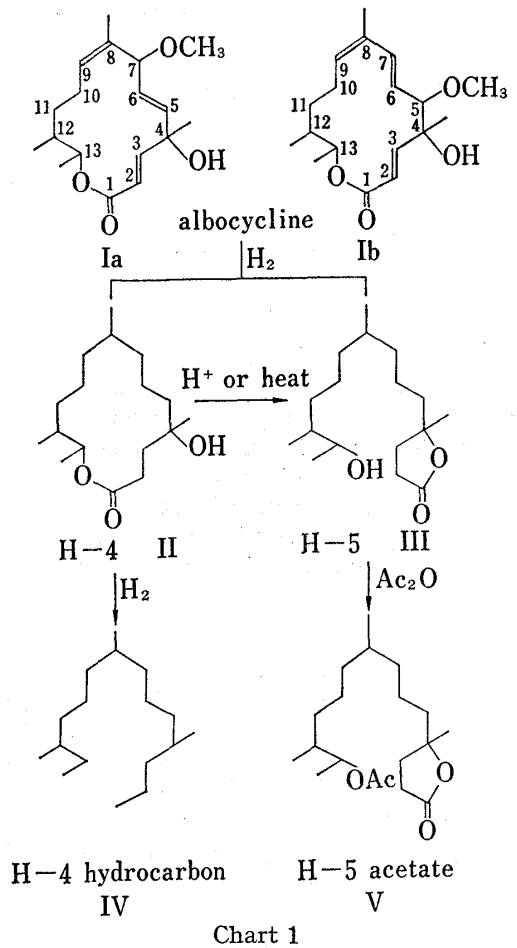


Chart 1

was further reduced with lithium aluminium hydride followed by catalytic reduction using platinum as a catalyst. The product named H-4 hydrocarbon (IV) was isolated by preparative gas chromatography. Analytical data showed that IV was a kind of saturated hydrocarbon with the molecular of $C_{17}H_{36}$. As shown in Fig. 1, mass spectrum of IV exhibited the fragmentation pattern of hydrocarbon. In addition to the molecular-ion peak at *m/e* 240 which corresponded to $C_{17}H_{36}$, the strong peaks at *m/e* 141, 127, 71 and 57 were observed. These peaks indicated that IV was 4,8,12-trimethyltetradecane (Chart 2). The present data together with precedingly reported date[1] suggested the structure of albocycline to be a 14-membered lactone.

Among many products by catalytic hydrogenation, II and III were the completely saturated products of three ethylenic linkage in albocycline. Though they were clearly distinguished from each other by thin-layer chromatography, they gave the same molecular formula, $C_{17}H_{32}O_3$, and the same retention time in gas chromatography. Their infrared (IR) and NMR data suggested the lack of a methoxy moiety which was noticed in albocycline by the absorption at 1100 cm^{-1} in IR and the signal at 6.78 τ in NMR.

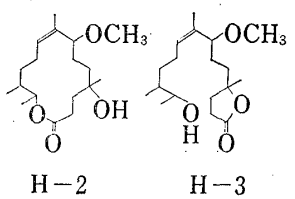
Isomerism between II and III was thought to be ascribed to the difference of a lactonic structure. Namely, II was supposed to be 14-membered lactone structure by the absorption at 1720 cm^{-1} in IR and the methine signal at 5.4τ in NMR, which was assigned in previous paper to the proton attached to a carbon bearing a lactonic oxygen. On the other hand, the

Table I. Catalytic Hydrogenation Products

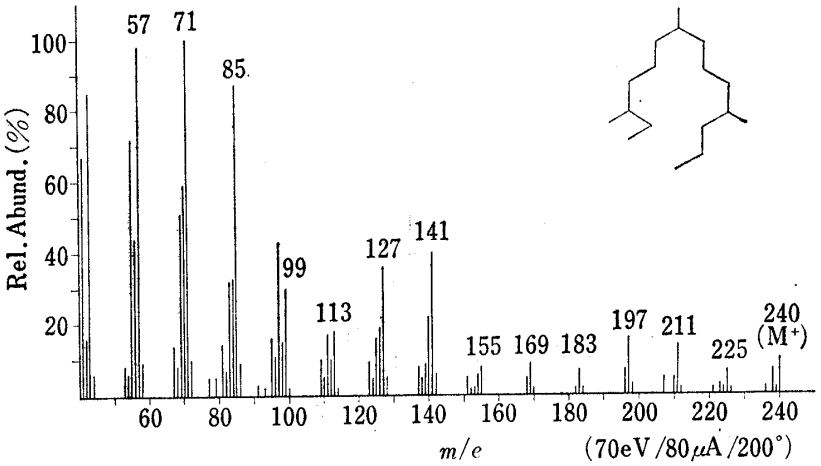
Product name	Mol. formula (M.W.)	M.P. or B.P.		*Anal.* (%) Found	Calcd.	UV	GC[a] (min)	TLC[b] (*Rf* value)
H-2[c]	$C_{18}H_{32}O_4$ (312)	liquid 175° (5 mmHg)	C	69.01	69.29	end	11.7	0.3
			H	10.41	10.34			
H-3[c]	$C_{18}H_{32}O_4$ (312)	prism 84—87°	C	69.56	69.29	end	11.7	0.4, 0.7[d]
			H	10.27	10.34			
H-4	$C_{17}H_{32}O_3$ (284)	prism 50—53°	C	71.41	71.89	end	7.7	0.15
			H	11.19	11.36			
H-5	$C_{17}H_{32}O_3$ (284)	liquid 180° (5 mmHg)	C	71.56	71.89	end	7.7	0.4, 0.6[d]
			H	10.89	11.36			

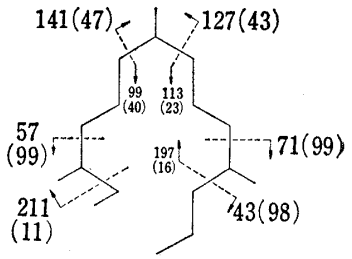
a) SE-52, 5%, 1.5m, N_2 90 ml/min, 180°
b) Silica gel G, *n*-hexane–EtOAc (1:1)
d) Alumina G, isopropyl ether–ethyl ether (1:1)

c) tentative structure



H−2 H−3



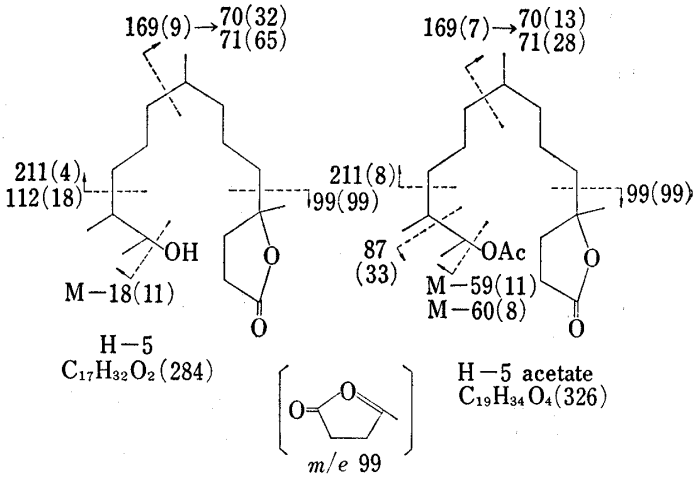


H−4 hydrocarbon
$C_{17}H_{36}$ (240)

Chart 2

Fig. 1. Mass Spectrum of H-4 Hydrocarbon (IV)

presence of 5-membered lactone moiety was suggested in III by the IR absorption at 1760 cm^{-1}. This moiety was definitely confirmed by a predominant peak at *m/e* 99 in mass spectrum (Chart 3). By acid or by heating II was caused isomerization. This phenomenon was easily detected by IR spectrum, that is, by the treatment described above the absorption at 1720 cm^{-1} due to a 14-membered lactone gradually disappeared to give the absorption at 1760 cm^{-1} due to a 5-membered lactone. The substance changed from II was identified with III in all respects of the chemical, spectrometric and chromatographic behaviors. The formation of a 5-membered lactone structure meant that the position of the hydroxy function was at C-4 of albocycline structure.



H−5
$C_{17}H_{32}O_2$ (284)

H−5 acetate
$C_{19}H_{34}O_4$ (326)

Chart 3

The position of hydroxy function in III was determined by mass spectrum of H-5 acetate (V) which was easily derived from III by the common method. Fragment ions at *m/e* 297 (M-59), 296 (M-60), 115 and 87 verified position of an acetoxyl moiety at C-13 as illustrated in Chart 3.

On the basis of these present data and the partial structures reported in previous paper, the structure of albocycline was suggested Ia or Ib . In order to confirm which structure was rational to albocycline, the precise re-examination of 100 MHz NMR spectra of albocycline and its acetate (Fig. 2, 3) was undertaken.

In spin-decoupling experiments in the spectrum, the irradiation at H_G signal attached to the carbon bearing a methoxy group caused the doublet-doublet pattern of H_D to reduce to a doublet, and changed the broad singlet methyl signal (8.36τ), which showed long range coupling to sharp splitting pattern. These data indicated that H_G was located on the carbon which situated between the ethylenic carbon bearing H_D and the ethylenic carbon bearing a methyl group. By irradiation of the methyl signal (8.29τ) on C-4 carbon in the spectrum of albocycline acetate, H_A and H_C signals gave clear splitting pattern. H_A was coupling to H_B (J=15 Hz) and H_C was coupling to H_D (J=16 Hz). It thus appeared that H_A and H_C were respectively located on carbons at C-3 and C-5 position. Furthermore, considering from coupling constants, it seemed that H_A and H_B were situated in trans and H_C and H_D were situated in trans.

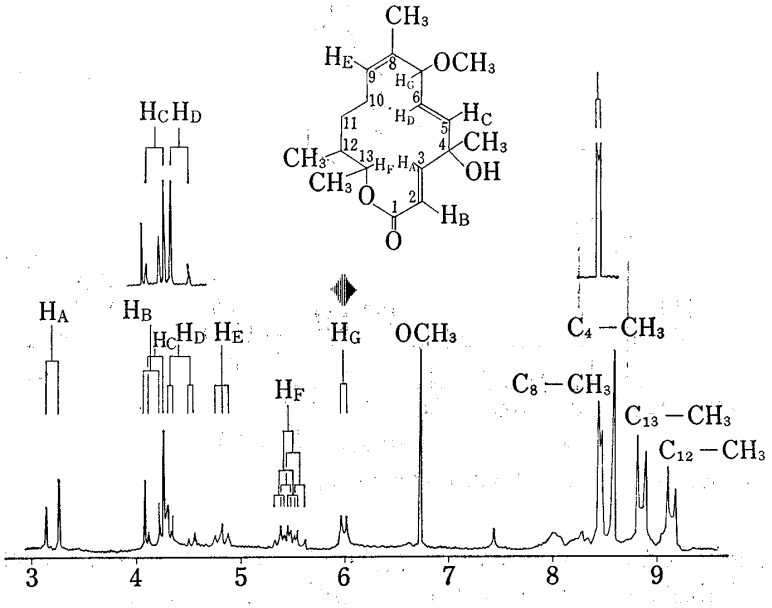


Fig. 2. NMR Spectrum of Albocycline (100 MHz, $CDCl_3$)

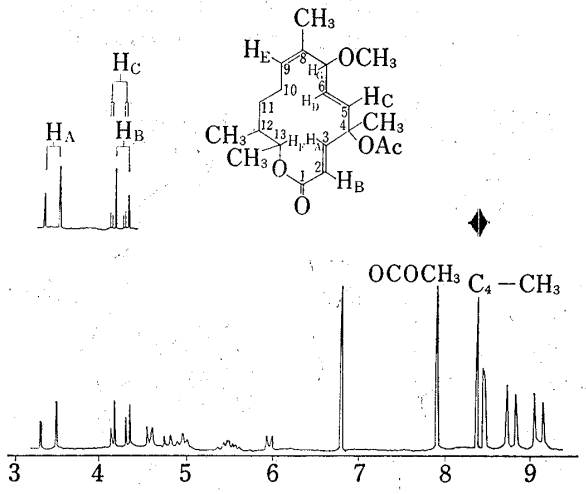


Fig. 3. NMR Spectrum of Albocycline Acetate (100 MH_2, $CDCl_3$)

From these results it was verified that a methoxy group was situated on the C-7 carbon and an olefinic linkage existed between C-5 and C-6. Thus, the structure Ia was assigned to albocycline structure.

Based on the previous data concerning periodate oxidation of cineromycin B,[9] the authors presented Ib as the structure of albocycline in former time.[10] Now, they stood for the structure Ia. Though Ia is inconsistent with the periodate oxidation data, it will be discussed in following paper.

Experimental

Catalytic Hydrogenation of Albocycline——To 100 ml of EtOH containing 5% Pd/C (300 mg), albocycline (3g) was added and shaken in H_2 at room temperature. 2.5 moles of H_2 were rapidly uptaken during 30 minutes, and adsorption ceased. After the catalyst was removed, the solution was evaporated *in vacuo* to give an oil which showed eight spots on TLC (Silicagel G, Merck) using the solvent system of *n*-hexane–AcOEt (1:1). The hydrogenation mixture was separated by column chromatography with 180 g of Silica gel H (Merck). Four main substances, H-2, H-3, H-4 and H-5, were isolated as shown in Table I.

H-4 Hydrocarbon (IV)——A solution of II (300 mg) in 50 ml of THF was dropwise added in 150 ml of THF containing $LiAlH_4$ (566 mg). After refluxing for 10 hours, AcOEt was added until no bubbles were recognized and, furthermore, the saturated aq. solution of Na_2SO_4 was added into the reaction mixture drop by drop under continuous stirring until the white precipitate no more increased. The solvent layer was separated and dried with Na_2SO_4. The aqueous layer containing the precipitate was filtered and the cake was extracted three times with each 30 ml of $CHCl_3$. The extracts were combined to the above solvent part and evaporated to afford an oil (280 mg), which was dissolved in 12 ml of hydriodic acid containing red-phosphorus (600 mg). The solution was refluxed for 24 hours on the water bath and, after cooling, extracted

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Product	Elution solvent	Fraction No.[a)]	Weight (mg)
H-5	20% AcOEt/*n*-hexane	129—139	24
H-4	20% AcOEt/*n*-hexane	148—161	73
H-3	40% AcOEt/*n*-hexane	253—271	270
H-2	50% AcOEt/*n*-hexane	333—393	179

a) volume of one fraction: 5 ml

three times with ether, which was then evaporated to dryness give an oil (356 mg). This substance was dissolved in 36 ml of tetrahydrofuran containing $LiAlH_4$ (600 mg) and refluxed for 15 hours on the water bath. After cooling, the mixture was worked up in the same manner as discribed above to leave an oil which was taken up in 200 ml of ether. The ether solution was washed with 100 ml of 10% NaCl aq. solution, 100 ml of 2% $Na_2S_2O_3$ aq. solution and finally 100 ml of water two times, and dried over Na_2SO_4 and evaporated to dryness to give a residue (112 mg). The residue was again dissolved in 10 ml of *n*-hexane and hydrogenated in H_2 stream using PtO_2 (12 mg) as a catalyst. After filtration, the solvent was evaporated *in vacuo* and the residue was chromatographed over alumina (Act. I, Woelm, 10 g), followed by developing the solvent from *n*-hexane to ether gradiently. Elution with 2% ether/*n*-hexane afforded 59 mg of IV as an oil. *Anal.* Calcd. for $C_{17}H_{36}$: C, 85.00; H, 15.00. Found: C, 86.11; H, 15.97. IR: ν_{max}^{liquid} (cm^{-1}); 2980—2900, 1460, 1380, 1150, 950. Mass Spectrum *m/e*: 240 (M^+), 141, 127, 71, 57, 43. Gas chromatography, retention time: 44 min (5% SE-52, 3 m, N_2, 60 ml/min, 80°).

Conversion from H-4 (II) to H-5 (III)——After 20 mg of II dissolved in 10 ml of $CHCl_3$ and refluxed for 1 hour, the solvent was evaporated off *in vacuo* to give oil (18 mg). Spectral and TLC data of this oil were completely identical with III. This conversion similarly took place by addition of acid, namely, by adding a drop of conc. HCl to solution of II (20 mg) in MeOH (10 ml). IR: ν_{max}^{liquid} (cm^{-1}); 3320—3400, 1760, 1300, 1170. NMR ($CDCl_3$) τ: 8.94 (3H, d, J=6.0 Hz), 8.63 (3H, s), 6.47 (1H, m). TLC: *Rf* value; 0.4 (Silica gel G, *n*-hexane–AcOEt 1:1), 0.6 (Alumina G, isopropyl ether–ether 1:1).

H-5 Acetate (V)——A solution of II (100 mg) in Ac_2O (1 ml) and pyridine (1 ml) was allowed to stand over night at room temperature. The reaction mixture was poured into ice water, and then extracted with $CHCl_3$. The extract was evaporated *in vacuo* to dryness. The residue was dissolved in a small volume of *n*-hexane and passed through the column packed with 5 g of Silica gel H (Merck), The elution with *n*-hexane–AcOEt (8:1) was collected and evaporated to yield V (20 mg) as an oil. *Anal.* Calcd. for $C_{19}H_{34}O_4$: C, 70.00; H, 10.50. Found: C, 70.75; H, 11.05. IR: ν_{max}^{liquid} (cm^{-1}); 1770, 1730, 1250, 1160, 940. NMR ($CDCl_3$) τ: 9.13 (6H, d, J=6.0 Hz, C-CH_3 at C-8 and C-12), 8.88 (3H, d, J=6.0 Hz), 8.04 (3H, s), 5.30(1H, m).