STUDIES ON AN ANTIBIOTIC, ALBOCYCLINE

VII.[1] MINOR COMPONENTS OF ALBOCYCLINE

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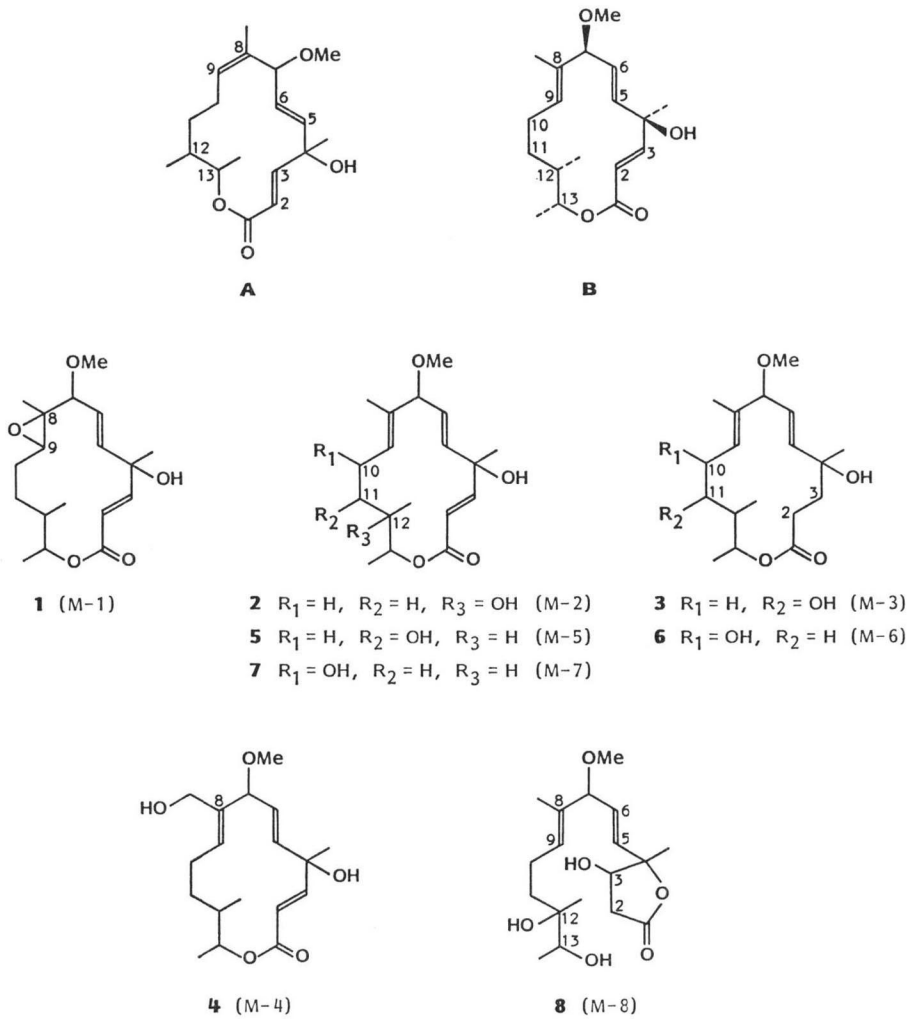
As an approach to the search for new potentially useful macrolide antibiotics, we explored the minor components of albocycline (ALB) from the culture broth of *Streptomyces bruneogriseus*. Eight minor components were isolated and their structures were confirmed as **1**~**8**. Unexpectedly, they were not glycosidic compounds but only oxidation or reduction products of ALB. Three or four of them will serve as a useful intermediate to introduce amino sugar moiety into ALB skeleton.

Albocycline (ALB) is a macrolide antibiotic with a 14-membered macrocyclic lactone ring structure, whose skeleton is different from those of representative 14-membered macrolide antibiotics such as erythromycin and oleandomycin. Although it does not possess any carbohydrate moiety, it is active against *Staphylococcus aureus*.[2,3] The planar structure of ALB was proposed as **A** by degradative reactions and spectroscopic studies.[4~6] However, it has been suggested that its structure is not **A** but **B** whose 8,9-double bond is corrected to *E*-stereochemistry by the nuclear Overhauser effect (NOE) experiments. (HARADA, K.-I.; F. NISHIDA, H. TAKAGI, M. SUZUKI & T. IWASHITA: Unpublished results). Recently, we have shown the correct structure for ALB as **B** by the X-ray crystal analysis.[1] Moreover, the absolute configuration has been determined by the X-ray crystal analysis of its *p*-bromobenzoate by the Upjohn's research group.[7] As an approach to the search for new potentially useful macrolide antibiotics, we explored the minor components present in an ALB fermentation. In this paper we wish to describe eight minor components of ALB (Fig. 1).

ALB has been produced by four strains, *Streptomyces bruneogriseus*,[2] *S. roseocinereus*,[3] *S. roseochromogenes*[3] and *S. maizeus*.[8~10] In this study we isolated the minor components from the culture broth of *S. bruneogriseus*. Because amino sugars such as desosamine and mycaminose are generally required for strong antimicrobial activity of macrolide antibiotics,[11] we sought to isolate more polar components rather than ALB. From the fermentation complex of antibiotics, each minor component was isolated in a pure state by use of repeated silica gel chromatographies (Fig. 2). They were tentatively designated as ALB-M-1 (**1**) ~ ALB-M-8 (**8**) as shown in Fig. 3. The physico-chemical properties, as well as the chemical ionization (CI) and desorption chemical ionization (D/CI) mass spectra are summarized in Tables 1 and 2, respectively.

The ^{13}C NMR spectral data of all minor components suggest that they have the same carbon skeleton (18 carbon atoms) as ALB. Therefore, in preference to structural elucidation of the minor com-

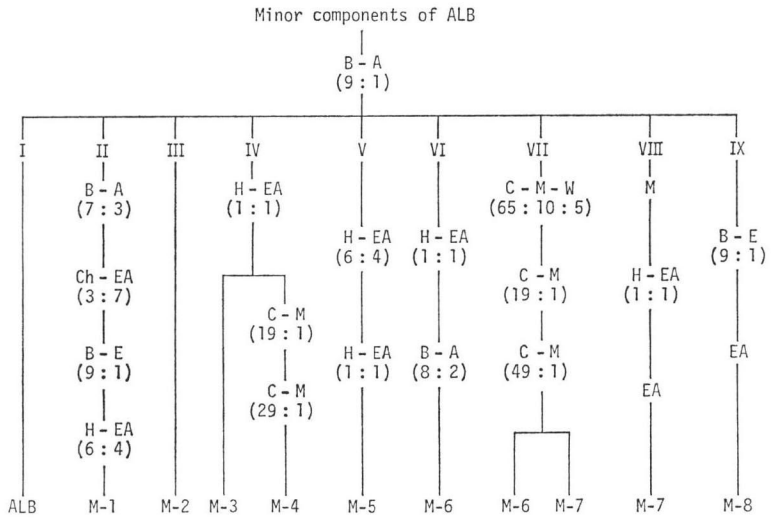
Fig. 1.



ponents, we correctly assigned the signals in the ^{1}H NMR and ^{13}C NMR spectra of ALB. The assignments were greatly facilitated by use of two dimensional (2D) proton-proton and proton-carbon shift correlation spectroscopy. Their spectra are shown in Figs. 4 and 5, and the assignments of ^{1}H NMR and ^{13}C NMR signals are summarized together with those of minor components in Tables 3 and 4, respectively.

The high resolution mass spectral data of M-1, M-2, M-4, M-5 and M-7 show that their formulae are $C_{18}H_{28}O_5$, indicating that an additional oxygen atom is introduced into ALB skeleton. The least polar component, M-1 (**1**) was obtained as a colorless oily substance. The disappearance of the two olefinic signals of ALB in the ^{13}C NMR spectrum of M-1 suggests that the double bond at C-8 and C-9 was oxidized to give a epoxy derivative. Actually, the upfield shifts of H-9 (5.35→3.02 ppm), and C-8 (136.1→61.7 ppm) and C-9 (129.1→57.7 ppm) are clearly observed. In order to correlate M-1 with ALB, epoxidation of ALB with *m*-chloroperbenzoic acid was performed in chloroform at room temperature. The reaction mixture was separated by silica gel chromatography to give two epoxides. All physico-chemical properties of the less polar product are completely identical with those of M-1.

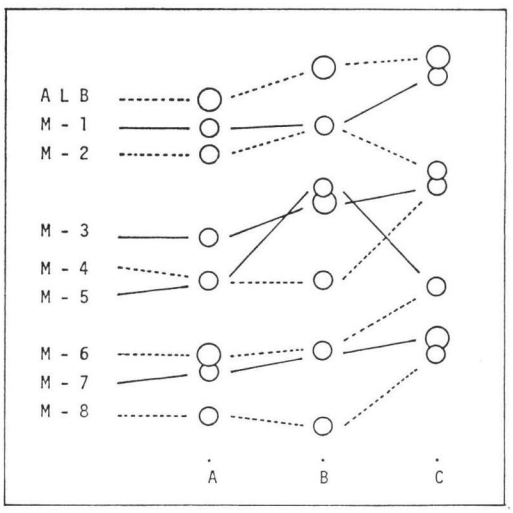
Fig. 2. Isolation procedure for minor components of albocycline.
Abbreviation: A: acetone, B: benzene, C: $CHCl_3$, Ch: cyclohexane, E: EtOH, H: *n*-hexane, M: MeOH, EA: EtOAc, W: H_2O.



Consequently, the structure of M-1 is given as **1**. An alternative epoxide was obtained as colorless needles, mp 146.5~148°C, whose spectral behavior is closely similar to that of M-1, showing that the epoxide is found to be a stereoisomer of 8,9-epoxyalbocycline (M-1). But, it could not be detected in the culture broth.

Subsequently, four hydroxylated components, M-2, M-4, M-5 and M-7 are discussed. They are all oily compounds. Comparison of the ^{13}C NMR chemical shifts of M-2 with those of ALB indicates that M-2 is 12-hydroxyalbocycline (**2**). Further evidence for the structure **2** is obtained by the ^1H NMR spectrum in which the signal of C_{12}-CH_3 appears at 1.10 ppm as a singlet.

Fig. 3. Thin-layer chromatograms of minor components of albocycline.
A : benzene - acetone, 6: 4.
B : EtOAc - *n*-hexane, 1: 1.
C : $CHCl_3$ - MeOH, 9: 1.



The structure of M-4 was also deduced through total assignment of the ^{13}C and ^1H NMR spectra. The appearance of hydroxymethyl group (59.9 ppm) at C-8 instead of C_8-CH_3 in ALB is evident from comparison of the ^{13}C NMR spectrum of M-4 with that of ALB. An AB-type signal is correspondingly observed at 4.10 and 4.26 ppm in the ^1H NMR spectra. The structure of M-4 is established as **4** which is oxidized at the allylic methyl group of ALB.

There are two resonances (24.7 and 34.2 ppm) showing triplets in the off-resonance spectrum of ALB, which are assigned to C-10 and C-11, respectively. The presence of only one signal due to a methylene group in the off-resonance spectra of M-5 and M-7 indicates that hydroxylation has occurred at C-10 or

Table 1. Physico-chemical properties of ALB and its minor components.

Compound	MP (°C)	Appearance (Recrystallization solvent)	Formula (MW)	*Anal* (%) Calcd (Found)		CI high resolution data for MH^+ Calcd (Found)	$[\alpha]_D$	IR (cm^{-1}, $CHCl_3$)	UV (nm, EtOH)
				C	H				
ALB	86~87	Colorless plate (Cyclohexane)	$C_{18}H_{28}O_4$ (308)	70.15 (70.38)	9.09 (8.78)		−90.4° (*c* 0.5, MeOH) −100.0° (*c* 0.5, $CHCl_3$)	3425 (OH), 1705 (CO)	212 (sh) (log ε 3.83)
ALB-M-1		Oily	$C_{18}H_{28}O_5$ (324)			325.2013 (325.1990)	−10.0° (*c* 0.35, MeOH)	3590, 3450 (OH), 1700 (CO)	220 (sh) (log ε 4.20)
ALB-M-2		Oily	$C_{18}H_{28}O_5$ (324)			325.2013 (325.1980)	−112.0° (*c* 0.5, MeOH)	3590, 3440 (OH), 1705 (CO)	End absorption
ALB-M-3	123~124.5	Colorless needles (Benzene - cyclohexane)	$C_{18}H_{30}O_5$ (326)	66.23 (66.13)	9.26 (9.59)		−25.6° (*c* 0.5, MeOH)	3580, 3420 (OH), 1710 (CO)	End absorption
ALB-M-4		Oily	$C_{18}H_{28}O_5$ (324)			325.2013 (325.2035)	−55.1° (*c* 0.15, MeOH)	3580, 3440 (OH), 1700 (CO)	220 (sh) (log ε 3.78)
ALB-M-5		Oily	$C_{18}H_{28}O_5$ (324)			325.2013 (325.2005)	−90.0° (*c* 0.2, MeOH)	3580, 3440 (OH), 1690 (CO)	End absorption
ALB-M-6	86~87.5	Colorless needles (*i*-Propylether - cyclohexane)	$C_{18}H_{30}O_5 \cdot \frac{1}{2} H_2O$ (326)	64.45 (64.20)	9.01 (9.16)		+6.9° (*c* 0.35, MeOH)	3580, 3420 (OH), 1720 (CO)	End absorption
ALB-M-7		Oily	$C_{18}H_{28}O_5$ (324)			325.2013 (325.1953)	−13.5° (*c* 0.1, MeOH)	3580, 3420 (OH), 1700 (CO)	215 (sh) (log ε 3.77)
ALB-M-8		Oily	$C_{18}H_{30}O_6$ (342)			343.2119 (343.2094)	−35.5° (*c* 0.2, MeOH)	3550, 3400 (OH), 1765	End absorption

Table 2. Diagnostic ions (m/z) from CI (i-C_4H_{10}) mass spectra of ALB and its minor components.

Compound	D/CI (i-C_4H_{10})			Conventional CI (i-C_4H_{10})		
	MH^+	MH^+-H_2O	MH^+-MeOH	MH^+	MH^+-H_2O	MH^+-MeOH
ALB	309 (100)	291 (42.0)	277 (99.0)	309 (57.8)	291 (41.6)	277 (42.0)
ALB-M-1	325 (100)	307 (72.1)	293 (7.6)	325 (2.2)	307 (10.0)	293 (—)
ALB-M-2	325 (100)	307 (13.5)	293 (42.0)	325 (9.3)	307 (19.3)	293 (18.3)
ALB-M-3	327 (2.7)	309 (100)	295 (83.6)	327 (0.3)	309 (2.0)	295 (0.1)
ALB-M-4	325 (15.0)	307 (11.3)	293 (16.0)	325 (0.2)	307 (1.9)	293 (0.9)
ALB-M-5	325 (14.3)	307 (4.5)	293 (4.5)	325 (8.4)	307 (13.0)	293 (4.8)
ALB-M-6	327 (8.2)	309 (100)	295 (59.2)	327 (—)	309 (5.0)	295 (0.2)
ALB-M-7	325 (7.0)	307 (24.8)	293 (5.4)	325 (1.2)	307 (3.8)	293 (5.0)
ALB-M-8	343 (—)	325 (—)	311 (44.4)	343 (0.1)	325 (1.3)	311 (2.7)

Relative intensity, % in parentheses.

C-11. Distinction between M-5 and M-7 was achieved by comparison of ^1H NMR spectrum of M-7 with that of ALB. The H-9 triplet at 5.35 ppm in ALB alters the doublet at 5.32 ppm in M-7. This result shows that M-7 is 10-hydroxyalbocycline (**7**) and M-5 is 11-hydroxyalbocycline (**5**). As expected, acetylation of M-5 and M-7 with acetic anhydride in pyridine in the usual manner gave the corresponding monoacetate.

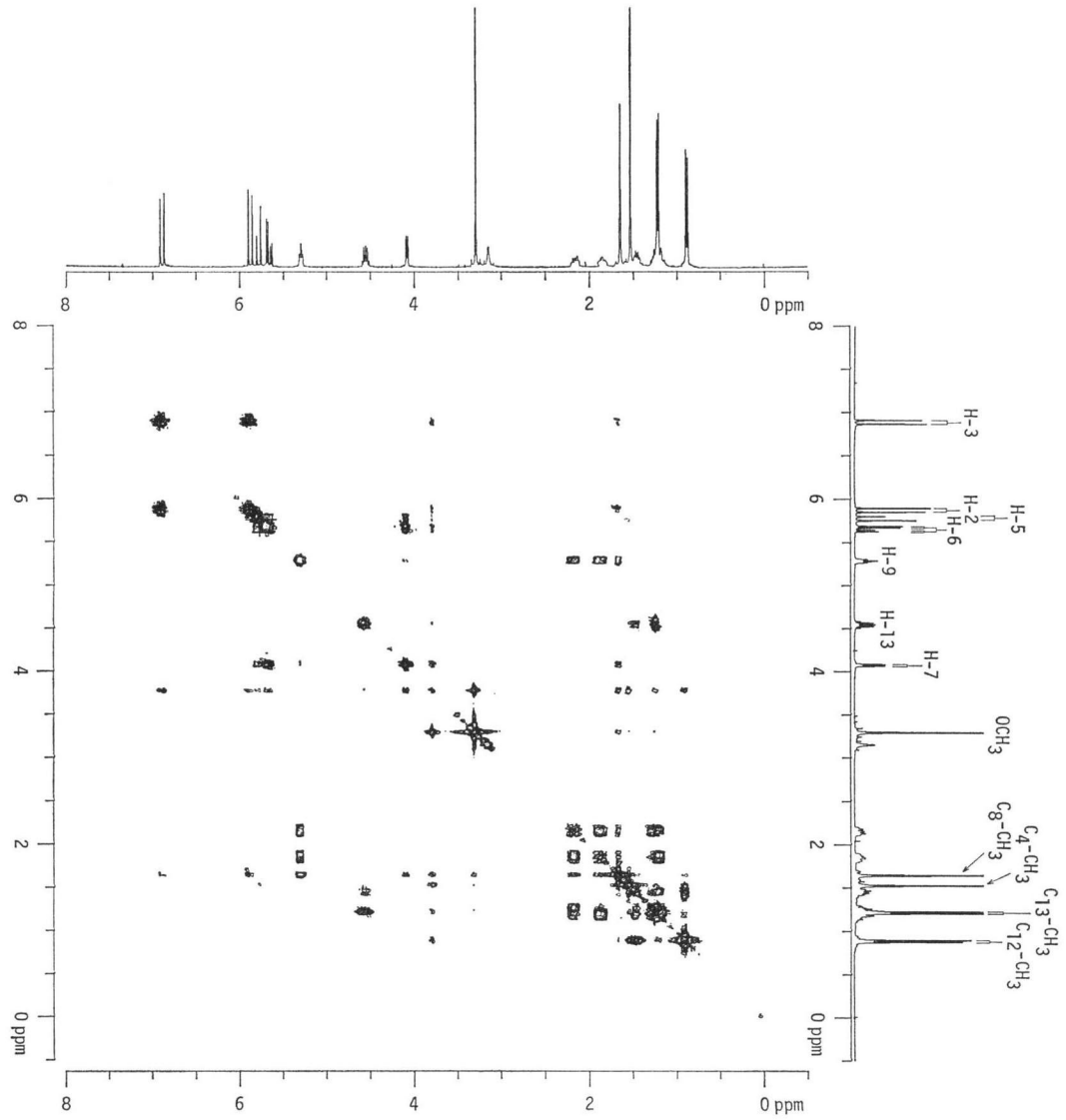
The same formula $C_{18}H_{30}O_5$ for M-3 and M-6 was established by elemental analysis and CI mass spectral data, indicating that an oxygen atom and a hydrogen molecule are additionally contained in the ALB skeleton. Comparison of ^{13}C NMR spectra of both the components with that of ALB leads to the conclusion that they are 2,3-dihydroalbocycline derivatives. That is, the resonance of lactone carbonyl carbon (166.4 ppm) is shifted to 173.5 ppm for M-3 and 173.2 ppm for M-6 and the olefinic carbon signals (115.4 and 155.0 ppm) due to C-2 and C-3 completely disappear. Because there are three methylene signals of which two are assigned to C-2 and C-3 in the ^{13}C NMR spectra of both the components, the same discussion can be applied to determine the locations of oxidation as in the cases of M-5 and M-7. The presence of the H-9 doublet (J=8 Hz) in the ^1H NMR spectrum of M-6 indicates that the secondary hydroxyl group is located at C-10, so that hydroxylation occurred at C-11 in the case of M-3. Therefore, M-3 is 2,3-dihydro-11-hydroxyalbocycline (**3**) and M-6 is 10-hydroxyl analogue (**6**).

The most polar component, M-8 was obtained as a labile oily substance. The molecular formula $C_{18}H_{30}O_6$ was obtained by high resolution mass spectral data under CI conditions. The IR spectrum shows a very strong carbonyl band at 1765 cm^{-1} and suggests the presence of a γ-lactone in place of 14-membered lactone ring. Although the presence of two double bonds of C-5, 6 and C-8, 9 could be recognized, the resonances of a double bond due to C-2 and C-3 could not be found in the ^{13}C NMR spectrum. In the ^1H NMR spectrum an ABX (A: 2.50 ppm (dd), B: 2.90 ppm (dd), X: 4.21 ppm (dd)) system is observed and they are assigned to H-2_a, H-2_b and H-3, respectively. The spectral pattern of H-5 ~ H-9 is very similar to that of M-3. The signals of H-13 at 3.66 ppm and C_{12}-CH_3 at 1.13 ppm appear as a quartet and a singlet, respectively. Moreover, acetylation of M-8 with acetic anhydride in pyridine at room temperature gave expectedly a diacetate. These results clearly indicate that M-8 has the structure **8**.

Unfortunately, antimicrobial activity of all isolated components was much weaker than that of the parent antibiotics.

In summary, we isolated eight minor components of ALB and their structures were confirmed in

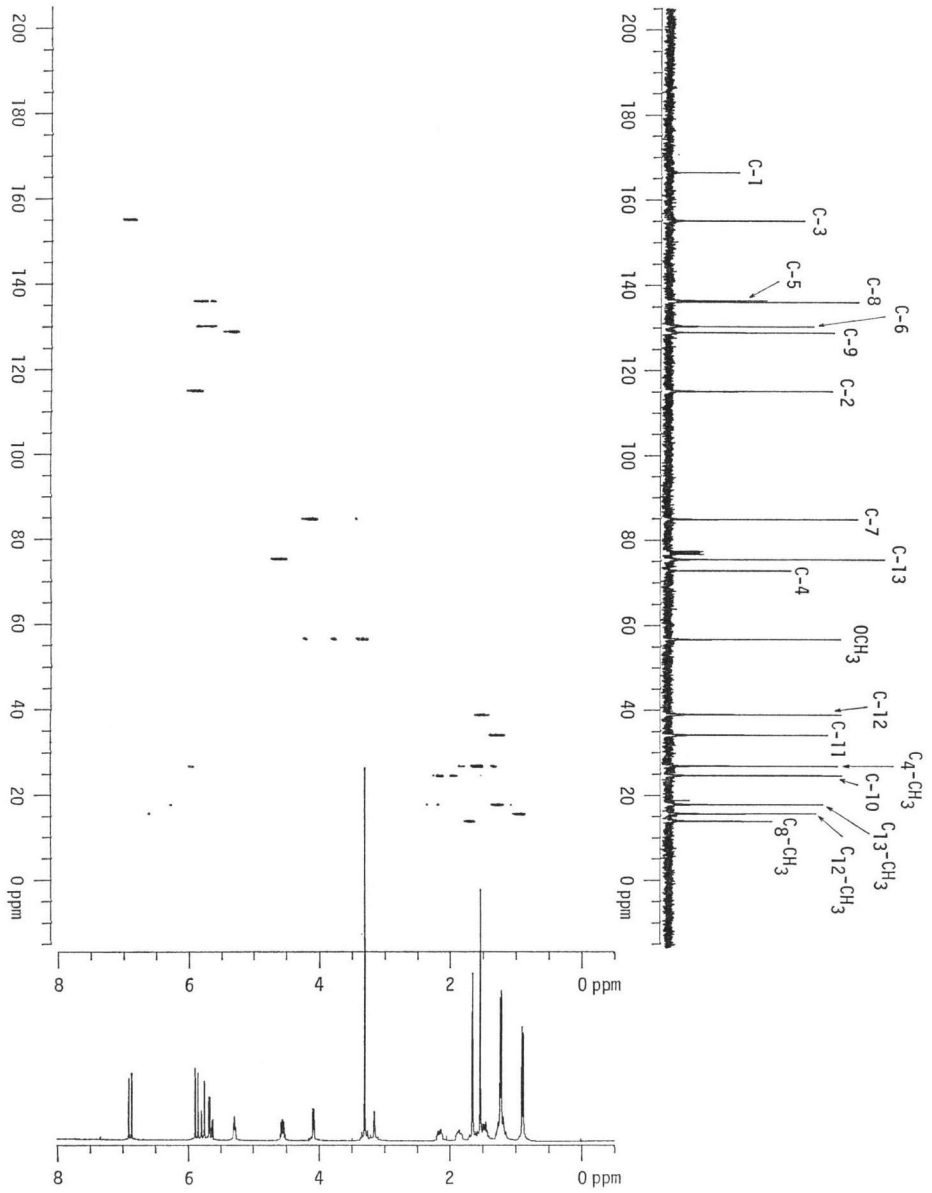
Fig. 4. Contour plot of 2D proton-proton shift correlation spectrum (COSY; $90°$-t_1-$90°$-t_2) of albocycline. The size of data matrix was $1K \times 512$, and the data processing was carried out with standard Nicolet software.



this study. Unexpectedly, they are not glycosidic compounds but only oxidation or reduction products of ALB. Although it is impossible to distinguish at present whether they are formal metabolites of this strain or artifacts, the occurrence of M-8 is very interesting with respect to biosynthesis of macrolide antibiotics. As mentioned above, albocycline and its minor components have little antimicrobial activity. It is considered to be caused by the lack of an amino sugar in the molecule. Introduction of an amino sugar into their molecules is practically significant problem. Since glycosidation at the tertiary hydroxyl group of C-4 in ALB is considered to be very hard, the isolated primary or secondary alcohols will serve as a useful intermediate to accomplish the purpose.

Fig. 5. Contour plot of 2D proton-carbon shift correlation spectrum (90°[H]-t_1/2-180°[C]-t_1/2-Δ_1-90°[H]-90°[C]-Δ_2-t_2) of albocycline.

The size of data matrix was 2K × 256. Delays Δ_1 and Δ_2 were set to 3.3 milliseconds and 20 milliseconds, respectively. The data processing was carried out with standard Nicolet software.



Experimental

All melting points were determined on a micro-melting point apparatus (hot-stage type, Yanagimoto MP-S3) and were uncorrected. Optical rotations were measured with a Jasco DIP-181 polarimeter. IR spectra were determined with a Hitachi IR-215 spectrometer. UV spectra were taken on a Hitachi 200-10 double beam spectrometer. ^{1}H NMR spectra were recorded on a Hitachi R-24B (60 MHz), a Jeol FX-100 (100 MHz) and a Nicolet NT-360 spectrometers, and ^{13}C NMR spectra were recorded on a Jeol FX-100 (25 MHz) spectrometer using tetramethylsilane as an internal standard. CI mass spectra

Table 3. ^{1}H NMR chemical shifts (ppm value) and coupling constants (Hz, in parentheses) of ALB and its minor components.[a]

Compound	H-2 ($J_{2,3}$)	H-3	H-5 ($J_{5,6}$)	H-6 ($J_{6,7}$)	H-7	H-9 ($J_{9,10}$)	H-10	H-11	H-12	H-13 ($J_{12,13}$)	OCH_3	C_4-CH_3	C_8-CH_3	C_{12}-CH_3 ($J_{CH_3,12}$)	C_{13}-CH_3 ($J_{CH_3,13}$)
ALB	5.94 d (16)	6.98 d	5.90 d (16)	5.64 dd (5)	4.12 d	5.35 t (6)				4.62 dq (8)	3.34 s	1.55 s	1.67 s	0.89 d (6)	1.22 d (6)
ALB-M-1	5.98 d (16)	6.80 d	5.96 d (16)	5.50 dd (6)	3.80 d	3.02 m				4.60 dq (9)	3.30 s	1.24 s	1.54 s	0.88 d (8)	1.30 d (8)
ALB-M-2	5.88 d (16)	6.72 d	5.84 d (16)	5.66 dd (6)	4.16 m	5.48 t (7)				4.73 q	3.26 s	1.53 s	1.58 s	1.10 s	1.29 d (6)
ALB-M-3			5.45 d (16)	5.86 dd (6)	4.02 d	5.18 t (8)		3.85 m		4.70 quintet (6)	3.20 s	1.25 s	1.65 s	1.02 d (6)	1.20 d (6)
ALB-M-4	5.88 d (16)	6.88 d	5.84 d (16)	5.73 dd (6)	4.38 d	5.32 t (6)				4.59 dq (8)	3.36 s	1.56 s	[b]	0.88 d (8)	1.22 d (8)
ALB-M-5	5.84 d (16)	6.89 d	5.80 d (17)	5.52 dd (6)	4.08 d	5.08 t (7)		3.21 m		4.94 dq (8)	3.28 s	1.51 s	1.64 s	0.88 d (8)	1.22 d (8)
ALB-M-6			5.48 d (16)	5.90 dd (6)	4.08 d	5.34 d (8)	4.57 m			4.57 m	3.20 s	1.25 s	1.70 s	0.97 d (7)	1.08 d (7)
ALB-M-7	5.90 d (16)	6.85 d	5.80 d (16)	5.52 dd (6)	4.02 d	5.32 d (9)	4.12 m			4.62 quintet (6)	3.34 s	1.51 s	1.83 s	0.94 d (6)	1.18 d (6)
ALB-M-8	[c]	4.21[d] dd	5.90 d (16)	5.70 dd (2)	4.08 d	5.48 t (8)	2.12 m			3.66 q	3.24 s	1.48 s	1.56 s	1.13 s	1.19 d (6)

[a] Measured in $CDCl_3$ at 100 MHz with TMS as an internal standard.

[b] C_8-CH_2OH; H_a: 4.26 ppm, d, J=12 Hz, H_b: 4.10 ppm, d, J=12 Hz.

[c] H-2$_a$: 2.90 ppm, dd, $J_{2a,2b}$=18, $J_{2a,3}$=6 Hz, H-2$_b$: 2.50 ppm, dd, $J_{2a,2b}$=18, $J_{2b,3}$=4 Hz.

[d] $J_{3,2a}$=6, $J_{3,2b}$=4 Hz.

Table 4. ^{13}C NMR chemical shifts of ALB and its minor components (ppm value).[a]

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	OCH_3	C_4-CH_3	C_8-CH_3	C_{12}-CH_3	C_{13}-CH_3
ALB	166.4 s	115.4 d	155.0 d	73.1 s	136.6 d	130.6 d	85.0 d	136.1 s	129.1 d	24.7 t	34.2 t	39.0 d	75.6 d	56.9 q	27.0 q	13.7 q	15.7 q	17.9 q
ALB-M-1	166.4 s	117.5 d	154.6 d	72.6 s	138.4 d	132.6 d	80.3 d	61.7 s	57.7 d	25.1 t	28.9 t	41.7 d	76.4 d	57.7 q	26.2 q	15.4 q	16.1 q	18.9 q
ALB-M-2	166.3 s	115.2 d	154.8 d	73.0 s	135.9 d	131.6 d	85.7 d	135.3 s	129.8 d	21.0 t	38.2 t	74.9 s	76.1 d	56.4 q	26.4 q	13.0 q	23.3 q	14.0 q
ALB-M-3	173.5 s	32.3[c] t	30.8[c] t	72.0 s	137.2 d	123.4 d	87.5 d	137.8 s	128.5 d	32.3[c] t	71.5 d	41.9 d	73.1 d	55.7 q	30.1 q	10.5 q	12.0 q	17.7 q
ALB-M-4	166.2 s	115.2 d	154.9 d	73.2 s	137.5 d	133.2 d	83.0 d	138.8 s	130.2 d	24.5 t	34.2 t	39.5 d	75.4 d	57.0 q	26.8 q	59.9[b] t	15.9 q	18.1 q
ALB-M-5	166.8 s	114.7 d	156.3 d	73.4 s	136.3 d	130.3 d	84.7 d	137.8 s	125.0 d	34.5 t	71.8 d	43.6 d	73.3 d	57.0 q	26.4 q	8.1 q	14.2 q	18.3 q
ALB-M-6	173.2 s	30.5[d] t	37.4[d] t	72.1 s	137.5 d	128.7 d	87.8 d	139.7 s	129.9 d	65.5 d	40.2 t	34.9 d	71.5 d	55.6 q	30.5 q	10.9 q	13.9 q	15.3 q
ALB-M-7	166.5 s	115.1 d	155.7 d	73.2 s	136.6 d	129.9 d	83.8 d	140.8 s	132.4 d	66.4 d	44.2 t	36.4 d	75.6 d	57.4 q	26.7 q	16.0 q	16.8 q	17.7 q
ALB-M-8	174.9 s	38.5 t	72.8 d	88.5 s	132.0 d	128.5 d	86.3 d	133.4 s	129.6 d	21.9 t	37.6 t	75.1 s	74.0 d	55.6 q	24.4 q	11.2 q	20.5 q	17.6 q

[a] Measured in $CDCl_3$ at 25 MHz with TMS as an internal standard.

[b] C_8-CH_2OH.

[c,d] Assignments may be reversed.

were obtained using a Shimadzu Auto GCMS-6020 mass spectrometer. D/CI mass spectra were measured with a Shimadzu LKB 9000A spectrometer. Operating conditions were as follows: ion source temperature 250°C; electron energy 155 eV; accelerating voltage 3.5 kV; reagent gas (isobutane) pressure 0.3 Torr. High resolution CI mass spectra were recorded on a Jeol JMS-D-300 mass spectrometer online to a JEC-980B computer at a resolution of approximately 5000 under the following conditions: ion source temperature 235~240°C; electron energy 240 eV; accelerating voltage 3 kV; reagent gas pressure about 0.3 Torr. TLC was performed on Merck pre-coated plates (Kieselgel 60 F_{254}). For column chromatography, Merck Kieselgel 60 (Art. 7729 or 7734) was used.

Isolation and Purification of the Minor Components

The EtOAc fraction described in our earlier report[2] was evaporated under reduced pressure. The residue was separated as shown in Fig. 1 to give M-1 110 mg, M-2 596 mg, M-3 346 mg, M-4 50 mg, M-5 52 mg, M-6 96 mg, M-7 205 mg, M-8 27 mg.

Epoxidation of ALB

To a solution of ALB (300 mg) in $CHCl_3$ (10 ml) was added *m*-chloroperbenzoic acid (237 mg) in $CHCl_3$ (10 ml) with stirring and the mixture was stirred for 6 hours at room temperature. To the reaction mixture was added 239 mg of $Na_2S_2O_4$ and was stirred for 2 hours at room temperature. After filtration the filtrate was evaporated to dryness. The residue was dissolved in EtOAc (20 ml) and the solution was extracted three times with 5% $NaHCO_3$ (20 ml). The aqueous layer was extracted twice with EtOAc (20 ml) and the combined organic layer was dried over $MgSO_4$ (anhydrous) and then concentrated to dryness to give yellow oily substance (276 mg). The residue was chromatographed on silica gel column with *n*-hexane - EtOAc (7: 3) to afford ALB (121 mg), ALB-M-1 (68 mg) and the stereoisomer of ALB-M-1 (48 mg). ALB-M-1 was purified by preparative TLC (*n*-hexane - EtOAc, 1: 1) to yield viscous colorless oil (41 mg). All physico-chemical properties were completely identical with those of ALB-M-1 isolated naturally. The isomer of ALB-M-1 was recrystallized from *n*-hexane - cyclohexane to give colorless needles, mp 146.5~148°C; *Anal* Calcd for $C_{18}H_{28}O_5$: C 66.64, H 8.70. Found: C 66.60, H 9.04; CIMS *m/z* 325 (MH^+); $[\alpha]_D^{23}$ +0.81° (*c* 0.5, MeOH); UV λ_{max}^{EtOH} nm (log ε) 215 (4.66, sh); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3590, 3450 (OH), 1710 (CO); ^1H NMR ($CDCl_3$) ppm 6.92 (1H, d, *J*=16 Hz, H-3), 5.92 (1H, d, *J*=16 Hz, H-2), 5.86 (1H, d, *J*=16 Hz, H-5), 5.48 (1H, dd, *J*=16, 6 Hz, H-6), 4.61 (1H, dq, *J*=8, 6 Hz, H-13), 3.38 (3H, s, OCH_3), 3.32 (1H, d, *J*=6 Hz, H-7), 1.30 (3H, s, C_8-CH_3), 1.25 (3H, d, *J*=6 Hz, C_{13}-CH_3), 1.09 (3H, s, C_4-CH_3), 0.85 (3H, d, *J*=6 Hz, C_{12}-CH_3); ^{13}C NMR ($CDCl_3$) ppm 166.8 (s, C-1), 154.3 (d, C-3), 138.5 (d, C-5), 128. 5 (d, C-6), 116.1 (d, C-2), 86.6 (d, C-7), 75.9 (d, C-13), 72.6 (s, C-4), 62.5 (s, C-8), 58.9 (d, C-9), 57.1 (q, OCH_3), 39.8 (d, C-12), 28.9 (t, C-11), 26.7 (q, C_4-CH_3), 24.4 (t, C-10), 18.9 (q, C_{13}-CH_3), 15.6 (q, C_8-CH_3), 11.2 (q, C_{12}-CH_3).

General Procedure for Acetylation of the Minor Components

A solution of the minor component in pyridine was treated with acetic anhydride and the reaction mixture was allowed to stand overnight at room temperature. The mixture was concentrated to dryness. The residue was purified by chromatography using silica gel column.

ALB-M-5 acetate: white powder; mp 195~199°C; D/CIMS *m/z* 367 (MH^+); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3590, 3440 (OH), 1710 (CO); ^1H NMR ($CDCl_3$) ppm 6.86 (1H, d, *J*=16 Hz, H-3), 5.88 (1H, d, *J*=16 Hz, H-2), 5.84 (1H, d, *J*=15 Hz, H-5), 5.64 (1H, dd, *J*=15, 6 Hz, H-6), 5.26 (1H, t, *J*=8 Hz, H-9), 4.70 (1H, m, H-11), 4.43 (1H, dq, *J*=8, 7 Hz, H-13), 4.12 (1H, d, *J*=6 Hz, H-7), 3.30 (3H, s, OCH_3), 2.06 (3H, s, $OCOCH_3$), 1.68 (3H, s, C_8-CH_3), 1.52 (3H, s, C_4-CH_3), 1.24 (3H, d, *J*=7 Hz, C_{13}-CH_3), 0.96 (3H, d, *J*=7 Hz, C_{12}-CH_3).

ALB-M-7 acetate: oily; D/CIMS *m/z* 367 (MH^+); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3590, 3420 (OH), 1710 (CO); ^1H NMR ($CDCl_3$) ppm 6.78 (1H, d, *J*=16 Hz, H-3), 5.88 (1H, d, *J*=16 Hz, H-2), 5.84 (1H, d, *J*=16 Hz, H-5), 5.54 (1H, dd, *J*=16, 6 Hz, H-6), 5.30 (1H, d, *J*=8 Hz, H-9), 5.25 (1H, m, H-10), 4.56 (1H, quintet, *J*=8 Hz, H-13), 4.00 (1H, d, *J*=6 Hz, H-7), 3.28 (3H, s, OCH_3), 1.98 (3H, s, $OCOCH_3$), 1.74 (3H, s, C_8-CH_3), 1.53 (3H, s, C_4-CH_3), 1.20 (3H, d, *J*=8 Hz, C_{13}-CH_3), 0.96 (3H, d, *J*=8 Hz, C_{12}-CH_3).

ALB-M-8 acetate: oily; D/CIMS *m/z* 427 (MH^+); IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3580, 3510 (OH), 1770, 1730 (CO); ^1H NMR ($CDCl_3$) ppm 5.86 (1H, dd, *J*=16, 4 Hz, H-6), 5.66 (1H, d, *J*=16 Hz, H-5), 5.42 (1H, t, *J*=8 Hz, H-9), 5.24 (1H, dd, *J*=6, 4 Hz, H-3), 4.86 (1H, q, *J*=8 Hz, H-9), 4.00 (1H, d, *J*=4 Hz, H-7),

3.24 (3H, s, OCH_3), 3.06 (1H, dd, J=18, 6 Hz, H-2_a), 2.58 (1H, dd, J=18, 4 Hz, H-2_b), 2.12 (3H, s, $OCOCH_3$), 2.06 (3H, s, $OCOCH_3$), 1.52 (2×3H, s, C_4-CH_3 and C_8-CH_3), 1.28 (3H, d, J=6 Hz, C_{12}-CH_3), 1.24 (3H, s, C_{13}-CH_3).

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