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Studies on an Antibiotic, Albocycline. V.[1] High Pressure-High Temperature Hydrogenation Products, and Structure Proof of Albocycline

Noboru Nagahama, Isao Takamori and Makoto Suzuki

Microbial Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd.[2]

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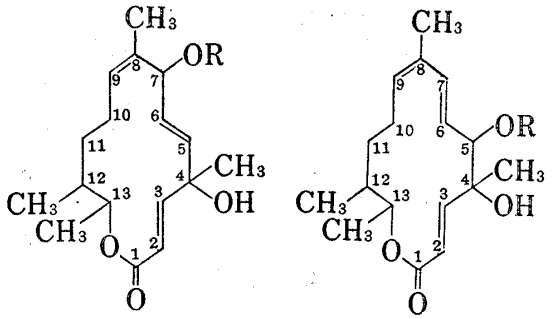
By the catalytic hydrogenation of albocycline in acetic acid under high pressure at elevated temperature, nine reaction products were obtained. They were classified into pairs by their infrared (IR), nuclear magnetic resonance (NMR) and mass spectral data. The eluci dation of structures of these pair products gave the conclusive answers about the positions of O-functions, especially that of a methoxy group, in albocycline molecule. Thus, the structure of albocycline was finally confirmed as Ia.

In the preceding paper, the authors proposed the structure of albocycline as Ia, based upon the spectrometric and chemical data on albocycline and its hydrogenation products together with hydrocarbon compound obtained by the exhaustive hydrogenation. The structure Ia, however, was inconsistent with the data on periodate oxidation of cineromycin B,[3] a closely related antibiotic with albocycline. The data of periodate oxidation suggested the presence of the vicinal hydroxy groups in the molecule and consequently supported the structure IIa and IIb for albocycline and cineromycin B respectively.



Ia : R=CH_3 IIa : R=CH_3
Ib : R=H IIb : R=H

Chart 1

In order to resolve this ambiguous problem, the experimental results on many hydrogenation products of albocycline under high temperature and high pressure was discussed on the present paper. This type of hydrogenation involving hydrogenolysis with palladium on alumina as a catalyst had been frequently applied to determine the position of O-functions in polyene-macrolide antibiotics.[4-8]

Albocycline was hydrogenated at 200° under 150 atm or 250 atm in glacial acetic acid for 20 minutes using palladium on alumina as a catalyst. The crude products were derived to the corresponding methyl esters using diazomethane and, then, investigated by thin-layer chromatography (TLC). Thus, the presence of a large number of hydrogenated compounds were indicated. Nine of them, here called HH-1 to HH-9, were successfully isolated by silica-gel chromatography and preparative gas chromatography as pure liquid materials. Their properties were summerized in Table I.

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2) Location: *50-2-2 Kawagishi, Toda, Saitama.*
3) N. Miyairi, M. Takashima, K. Shimizu and H. Sakai, *J. Antibiotics* (Tokyo), Ser. A, **19**, 56 (1966).
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7) A. C. Cope, U. Axen, E. P. Burrows and J. Weinlich, *J. Am. Chem. Soc.*, **88**, 4228 (1966).
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Table I. High-Pressure Hydrogenation Products

Product	Mol. formula (M.W.)		Anal. Found	Anal. Calcd.	GC[a] (min)	TLC.[b] (*Rf* value)
HH-1	$C_{20}H_{36}O_5$ (356)	C	67.13	67.47	15.3	0.2
		H	9.83	10.19		
HH-2	$C_{19}H_{34}O_4$ (326)	C	69.58	70.00	11.5	0.5
		H	10.23	10.51		
HH-3	$C_{20}H_{36}O_5$ (356)	C	66.98	67.47	14.0	0.8
		H	10.90	10.19		
HH-4	$C_{21}H_{40}O_5$ (372)	C	67.35	67.80	9.1	0.2
		H	10.49	10.84		
HH-5	$C_{17}H_{30}O_3$ (282)	C	72.90	72.40	2.4	0.3
		H	10.88	10.72		
HH-6	$C_{20}H_{38}O_4$ (342)	C	70.88	70.23	6.1	0.5
		H	11.16	11.20		
HH-7	$C_{19}H_{38}O_3$ (314)	C	72.12	72.67	2.8	0.65
		H	11.02	10.24		
HH-8	$C_{18}H_{34}O_3$ (298)	C	72.36	72.54	2.6	0.8
		H	11.71	11.50		
HH-9	$C_{18}H_{36}O_2$ (284)	C	76.44	76.12	2.1	0.85
		H	12.68	12.78		

a) SE-52, 5%, 1.5m, 180°, N_2 90 ml/min
b) HH-1—HH-3: Silicagel G, ether–petroleum ether (1:1)
HH-4—HH-9: Silicagel G, ether–petroleum ether (1:4)

Using milder condition in above trials, namely at 200° under 150 atm, HH-1 and HH-2 were mainly obtained, and in another condition, at 200° under 250 atm, HH-7, HH-8 and HH-9 were predominant. Their differences in structure were primarily due to existence or non-existence of a methoxy group, an end-acetyl moiety, and a five-membered lactone ring. Thus, the hydrogenation products were classified into five groups concerning the oxygen function which was characterized by IR, NMR and mass spectra.

The first group, HH-1 and HH-2 (Chart 2), had a five-membered lactone and an acetoxy moieties. The γ-lactone moiety was suggested by characteristic absorption of 1770 cm^{-1} in infrared (IR) and by the signals at 7.50 and 7.94 τ of methylene protons at C-2 and C-3 in nuclear magnetic resonance (NMR) spectrum. Furthermore, the predominant peak of *m/e* 99 in mass spectrum (Fig. 1) was obviously due to a five membered lactone containing methyl group. This result supported the presence of the tertiary hydroxy group at C-4 in albocycline structure. The presence of an acetyl group in HH-1 and HH-2 was supported by the signal at 8.00 τ (three protons, singlet) in NMR and the fragments of *m/e* 87, 115 and the peaks at (M-59) and (M-60) in mass spectrum. HH-1 was different from HH-2 in respect that HH-1 had a methoxy group, which was detected by molecular weight determination, NMR (6.8 τ, three protons, singlet), IR (1100 cm^{-1}) and mass spectra (Fig. 1). The fragment peaks at

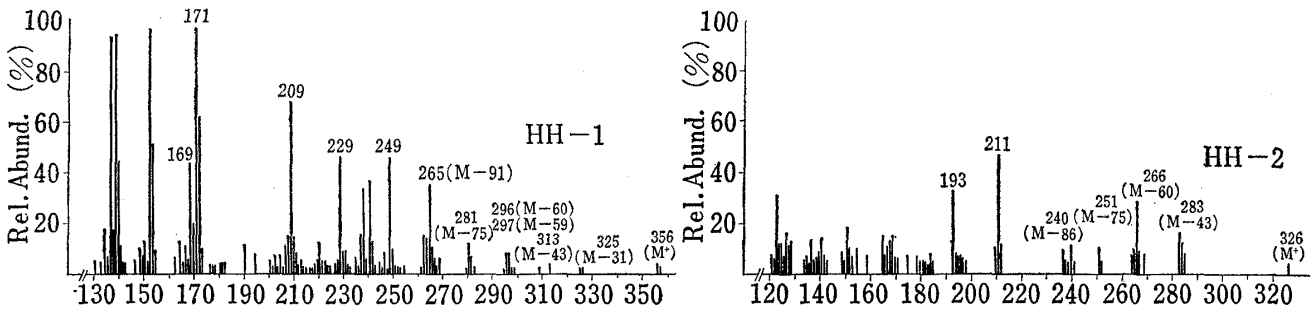
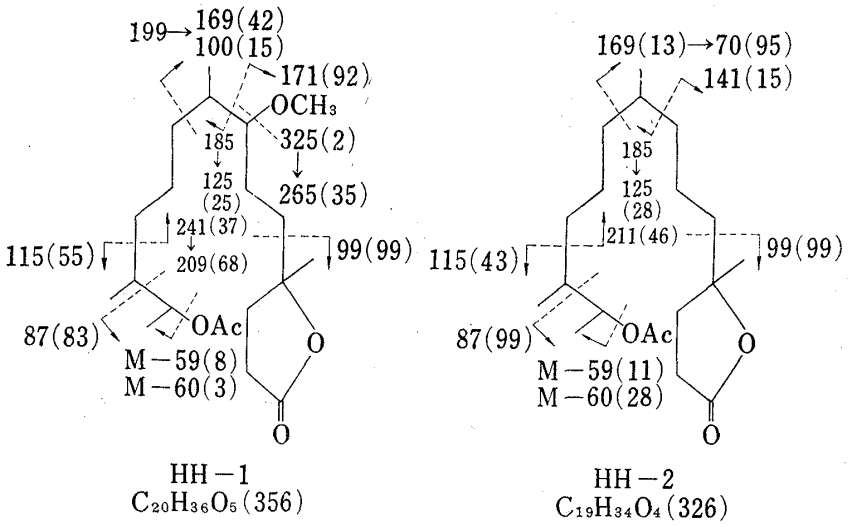


Fig. 1. Mass Spectra of HH-1 and HH-2



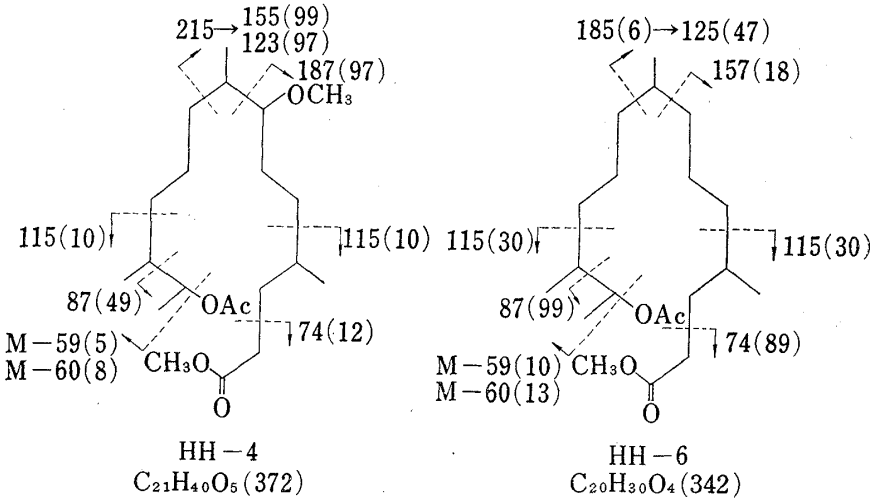
HH−1
$C_{20}H_{36}O_5$ (356)

HH−2
$C_{19}H_{34}O_4$ (326)

Chart 2

m/e 325 (M-31) and *m/e* 265 (M-31-60) in HH-1 strongly suggested the presence of a methoxy group.

The second group, HH-4 and HH-6, was assumed to be methyl esters of long-chained fatty acid containing an acetoxy moiety from the following data. In NMR spectra, they showed the signals at 6.34 and 8.00 τ respectively based on a methyl ester and acetyl groupings and in IR spectra they gave the absorption at 1740 cm^{-1} and 1240 cm^{-1} due to an acetyl group, but they did not show the absorption due to a hydroxy function. Mass spectral data were illustrated in Chart 3. As in the case of the first group, HH-4 was differenciated from HH-6 by the presence of a methoxy substituent. The intense peaks at *m/e* 187 in HH-4 might be rationally ascribed to the α-fission of a methoxy group in position at C-7.

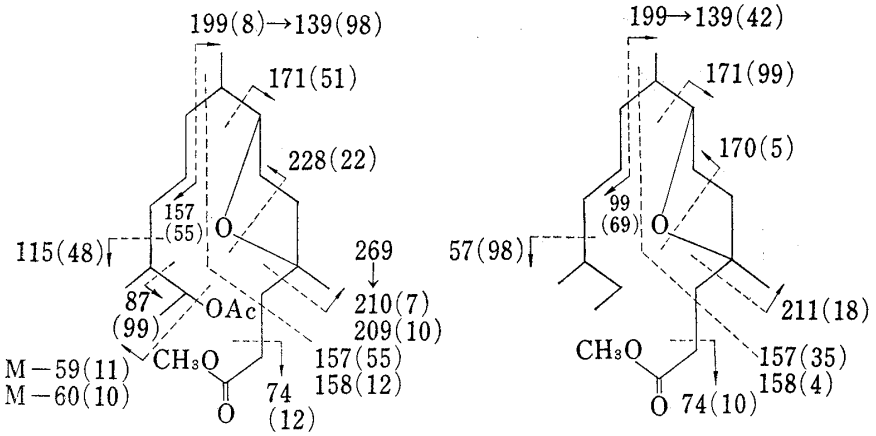


HH−4
$C_{21}H_{40}O_5$ (372)

HH−6
$C_{20}H_{30}O_4$ (342)

Chart 3

The third group, HH-3 and HH-8 (Chart 4), was methyl esters of fatty acids and they did not contain methoxy and hydroxy functions. They were differentiated from each other by the fact whether an acetoxy function was present or not. The abundant peaks of *m/e* 87 and *m/e* 115 depending on the acetoxy moiety at C-13 were observed only in HH-3. The most characteristic feature in both compounds was the presence of the oxylane ring in the molecule happened by hydrogenolysis during hydrogenation. It was confirmed by the newly born methine signal on C-7 at 6.60 τ in NMR, and an abundant peak at *m/e* 171 in mass spectrum.

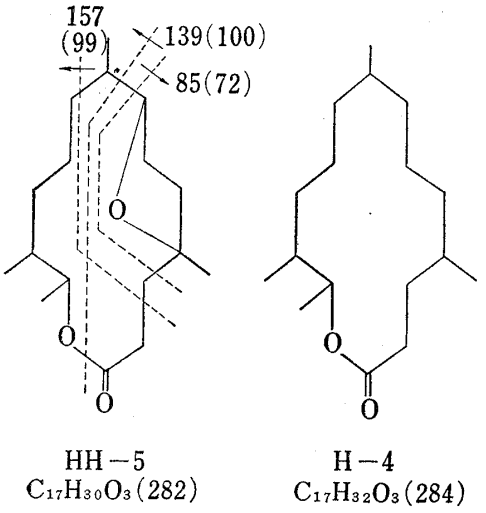


HH−3
$C_{20}H_{36}O_5$ (356)

HH−8
$C_{18}H_{34}O_3$ (298)

Chart 4

HH-5 was the only one product remaining a 14-membered lactone ring and should be compared with H-4 which was the product obtained by catalytic hydrogenation at an atomospheric pressure as described in the previous paper (Chart 5). In the IR spectrum of HH-5 absorptions based on a lactone at 1730 and 1250 cm^{-1} were remarkably observed and the characteristic peak at *m/e* 85 in mass spectrum corresponded to α-methyltetrahydrofuran ion which was produced by intramolecule oxylane–ring formation as shown in Chart 5.



HH−5
$C_{17}H_{30}O_3$ (282)

H−4
$C_{17}H_{32}O_3$ (284)

Chart 5

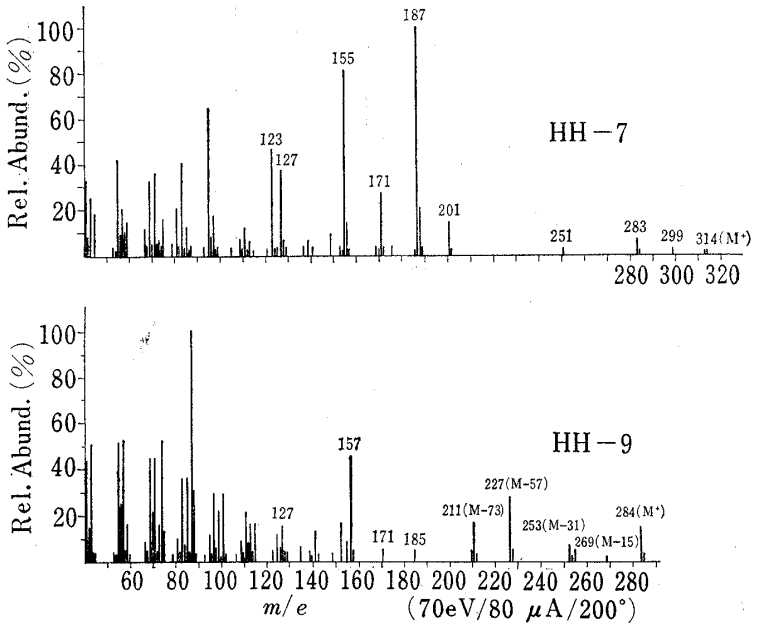


Fig. 2. Mass Spectra of HH-7 and HH-9

The last group of HH-7 and HH-9 (Chart 6) was compared in the same manner. On the basis of spectrometric data, both were determined to be methyl esters of fatty acids, which lost two oxygen function at C-4 and C-13. HH-7 was different from HH-9 on the point that HH-7 remained a methoxy group which was proved by a signal at 6.65 τ (three protons, singlet) in NMR spectrum. The fragment of *m/e* 187 of HH-7 was clearly explained by α-fission of methoxy group as in the case of HH-1 and HH-4.

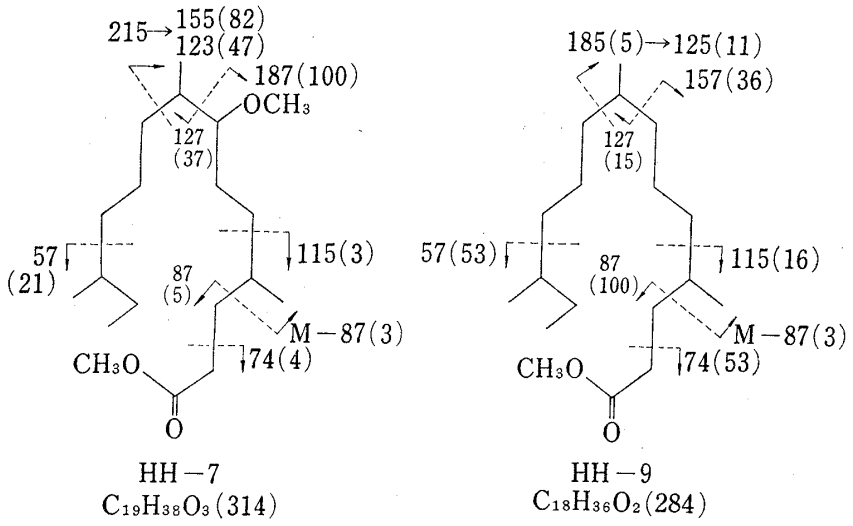


Chart 6

The mass spectral fragmentation patterns of high-pressure hydrogenation products have finally pointed out the position of a methoxy at C-7, a hydroxy at C-4 and a lactonic oxygen at C-13 in albocylcine. These results were fully consistent with the data of a high resolution mass spectrum of albocycline illustrated in Fig. 3 and Table II.

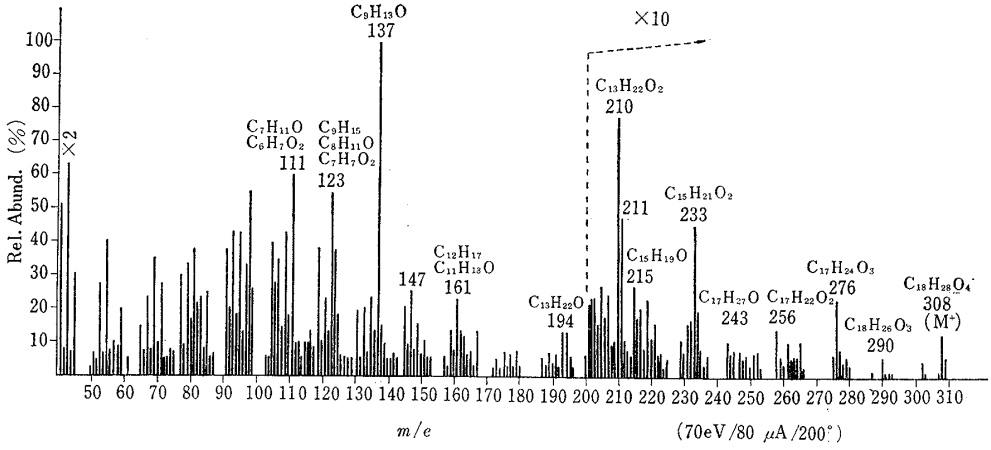


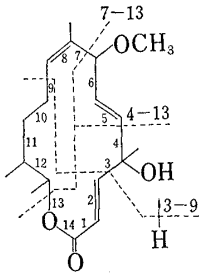
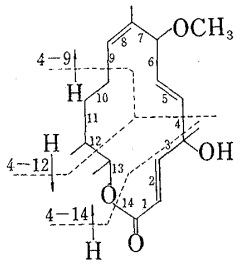
Fig. 3. High Resolution Mass Spectrum of Albocycline

Based on the experimental result on periodate oxidation of cineromycin B which was assumed to be des-O-methyl albocycline, the authors once presented the structures of albocycline and cineromycin B as IIa and IIb respectively.[9] However, as described in the present and preceding papers, the detailed spectrometric examination of albocycline and its numerous hydrogenation products, led to the conclusion that Ia was more plausible to the structure of albocycline than IIa, though Ia was incompatible to the result on periodate oxidation experiments.

The possibility that the structure of cineromycin B might be shown in IIb would be neglected by a close similarities of the structure reflected by NMR and mass spectra of both antibiotics. The second was unexpected consumption on periodate oxidation in case of cineromycin B. Concerning the abnormal periodate reaction in cineromycin B, the following reasons

9) M. Suzuki, N. Nagahama and I. Takamori, "Jap. Antibiot. Res. Assoc.," 157th Meeting, Sept., 1967.

Table II

Two-bond fission pattern	Fission type	Ion species in medium mass region	
	4—13	*m/e* 194 $C_{13}H_{22}O^+$-MeOH	*m/e* 162 ($C_{12}H_{18}^+$)
		m/e 193 $C_{13}H_{21}O$-MeOH	*m/e* 161 ($C_{12}H_{17}^+$)
	7—13	C_9H_{16} (124)	*m/e* 123 ($C_9H_{15}^+$)
	3— 9	$C_9H_{15}O_2$-H_2O (155)	*m/e* 137 ($C_9H_{13}O^+$)
		-MeOH	*m/e* 123 ($C_9H_{11}O^+$)
		-MeCHO	*m/e* 111 ($C_7H_{11}O^+$)
	4— 9	*m/e* 111 $C_7H_{11}O^+$-MeOH	*m/e* 79 ($C_6H_7^+$)
	4—12	$C_{11}H_7O^+$	*m/e* 161 ($C_{11}H_{13}O^+$)
	4—14	*m/e* 210 $C_{13}H_{22}O_2^+$	*m/e* 211 $C_{13}H_{23}O_2^+$

would be considered. It would be supposed that the hydroxy group at C-7 in cineromycin B was sensitive to the oxidizing agents and, consequently, occured the allylic rearrangement through periodate ester formation of C-7-ol. It was regretful that, because of unavailability of the necessary amount of cineromycin B, the authors were not able to draw final conclusions. Fundamental experiments on the matter would be problems in future.

Experimental

High Pressure-High Temperature Hydrogenation——2 g of albocycline were dissolved in 30 ml of glacial acetic acid and hydrogenated in an autoclave at 167 atm and 200° for 20 min in the presence of 1 g of 5% paladium on alumina. After standing for 18 hours at room temperature, the reaction mixture was filtered. The catalyst cake was extracted by refluxing with methanol two times. The extract was combined to the filtrate and evaporated in reduced pressure to yield an oily substance. It was esterified by diazomethane in ethereal solution and evaporated to dryness *in vacuo*. 1.78 g of oily substance were obtained. It gave eight spots on TLC; Silica gel G, ethyl ether–petroleum ether (1:1). These components were isolated by column chromatography on Silica gel H (Merck) using the solvent system of ethyl ether and petroleum ether as following.

Component name	Eluted solvent	Fraction No.[a)]	Yield (mg)
HH-9 and HH-8	5% (ether/pet. ether)	11— 18	32.5
HH-7	5% (ether/pet. ether)	29— 42	86.9
HH-6	10% (ether/pet. ether)	49— 53	148.3
HH-5	10% (ether/pet. ether)	57— 62	58.5
HH-4	20% (ether/pet. ether)	79—105	517.0
HH-3	40% (ether/pet. ether)	116—126	21.0
HH-2	60% (ether/pet. ether)	166—191	29.5
HH-1	60% (ether/pet. ether)	231—286	530.7

a) One fraction was collected by 1 ml.

The purity of each components was confirmed by TLC and gas chromatography. Through the portion of fraction 11—18 gave one spot on TLC, it was mixture of HH-8 and HH-9 by gas chromatography. By preparative gas chromatography (1.5 m, 5% SE-52, N_2, 90 ml/min, 180°), 3 mg of HH-8 and 5 mg of HH-9 were, respectively, obtained from 22 mg of the mixture.

When it was hydrogenated in condition at 250 atm and 200°, the following products were obtained from 2 g of the starting material.

Component name	Yield (mg)	Component name	Yield (mg)
HH-9	1128	HH-6	34
HH-8	64	HH-5	trace
HH-7	368		

HH-1——Liquid. *Anal.* Calcd. for $C_{20}H_{36}O_5$: C, 67.47; H, 10.19. Found: C, 67.13; H, 9.83. IR ν_{max}^{liquid} (cm^{-1}): 1770, 1730, 1250, 1100. NMR τ ($CDCl_3$): 9.20 (3H, d, J=6 Hz, –CH–C$\underline{H}_3$), 9.15 (3H. d, J=6 Hz, –CH–C$\underline{H}_3$), 8.90 (3H, d, J=6 Hz, –CH–C$\underline{H}_3$), 8.65 (3H, s, C–C$\underline{H}_3$), 8.00 (3H, s, -OCOC$\underline{H}_3$), 7.10 (1H,m), 6.64 (3H, s, -OC$\underline{H}_3$), 5.25 (IH, m). Mass Spectrum *m/e*: 356 (M^+), 325 (M-31), 313 (M-43), 296 (M-59), 295 (M-60), 281, 265, 249, 229, 209.

HH-2——Liquid. *Anal.* Calcd. for $C_{19}H_{34}O_4$: C, 70.00; H, 10.51. Found: C, 69.58; H, 10.23. IR ν_{max}^{liquid} (cm^{-1}): 1770, 1730, 1250. NMR τ ($CDCl_3$): 9.13 (6H, d, J=6 Hz, –CH–C$\underline{H}_3$×2), 8.88 (3H, d, J=6 Hz, –CH–C$\underline{H}_3$), 8.65 (3H, s, C–C$\underline{H}_3$), 8.04 (3H, s, -OCOC$\underline{H}_3$), 5.30 (1H, m). Mass Spectrum *m/e*: 326 (M^+), 283 (M-43), 266 (M-60), 251, 240, 211, 193.

HH-3——Liquid. *Anal.* Calcd. for $C_{20}H_{36}O_5$: C, 67.47; H, 10.19. Found: C, 66.98; H, 10.90. IR ν_{max}^{liquid} (cm^{-1}): 1735, 1240. NMR τ ($CDCl_3$): 9.15 (6H, d, J=6 Hz, -CH-C$\underline{H}_3$×2), 8.89 (3H,d, J=6 Hz, -CH-C$\underline{H}_3$), 8.77 (3H, s, C–C$\underline{H}_3$), 8.00 (3H, s, -OCOC$\underline{H}_3$), 6.60 (1H, m), 6.37 (3H, s, -OCOC$\underline{H}_3$), 5.25 (1H, m). Mass Spectrum *m/e*: 356 (M^+), 341 (M-15), 297 (M-59), 296 (M-60), 282, 269.

HH-4——Liquid. *Anal.* Calcd. for $C_{21}H_{40}O_5$: C, 67.80; H, 10.84. Found: C, 67.35; H, 10.49. IR ν_{max}^{liquid} (cm^{-1}): 1735, 1240, 1100. NMR τ ($CDCl_3$): 9.16 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 9.13 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 9.12 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 8.86 (3H, d, J=6 Hz, –CH–C$\underline{H}_3$), 8.00 (3H, s. –OCOC$\underline{H}_3$), 7.68 (2H, t, J=6 Hz). 7.18 (1H, m), 6.69 (3H, s, -OCH_3), 6.34 (3H, s, –COOC$\underline{H}_3$), 5.18 (1H, m). Mass Spectrum *m/e*: 372 (M^+), 357 (M-15), 341 (M-29), 313 (M-59), 312 (M-60), 297, 281.

HH-5——Liquid. *Anal.* Calcd. for $C_{17}H_{30}O_3$: C, 72.40; H, 10.72. Found: C, 72.90; H, 10.88. IR ν_{max}^{liquid} (cm^{-1}): 1730, 1250. NMR τ ($CDCl_3$): 9.22 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 9.12 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 8.86 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 8.80 (3H, s, C-C$\underline{H}_3$), 6.00 (1H, m), 5.20 (1H, m). Mass Spectrum *m/e*: 282 (M^+), 267 (M-15), 249, 238, 209, 157.

HH-6——Liquid. *Anal.* Calcd. for $C_{20}H_{38}O_4$: C, 70.23; H, 11.20. Found: C, 70.88; H, 11.16. IR ν_{max}^{liquid} (cm^{-1}): 1740, 1250, 1170. NMR τ ($CDCl_3$): 9.16 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 9.12 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 8.90 (3H, d, J=6 Hz, -CH-C$\underline{H}_3$), 7.97 (3H, s, -OCOC$\underline{H}_3$), 6.34 (3H, s, -OCOC$\underline{H}_3$), 5.20 (1H, m). Mass Spectrum *m/e*: 342 (M^+), 327 (M-15), 311 (M-31), 299 (M-43), 283 (M-59), 282 (M-60), 267, 251, 250, 227.

HH-7——Liquid. *Anal.* Calcd. for $C_{19}H_{38}O_3$: C, 72.67; H, 10.24. Found: C, 72.12; H, 11.02. IR ν_{max}^{liquid} (cm^{-1}): 1740, 1170, 1100. NMR τ ($CDCl_3$): 9.1—9.3 (12H), 7.05 (1H, m), 6.65 (3H, s, -OC$\underline{H}_3$), 6.31 (3H, s, -COOC$\underline{H}_3$). Mass Spectrum *m/e*: 314 (M^+), 299 (M-15), 283 (M-31), 251, 201, 187.

HH-8——Liquid. *Anal.* Calcd. for $C_{18}H_{34}O_3$: C, 72.54; H, 11.50. Found: C, 72.36; H, 11.71. IR ν_{max}^{liquid} (cm^{-1}): 1735. Mass Spectrum *m/e*: 298 (M^+), 283 (M-15), 267 (M-31), 254, 237, 211, 172, 171.

HH-9——Liquid. *Anal.* Calcd. for $C_{18}H_{36}O_2$: C, 76.12; H, 12.78. Found: C, 76.44; H, 12.68. IR ν_{max}^{liquid} (cm^{-1}): 1740, 1170. NMR τ ($CDCl_3$): 9.25—9.00 (16H, -CH_3×4), 9.0—8.3 (-C$\underline{H}_2$-), 7.65 (2H, t, J=6 Hz, -C$\underline{H}_2$-), 6.30 (3H, s, -COOC$\underline{H}_3$). Mass Spectrum *m/e*: 284 (M^+), 269 (M-15), 253 (M-31), 227, 211, 185, 171, 157.

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