Chemical Structure of Piericidin A

Part III. Structures of Piericidin A and Octahydropiericidin A

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Octahydropiericidin A (HPA) was degraded to C₁₁-, C₁₂- and C₁₄-dioic and C₁₈-hydroxy acids, *via* C₁₈-keto acid, to which structures XI, X, VIII and VII were proposed respectively through analyses of their NMR and mass spectra. Consideration of these results together with the NMR spectrum of piericidin A (PA) and other chemical evidences led to assign structures IIa and IVa to PA and HPA, respectively.

Piericidin A (PA) is a new insecticidal metabolite of *Streptomyces mobaraensis*¹⁾ and its isolation,¹⁾ physiological activities,²⁾ functional groups and partial structure³⁾ Ia or Ib have been presented in the previous papers. Recently we preliminary proposed⁴⁾ structure IIa to PA and IVa to octahydropiericidin A. This report deals with full description on the evidences which allow these assignments.

One of the possible formulas proposed to PA previously, C₂₅H₃₇NO₉ is compatible with degradation evidences described in this paper. Further this was confirmed by the presence of molecular ion at m/e 507 in the mass spectrum of HPA diacetate, C₂₉H₄₉NO₈.

The NMR spectrum of HPA shows that its side chain contains a secondary hydroxyl group (1H, m, δ 3.00,⁵⁾ which shifts to δ 4.60 on acetylation) and five methyl groups (15H in the region δ 0.8~1.2). Oxidation of HPA with nitric acid gave 2-methylbutyric acid, which was identified by means of paper and gas chromatography and also by mixed melt-

ing point of its amide derivative. This suggests the presence of partial structure (A) in

the side chain of HPA. C₁₈-keto acid, C₁₈H₃₄O₈ (VI), which was obtained from HPA through ozonolysis, hydrolysis and chromium trioxide

1) S. Tamura, N. Takahashi, S. Miyamoto, R. Mori, S. Suzuki and J. Nagatsu, *This Journal*, **27**, 576 (1963).

2) N. Takahashi, A. Suzuki, S. Miyamoto, R. Mori and S. Tamura, *ibid.*, **27**, 583 (1963).

3) N. Takahashi, A. Suzuki and S. Tamura, *ibid.*, **27**, 798 (1963).

4) N. Takahashi, A. Suzuki and S. Tamura, *J. Am. Chem. Soc.*, **87**, 2066 (1965).

5) Chemical shifts are expressed in δ-value (p.p.m.) from tetramethylsilane as internal standard.

oxidation, was oxidized in methylene chloride by Baeyer-Villiger reaction using trifluoroperoacetic acid to give oily ester-acid, $C_{18}H_{34}O_4$. After hydrolysis of this C_{18} -ester-acid with alkali, the product was separated into volatile and unvolatile acidic fractions. In the volatile fraction, the presence of 2-methylbutyric acid was confirmed by paper chromatography. The unvolatile fraction shows two spots at R_F 0.71 and 0.47 on its paper chromatogram using solvent system of 7N ammonia-isopropanol (2:8). Each acid was separated by preparative paper chromatography. In large scale preparation, the unvolatile acidic mixture was esterified, and the ester mixture was separated by silicic acid column. Examination of infrared and NMR spectra, retention time on gas chromatography and microanalytical data of each ester reveals that acid of R_F 0.71 must be C_{13} -hydroxy acid, $C_{13}H_{26}O_3$ (VII), and acid of R_F 0.47 to be C_{14} -dioic acid, $C_{14}H_{26}O_4$ (VIII). This result indicates that C_{18} -ester-acid should be a mixture of two isomers which were produced by introduction of oxygen atom to each side of the keto group with equal probability. Then, C_{13} -hydroxy acid (VII) was oxidized to yield C_{13} -keto acid, $C_{13}H_{24}O_3$ (IX), which shows positivity for iodoform reaction, suggesting the presence of methyl keto group. Oxidation of IX with sodium hypobromite gave C_{12} -dioic acid, $C_{12}H_{22}O_4$ (X). These evidences make it possible to assign the position of three methyl groups out of the five contained in the side chain of HPA as the following structure (B). Positions of remaining two

methyl groups were decided as follows. Treatment of IX with trifluoroperoacetic acid followed by hydrolysis and chromium trioxide oxidation gave C_{11} -dioic acid, $C_{11}H_{20}O_4$ (XI). Comparison of NMR spectra was made be-

tween methyl esters of X and XI as shown in Fig. 1. In the spectrum of X ester, six protons triplet due to two methyl groups was observed in the region of δ 0.8~0.9. On the

FIG. 1. NMR Spectra of Methyl Esters of C_{11} - and C_{12} -dioic Acids.

(A) C_{11} -diester (B) C_{12} -diester

other hand, in that of XI ester, two doublets due to two methyl groups were observed at δ 0.86 and 1.09. Signal at δ 1.09, showing shift to lower field by 0.2 p.p.m. from chemical shift of methyl group in X ester, suggests the presence of a methyl group at α -carbon to a carbomethoxyl group in XI ester. In good agreement with this observation, four protons signal due to protons attached to α -carbon to carbomethoxyl groups was observed in the range of δ 2.0~2.5 in X ester, while three protons signal in the range of δ 2.0~2.5 in XI ester. The position of the last methyl group was assigned in the following way. C_{14} -dioic acid (VIII) was converted to acyl chloride on heating with excess of thionyl chloride. After removal of sulfur dioxide in vacuo, the solution was treated with bromine to give C_{14} - α,α' -dibromodioic acid. After hydrolysis of this bromo acid with alkali, the product was esterified and oxidized with chromium trioxide

FIG. 2. NMR Spectrum of Methyl Ester of C_{14} - α -oxo- α' -hydroxydioic Acid.

to give C_{14} - α -oxo- α' -hydroxydioic acid (XII) methyl ester. As shown in Fig. II, the NMR spectrum of XII ester* reveals an octet of AB protons of ABX type^{6†} at δ 2.2~3.0 (J_{AB} = 16,^{7‡} J_{AX} = 6, J_{BX} = 7 c.p.s.), suggesting the presence of methylene group in the system (C). This indicates that the remaining one

methyl group attaches to the γ -carbon to the carboxyl group in VIII. Thus structures of C_{11} -dioic, C_{12} -dioic, C_{14} -dioic, C_{14} - α -oxo- α' -hydroxydioic, C_{13} -hydroxy and C_{18} -keto acids should be 2,6-dimethylnonanedioic (XI), 3,7-dimethyldecanedioic (X), 2,4,8-trimethylundecanedioic (VIII), 2-hydroxy-2,4,8-trimethyl-10-oxoundecanedioic (XII), 10-hydroxy-4,8-dimethylundecanoic (VII), and 11-oxo-4,8,10,12-tetramethyltridecanoic acids (VI), respectively. Outline of these degradation experiments is shown in Scheme I.

Mass spectra of these degradation products were also analysed. They are in very good agreement with these structural assignments. Details will be discussed in the succeeding paper.

For final confirmation of structure of C_{12} -dioic acid, dimethyl 3,7-dimethyldecanedioate was synthesized together with its isomers, dimethyl 4,5-dimethyl- and 3,6-dimethyldecanedioates. Synthetic routes are shown in

Scheme II. Infrared and mass spectra of these three esters show close similarity with each other but some clear differences were observed and spectra of C_{12} -dioic acid methyl ester from natural source were completely identical with those of dimethyl 3,7-dimethyldecanedioate.

Thus the positions of all substituents, *viz.* five methyl and a secondary hydroxyl groups in the side chain of HPA have been assigned definitely as shown in IV.

The remaining problem is how to put four double bonds in the side chain of PA having the substitution pattern (D). It has been

already shown that PA contains four double bonds, to which four methyl groups (12 H, δ 1.5~1.8) and five hydrogens (5 H, δ 5.0~6.2) attach, along with a group $\text{CH}_3-\text{CH}-$ (3 H, d, δ 0.78), from consideration of its NMR spectrum. Furthermore, evolution of acetaldehyde on ozonolysis of PA diacetate suggests that one of four allyl methyls exists in the form $\text{CH}_3-\text{CH}=\text{}$. As shown in Fig. III, three doublets are observed at δ 2.73, 3.30 and 3.53 in the NMR spectrum of PA. Two protons doublet at δ 3.30 must be due to the methylene group in the group $=\text{CH}-\text{CH}_2-\text{pyr}$. This accommodates the structure of Acid II (V), obtained on ozonolysis of PA diacetate, and two protons triplet at δ 2.60 in NMR spectrum of HPA. Since one proton doublet at δ 3.53 shifts to δ 4.90 on acetylation, this signal must be due to a methine group in the form $=\text{C}-\text{CH}(\text{OH})-\text{CH}-$. Two protons doublet at δ 2.73 can be associated with a methylene group in $=\text{CH}-\text{CH}_2-\text{C}=\text{}$ from its chemical shift and feature of coupling.

It was already reported that Acid II (V) was isolated, along with Acid I (acetate of Acid II), on ozonolysis of PA diacetate. Further investigation on the acidic fraction of ozonolysis products from PA diacetate resulted

* The spectrum was determined by Varian H-100 spectrometer at 100 megacycle.

† L. M. Jackman, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, New York, N. Y., 1959, p. 102.

‡ T. Takahashi, *Tetrahedron Letters*, 565 (1964).

Scheme I

in the isolation of a new acid, Acid III, C₈H₁₂O₅, mp. 98°C. It shows positivity for iodoform reaction, suggesting the presence of a methyl keto group. Two bands at 1740 (intensity corresponds to two carbonyl functions) and 1718 cm⁻¹ in the carbonyl region of its infrared spectrum show the presence of

acetoxyl, carboxyl and isolated keto groups. This was also confirmed by its NMR spectrum (3H, d, δ 1.30, **CH**₃-CH-; 6H, s, δ 2.33, acetoxyl and methyl keto groups; 1H, m, δ 3.1~3.5, -**CH**(CH₃)-COOH). The presence of these functional groups indicates that Acid III must have originated from a structural

1) Dimethyl 3,7-dimethyldecanedioate

2) Dimethyl 4,7-dimethyldecanedioate

3) Dimethyl 3,6-dimethyldecanedioate

Scheme II

part which is located between two double bonds and contains an acetylated secondary hydroxyl group. From the consideration of HPA structure and NMR spectrum of Acid III, this acid must be 3-acetoxy-2-methyl-4-oxopentanoic acid, (XIII). Contrary to the

earlier proposal, isolation of Acid III excludes the presence of two conjugated dienes in PA. Now only two kinds of side chain structure, II and III, accomodate all these data. Then

spin decoupling study⁸⁾ was undertaken. Spectrum changes at double resonance experiment and final assignment of each proton are shown in Fig. III. Multiplicity change and chemical shift of decoupled protons are summarized in Table I. Spin decoupling study not only affords rigorous proof to the assigned structures for PA but also reveals precise chemical shifts of "hidden" protons in olefinic region and a missing methinyl proton. NMR spectrum measured by 100 Mc instrument is also in good agreement with this proton assignment. Especially one proton doublet

⁸⁾ J. D. Baldeschwieler and E. W. Randall, *Chem. Rev.*, **63**, 81 (1963).

FIG. 3. NMR Spectrum of Piericidin A and Spectrum Change on Double Resonance Experiment.

TABLE I. MULTIPLICITY CHANGE AND PRECISE CHEMICAL SHIFT OF EACH PROTON IN SPIN DECOUPLING STUDY OF PIERICIDIN A

Position of protons, p.p.m.		Multiplicity change
Irradiated	Observed	
H-9 2.66	H-16 0.78	d→s
	H-10 3.53	d→s
	H-8 5.12	d→s
H-5 5.48	H-6 2.73	d→s
H-6 2.73	H-5 5.48	m→d
H-2 5.30	H-1 3.30	d→s
H-1 3.30	H-2 5.30	t→s
H-12 5.35	H-13 1.57	d→s
H-13 1.57	H-12 5.35	q→s

due to H-8 (δ 5.12) was observed separately from other olefinic protons. This proton which resonates at highest field among olefinic protons indicates that it belongs to the isolated

double bond,⁹⁾ not to the conjugated diene. Although all attempts to differentiate chemically II and III have been unsuccessful, the former should be more probable than the latter from this NMR evidence.

Evolution of diacetyl on ozonolysis of PA diacetate have been reported previously, but it became clear now that diacetyl cannot originate from the side chain of II. This is explainable only by decarboxylation of an intermediate, 3,4-dioxopentanoic acid (XIV), derived from decomposition of the pyridine ring of type (a) accompanying fission of the C-2 double bond in the side chain.

The presence of this novel polysubstituted pyridine ring was further confirmed by comparison of the ultraviolet spectra of HPA and

9) (a) ref. 6, p. 61; (b) J. A. Pople, W. G. Schneider and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance" McGraw Hill Book Co., Inc., New York, N. Y., 1959, p. 285.

Acid II with that of a model compound, 4-hydroxy-6-hydroxymethyl-2,3-dimethoxypyridine (XV), synthesized from 2-bromo-6-hydroxymethyl-4-oxo-3-methoxy-(4H)-pyran.¹⁰⁾ Shifts of ultraviolet spectra in neutral, acidic and basic solutions of these compounds were quite similar to each other. Synthesis and properties of this model compound will be reported in the separate paper.

Thus structures IIa and IVa has been assigned to PA and HPA, respectively.

Strong ultraviolet absorption of PA ($\lambda_{\max}$ 239 m μ , $\log \epsilon$ 4.6) and large coupling constant between H-5 and H-4 (16 c.p.s.) indicate that conjugated diene of PA must have *trans-trans* configuration. PA contains only two assymetric carbons, C-9 and C-10, which are involved in Acid III. Using this key compound, work on the elucidation of stereochemistry of these carbons is now in progress.

Biogenetically, the side chain of PA may be formed from acetate-propionate unit, but biogenesis of pyridine ring is not clear so far.

Relation between structure and physiological activity is also quite interesting, because PA contains a pyridine ring having similar substitution pattern to quinoid ring of coenzyme Q, and side chain is partly modified from isoprenoid chain of this conenzyme.

These problems may be solved in the future.

EXPERIMENTAL

Infrared spectra were measured in liquid films on oily samples and nujol mulls on crystals with Koken DS 301 spectrophotometer and ultraviolet spectra with Cary 14-PM spectrophotometer. NMR spectra of 60 megacycle were determined in carbon tetrachloride solution otherwise stated with Varian A-60 spectrometer. Spin decoupling study was conducted using JNM-C-60 spectrometer. Melting points are not corrected.

Nitric Acid Oxidation of HPA

A mixture of 750 mg of HPA and 10 ml of nitric acid (d 1.38) was heated for 20 min. at 140°C and, then, steam distilled. Residual unvolatile fraction was extracted with ether continuously. Evaporation of the solvent gave 100 mg of crude oxalic acid, which was purified by sublimation. Steam distillate was extracted with ether at pH 3. On evaporation of the solvent, 90 mg of crude volatile acid was obtained. Paper chromatography of this crude product using butanol saturated with 1.5 N ammonia revealed a main spot at R_F 0.48, which was identified as 2-methylbutyric acid by comparison with that of authentic sample.

Next, the crude acid was esterified with diazomethane in ether solution. The crude ester thus obtained was subjected to gas chromatography. Retention time was determined on Shimazu Seisakusho Model GC-1 B (hydrogen flame detector; stainless steel column, 225 cm $\times$ 6 mm, i.d.; column packing, ethyleneglycol succinate; column temperature, 118°C; carrier gas, nitrogen, 30 ml min⁻¹). Among four peaks observed, the main peak showing retention time of 2.7 min. was identical with that of authentic methyl 2-methylbutyrate. Further the crude acid was dissolved in thionyl chloride and refluxed for 2 hrs on a steam bath. The reaction mixture was poured into aq. ammonia cooled in an ice bath, followed by extraction with ether. After removal of the solvent, the residue was recrystallized from dil. ethanol into

10) (a) T. Yabuta, *J. Chem. Soc.*, **125**, 575 (1924); (b) I. Ichimoto, K. Miyagawa and C. Tatsumi, *Bull. Univ. Osaka Pref.*, B15, 61 (1964).

colorless rods, m.p. 109°C. Depression was not observed in mixed melting point with authentic 2-methylbutanamide. Infrared spectra of both samples showed complete identity.

Degradation Reaction of C_{18} -keto acid (VI)

a) Baeyer-Villiger Reaction of C_{18} -keto acid (VI)

To 1.6 g of trifluoroacetic anhydride in 6 ml of methylene chloride 0.14 g of 90% hydrogen peroxide was added under cooling with stirring. Then, 480 mg of C_{18} -keto acid (VI) in 7 ml of methylene chloride was added to the oxidation reagent with stirring at room temperature and kept at 26°C for 2 days. To the reaction mixture was added 50 ml of chloroform, which was washed three times with 10% aq. sulfuric acid solution and dried over anhydrous sodium sulfate. On evaporation of the solvent, 500 mg of residual syrup was obtained. This syrup was purified through 20 g of silicic acid column. Elution with benzene-ethyl acetate (100:0.7, v/v) afforded 130 mg of trifluoroacetate of C_{13} -hydroxy acid, which gave C_{13} -hydroxy acid (VII) on alkali hydrolysis. Successive elution with benzene-ethyl acetate (100:1.5, v/v) afforded 150 mg of C_{18} -ester-acid mixture. *Anal.* Found: C, 68.32; H, 10.80. Calcd. for $C_{18}H_{34}O_4$: C, 68.75; H, 10.90%.

b) Hydrolysis of C_{18} -ester-acid Mixture

Two hundred mg of C_{18} -ester-acid mixture was hydrolyzed with 10 ml of 1 N NaOH under reflux for 1.5 hrs. The reaction mixture was subjected to steam distillation at pH 3. The distillate was extracted with ether. Paper chromatography of the volatile acidic fraction obtained after evaporation of the solvent indicated the presence of 2-methylbutyric acid. The aqueous solution remained after distillation was extracted with ether. Evaporation of the solvent gave 110 mg of unvolatile acidic fraction, which showed two spots at R_F 0.47 and 0.71 on paper chromatogram using solvent system, isopropanol-7 N ammonia (8:2, v/v). Each acid was isolated by preparative paper chromatography using the same solvent system as above. Paper zone corresponding to R_F 0.71 gave 70 mg of C_{13} -hydroxy acid (VII) and that of R_F 0.47 45 mg of C_{14} -dioic acid. Each acid was esterified with diazomethane and the ester thus obtained was purified through 4 g of silicic acid column. C_{14} -diester was eluted with benzene-ethyl acetate (100:0.5, v/v). NMR spectrum: 6 H, d, δ 0.90; 3 H, d, δ 1.12; 12 H, δ 1.1~1.9; 3 H, t, δ 2.25; 6 H, s, δ 3.61. *Anal.* Found: C, 67.14; H, 10.52.

Calcd. for $C_{16}H_{30}O_4$: C, 67.09; H, 10.56%.

Elution with benzene-ethyl acetate (100:3, v/v) gave C_{13} -hydroxy ester. NMR spectrum: 6 H, d, δ 0.90; 3 H, d, δ 1.12; 12 H, δ 1.2~1.9; 1 H, s, δ 1.70 (OH); 2 H, t, δ 2.27; 3 H, s, δ 3.61; 1 H, m, δ 3.5~4.0. *Anal.* Found: C, 68.11; H, 11.49. Calcd. for $C_{14}H_{26}O_3$: C, 68.81; H, 11.55%. Treatment of C_{13} -hydroxy ester with acetic anhydride and sodium acetate under reflux gave its acetate, which was purified through silicic acid column. NMR spectrum: 6 H, d, δ 0.89; 3 H, d, δ 1.17; 12 H, δ 1.1~1.9; 3 H, s, δ 1.94; 2 H, t, δ 2.23; 3 H, s, δ 3.61; 1 H, m, δ 4.6~5.2. *Anal.* Found: C, 67.25; H, 10.59. Calcd. for $C_{16}H_{30}O_4$: C, 67.09; H, 10.56%.

In a large scale experiment, 500 mg of the unvolatile acidic fraction was esterified with diazomethane and a mixture of esters was separated through 15 g of silicic acid column. Elution with benzene-ethyl acetate (100:0.5, v/v) gave 110 mg of C_{14} -diester and benzene-ethyl acetate (100:3, v/v) gave 100 mg of C_{13} -hydroxy ester.

c) C_{13} -keto ester

To 270 mg of C_{13} -hydroxy ester in 10 ml of acetone was added 8 N chromium trioxide-sulfuric acid solution with stirring under cooling until red color of chromium trioxide began to remain. Stirring was continued for additional 30 min. The reaction mixture was poured onto water-ice mixture, followed by extraction with ether. Evaporation of the solvent gave 220 mg of C_{13} -keto ester which was purified through 10 g of silicic acid column. Elution with benzene-ethyl acetate (100:1, v/v) afforded pure C_{13} -keto ester. *Anal.* Found: C, 69.55; H, 10.84. Calcd. for $C_{14}H_{26}O_3$: C, 69.38; H, 10.81%.

d) C_{12} -diester

Three hundred mg of C_{13} -keto ester was hydrolyzed with 10 ml of 3 N NaOH at 120°C for 1.5 hrs. To this mixture, sodium hypobromite solution, prepared by adding 0.3 ml of bromine to 10 ml of 3 N NaOH, was added under cooling. After 1 hr, reaction mixture was acidified and extracted with ether. Evaporation of the solvent afforded 260 mg of crude product, which was esterified with diazomethane. The crude ester thus obtained was purified through 10 g of silicic acid column. Elution with benzene gave 100 mg of C_{12} -diester. NMR spectrum: 6 H, δ 0.8~1.0; 10 H, δ 1.1~1.8; 4 H, δ 2.0~2.4; 6 H, s, δ 3.58. *Anal.* Found: C, 65.16; H, 10.13. Calcd. for $C_{14}H_{26}O_4$: C, 65.08; H, 10.14%.

e) C₁₁-diester

To the oxidation reagent, prepared by adding 0.33 ml of 90% hydrogen peroxide to 10 ml of methylene chloride containing 3 g of trifluoroacetic anhydride, 780 mg of C₁₃-keto acid in 10 ml of methylene chloride was added and let to stand at room temperature for 48 hrs. This mixture was treated with 10% aq. sulfuric acid solution and extracted with chloroform. Evaporation of the solvent gave 700 mg of crude product, which was hydrolyzed with 30 ml of 3 N NaOH for 2 hrs at 120°C followed by acidification. Acidic fraction was extracted with ether and esterified with diazomethane, to give 620 mg of crude ester. This ester was purified through 10 g of silicic acid column. Elution with benzene-ethyl acetate (100:3, v/v) gave C₁₁-hydroxy ester. To acetone solution of this ester was added 8 N chromium trioxide-sulfuric acid solution. After stirring for 30 min. at 0°C, the reaction mixture was poured onto water-ice mixture and extracted with ether. Acidic product obtained on evaporation of the solvent was esterified with diazomethane to give 125 mg of crude C₁₁-diester. Purification through 6 g of silicic acid afforded 30 mg of pure C₁₁-diester using benzene-ethyl acetate (100:0.5, v/v) as eluent. NMR spectrum: 3 H, d, δ 0.86; 3 H, d, δ 1.09; 9 H, δ 1.1~1.8; 3 H, t, δ 2.24; 6 H, s, δ 3.36. Mass spectrum: 242 (M⁺).

f) C₁₄- α -oxo- α' -hydroxy-diester

A mixture of 1.4 g of C₁₄-dioic acid and 10 g of thionyl chloride was heated on a steam bath for 1 hr. After removal of sulfur dioxide in vacuo, 2.5 ml of bromine was added to this chloride solution in the period of 1 hr at 60°C and heating was continued for additional 2 hrs at 60~70°C. Removal of thionyl chloride in vacuo gave 1.2 g of crude bromo derivative, which was hydrolyzed with 50 ml of 4 N NaOH at 120°C for 4 hrs. The reaction mixture was acidified and extracted with ether. Evaporation of the solvent afforded 900 mg of crude dihydroxy acid, which was esterified with diazomethane. The crude ester was purified through 20 g of silicic acid column. Elution with benzene-ethyl acetate (100:1, v/v) gave 50 mg of unidentified oily byproduct containing double bond. Desired C₁₄-dihydroxy diester was obtained from eluate with benzene-ethyl acetate (100:3, v/v), yield 300 mg. A solution of 300 mg of C₁₄-dihydroxy diester in 20 ml of acetone was oxidized with 0.2 ml of 8 N chromium trioxide-sulfuric acid mixture under cooling. The reaction mixture was poured onto water-ice mixture and extracted with ether. Evapora-

tion of the ether afforded 250 mg of crude product, which was purified through 7 g of silicic acid column. Elution with benzene-ethyl-acetate (100:3, v/v) gave pure C₁₄- α -oxo- α' -hydroxy diester. NMR spectrum: 6 H, d, δ 0.90; 3 H, s, δ 1.30; 10 H, δ 1.1~1.8; 2 H; m, δ 2.2~3.0; 3 H, s, δ 3.72; 3 H, s, δ 3.80. *Anal.* Found: C, 60.98; H, 8.90. Calcd. for C₁₈H₂₈O₆: C, 60.74; H, 8.92%.

Isolation of Acid III from Ozonolysis Product of PA Diacetate

Ozonolysis of PA diacetate was conducted in the same way as that reported in the previous paper.³⁾ After oxidative decomposition of the ozonide, acidic fraction was extracted with ethyl acetate. Rapid and careful extraction did not cause spontaneous hydrolysis of the acetoxyl group in Acid I, the acetate of Acid II, giving a better yield of Acid I and, moreover, resulting in isolation of a new product, designated as Acid III, in the following way. Ozonolysis of 5.6 g of PA diacetate gave 840 mg of acidic fraction, which was dissolved in 15 ml of benzene. This solution was introduced into the column packed with 32 g of silicic acid pretreated with 22 ml of pH 5.8 1 mole phosphate buffer. At first elution was conducted with benzene, and 170 mg of unidentified syrup was obtained after evaporation of the solvent. Then, solvent composition was changed into benzene-butanol (100:1.5, v/v). On evaporating the solvents of fractions 4~10 (each fraction, 15 ml), 380 mg of Acid I was obtained as crystals. Fractions 12~45 and further elution with 300 ml of benzene-butanol (100:2, v/v) afforded 270 mg of a new acid, Acid III. In this case Acid II was not isolated. Crude Acid III was recrystallized from benzene-hexane into colorless plates, yield 200 mg. *Anal.* Found: C, 51.36; H, 6.46. Calcd. for C₈H₁₂O₅: C, 51.06; H, 6.43%. $[\alpha]_D^{MeOH} + 23^\circ$ (C=4.1).

Syntheses of three Isomers of Dimethyl Dimethyldecanedioate**a) Dimethyl 3,7-Dimethyldecanedioate**

2,6-Dimethylheptane-1,7-diol.—A solution of 10 g of dimethyl 2,6-dimethylheptanedioate¹¹⁾ in dry ether was added dropwise to 4 g of lithium aluminum hydride in the same solvent with stirring and refluxed for 8 hrs. After reaction was complete, 100 ml of saturated aqueous solution of sodium sulfate was added to the mixture. The ether layer was separated

11) P. Karrer, F. Benz, R. Morf, H. Raundnitz, M. Stoll and T. Takahashi, *Helv. Chim. Acta.*, **15**, 1399 (1932).

and dried over anhydrous sodium sulfate. The solvent was removed to give 8.5 g of 2,6-dimethylheptane-1,7-diol. This diol was acetylated in pyridine-acetic anhydride and the crude acetate thus obtained was purified through silicic acid column. Elution with benzene gave pure diol acetate as colorless oil. *Anal.* Found: C, 63.90; H, 9.87. Calcd. for $C_{13}H_{24}O_4$: C, 63.90; H, 9.90%.

1,7-Dibromo-2,6-dimethylheptane.—Dry hydrogen bromide was passed through 8 g of diol at 140°C for 1.5 hrs. Ice-cooled water was added to the reaction mixture, which was extracted with *n*-hexane. The extract was dried over anhydrous sodium sulfate and evaporated in vacuo to yield 11 g of crude bromide. Vacuum distillation of this bromide gave 9 g of pure product, b.p. 135°C/10 mm.

Dimethyl 3,7-dimethylnonanedioate.—A solution of 10 g of 1,7-dibromo-2,6-dimethylheptane in 60 ml of 95% ethanol was added dropwise to 10 g of potassium cyanide dissolved in 15 ml of 95% ethanol. The reaction mixture was heated on a steam bath for 4 hrs. Further, 5 g of potassium cyanide was added to the reaction mixture and heating was continued for additional 7 hrs. To the filtrate, concentrated to its half volume, 40 ml of water was added. Resulting aqueous solution was extracted with ethyl acetate, which was washed with water and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 6 g of 1,7-dicyano-2,6-dimethylheptane. Without further purification, this was hydrolyzed with 20 ml of conc. hydrochloric acid at 120°C for 5 hrs. After cooling, reaction mixture was extracted with ether, which was dried over anhydrous sodium sulfate. Residual syrup obtained on evaporation of the ether in vacuo was dissolved again in ether and treated with ethereal diazomethane. Crude dimethyl ester thus obtained was dissolved in a mixture of methanol-water (1:1) and extracted with *n*-hexane. After evaporation of the solvent, residue was subjected to vacuum distillation to give 3 g of dimethyl 3,7-dimethylnonanedioate, b.p. 131~133°C/3 mm. *Anal.* Found: C, 63.92; H, 9.79. Calcd. for $C_{13}H_{24}O_4$: C, 63.90; H, 9.90%.

3,7-Dimethylnonanedioic acid monomethyl ester.—To a mixture of 2.5 g each of 3,7-dimethylnonanedioic acid and its dimethyl ester in 2 ml of *n*-butyl ether, 0.2 ml of methanol and 0.5 ml of conc. sulfuric acid was added. This mixture was refluxed for 4 hrs in an oil bath and then distilled in vacuo. The distillate boiling between 100~155°C/1 mm was collected, and

dissolved in ether. The ether solution was extracted with aqueous sodium carbonate solution. After acidifying the alkaline solution, acidic fraction was extracted with ether. The solvent was evaporated and the residue was subjected to vacuum distillation to give 1 g of monomethyl ester.

Dimethyl 3,7-dimethyldecanedioate.—A mixture of 1 g of 3,7-dimethylnonanedioic acid monomethyl ester and 30 ml of thionyl chloride was refluxed on a steam bath for 2 hrs. After removal of thionyl chloride in vacuo, resulting crude acid chloride was dissolved in dry ether. This ether solution was added dropwise to cooled ethereal diazomethane at a rate that maintains internal temperature below 0°C. After standing at 5°C for 18 hrs, ether was removed by distillation to give diazoketone. Methanol solution of this diazoketone was added dropwise to a mixture of 2 g of silver oxide in 100 ml of methanol at 60°C over a period of 1 hr. The reaction mixture was refluxed with stirring for additional 4 hrs, during which 0.5 g of silver oxide was added portionwise. After the reaction was complete, precipitate was filtered off and filtrate was concentrated to its half volume. To this residual solution was added the same volume of water, and extracted with *n*-hexane. Evaporation of the solvent yielded 400 mg of oily product, which was purified through 10 g of silicic acid column. Elution with benzene gave pure dimethyl 3,7-dimethyldecanedioate. *Anal.* Found: C, 64.84; H, 10.00. Calcd. for $C_{14}H_{26}O_4$: C, 65.08; H, 10.14%.

b) Dimethyl 3,6-dimethyldecanedioate

1,4-Dimethylcyclohexanol.—To 3.4 g of magnesium in dry ether, a solution of 17 g of methyl iodide in 60 ml of ether was added with vigorous stirring. After magnesium was completely dissolved, 18.5 g of 4-methylcyclohexanone in 50 ml of ether was added dropwise to this Grignard reagent over a period of 2 hrs. at a rate that maintains internal temperature below 5°C. After reaction was complete, 100 ml of dil. hydrochloric acid (1:1) was added to the mixture, which was saturated with sodium chloride. Ether layer was separated and remaining aqueous solution was extracted with ether several times. The combined ether solutions were dried over anhydrous sodium sulfate. Evaporation of the solvent gave 15 g of sublimable crystals melting at 49°C. *Anal.* Found: C, 74.77; H, 12.41. Calcd. for $C_8H_{16}O$: C, 74.94; H, 12.58%.

Methyl 6-oxo-3-methylheptanoate.—A solution of 10 g of 1,4-dimethylcyclohexanol in dry benzene was

added dropwise to a viscous solution formed by mixing 8 ml of ethanol with 10 g of phosphorous pentoxide with vigorous stirring, and refluxed for 2 hrs. with successive stirring. Then, 100 ml of water was added to the mixture, which was extracted with ether. After evaporation of the solvent, the residue was distilled at ordinary pressure. The fraction boiling between 100~120°C was collected and redistilled in vacuo to give 6 g of olefin, b.p. 32~35°C/5 mm. This olefin was dissolved in chloroform and ozonolyzed under cooling in an ice bath. End point of the reaction was detected with potassium iodide solution. After reaction was complete, the chloroform was evaporated in vacuo at room temperature. To a residual syrup was added 100 ml of 3% hydrogen peroxide solution and refluxed for 1 hr. Manganese dioxide was added to the reaction mixture to decompose excess hydrogen peroxide, followed by extraction with ether. Evaporation of the solvent gave 4 g of crude 6-oxo-3-methylheptanoic acid, which was esterified with diazomethane. After two run experiments, 9 g of combined crude esters was subjected to vacuum distillation to give 5.5 g of pure methyl 6-oxo-3-methylheptanoate, b.p. 88°C/1 mm. *Anal.* Found: C, 62.42; H, 9.30. Calcd. for $C_9H_{16}O_3$: C, 62.76; H, 9.34%.

Dimethyl 3,6-dimethyldecanedioate.—A solution of 5.6 g of ethyl bromocrotonate in 20 ml of benzene was added dropwise to a mixture of 5.6 g of methyl 6-oxo-3-methylheptanoate and 1.5 g of activated zinc in 60 ml of dry benzene with vigorous stirring over a period of 1 hr. Refluxing and stirring were continued for additional 1.5 hrs. To the reaction mixture, 100 ml of water was added and extracted with ether. Evaporation of the solvent yielded 4 g of oily product, which was distilled under reduced pressure. The fraction boiling between 130~160°C/1 mm amounted to 2 g. Benzene solution of this oil was added to a viscous mixture of phosphorous pentoxide and ethanol with stirring and refluxed for 1 hr. Then, 100 ml of water was added to the reaction mixture, which was extracted with *n*-hexane. Evaporation of the solvent afforded 1.7 g of oily product. Methanol solution of this oil was hydrogenated over Adams' catalyst under hydrogen pressure of 30 kg/cm². After the catalyst was removed by filtration, the methanol solution was evaporated to leave 1.7 g of crude ester. This was hydrolyzed with 40 ml of 2 N NaOH under reflux. The reaction mixture was acidified and extracted with ether. After evaporation of the solvent, the

acid was esterified with diazomethane to yield 600 mg of crude ester. The ester was purified through 15 g of silicic acid column. Elution with benzene-ethyl acetate (100:0.5, v/v) gave 140 mg of pure dimethyl 3,6-dimethyldecanedioate. *Anal.* Found: C, 65.39; H, 10.09. Calcd. for $C_{14}H_{26}O_4$: C, 65.08; H, 10.14%.

c) Dimethyl 4,7-dimethyldecanedioate

3,6-Dimethyloctanedioic acid.—To a mixture of dry benzene-toluene (1:1) 32.2 g of acetonyl acetone and activated zinc were added successively. Then 91 g of ethyl bromoacetate was added dropwise under reflux with vigorous stirring. The reaction mixture was refluxed for additional 2 hrs. with vigorous stirring. After cooling, 200 ml of water was added and organic layer was separated. Evaporation of the solvent yielded 51.8 g of oily product, which was distilled under reduced pressure. The fraction boiling between 110~130°C/0.8 mm amounted to 27.7 g. This oil was dissolved in dry benzene to which 20 g of phosphorous oxychloride was added and refluxed for 1 hr. The residue obtained after evaporation of the solvent was dissolved in a mixture of methanol-water (1:1), and extracted with *n*-hexane. The extract was decolorized with activated carbon and evaporated to give 13 g of oil. This oil was dissolved in methanol and hydrogenated over 1.3 g of Adams' catalyst under hydrogen pressure of 80 kg/cm². The catalyst was removed by filtration and the filtrate was evaporated. Vacuum distillation of the residue gave 7 g of fraction boiling between 125~130°C/0.8 mm. This was purified through 60 g of silicic acid column. Elution with benzene-ethyl acetate (100:0.5, v/v) afforded 2 g of diethyl 3,6-dimethyloctanedioate. *Anal.* Found: C, 64.81; H, 9.87. Calcd. for $C_{14}H_{26}O_4$: C, 65.08; H, 10.14%.

Further elution with benzene-ethyl acetate (100:1, v/v) gave 4.5 g of unidentified oil.

Two g of dimethyl 3,6-dimethyloctanedioate was hydrolyzed with 50 ml of 3 N NaOH for 2 hrs. under reflux. The solution was acidified and extracted with ethyl acetate. On evaporation of the solvent, 1.2 g of 3,6-dimethyloctanedioic acid was obtained. Recrystallization from ethyl acetate-benzene gave rods melting at 86.5°C. *Anal.* Found: C, 59.00; H, 8.90. Calcd. for $C_{10}H_{18}O_4$: C, 59.38; H, 8.97%.

Dimethyl 4,7-dimethyldecanedioate.—Into 10 g of thionyl chloride 3.6 g of 3,6-dimethyloctanedioic acid was dissolved on heating and refluxed for 1 hr. Excess thionyl chloride was removed in vacuo to give 4.5 g of chloride. This chloride was dissolved in ether.

and the cooled solution was added dropwise to ethereal diazomethane below 0°C. After keeping at 5°C for 24 hrs., the solvent was evaporated to give 5.2 g of diazoketone. Methanol solution of this diazoketone was added dropwise to a mixture of 2 g of silver oxide and 100 ml of methanol at 60°C for 1 hr., and refluxed for additional 1 hr. with stirring. After cooling, resulting precipitate was removed by filtration. To the filtrate, concentrated to 50 ml in vacuo, 50 ml of water was added. This aqueous solution was extracted with *n*-hexane. Evaporation of the solvent gave 2.5 g of crude ester, which was purified through 20 g of silicic acid column. Elution with benzene-ethyl acetate (100:0.5, v/v) gave pure dimethyl 4,7-dimethyldecanedioate. *Anal.* Found: C, 64.59; H, 10.00. Calcd. for $C_{14}H_{26}O_4$: C, 65.08; H, 10.14%.

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