Chemical Structure of Piericidin A

Part VI. Stereochemistry

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Absolute configurations of C-2 and C-3 in "Acid III" (IIa), a degradation product of piericidin A diacetate, was determined as *R* and *S*, respectively. Consequently both C-9 and C-10 in piericidin A were confirmed to have *S* configurations. On the basis of UV and NMR spectra, configurations of the double bonds in piericidin A were assigned as shown in Ia.

Piericidin A (PA)^{1,2)} is a natural insecticide as well as a potent inhibitor³⁾ of mitochondrial electron transport. In our preliminary report,⁴⁾ *S* configurations have been proposed to the assymmetric centers, C-9 and C-10, contained in the compound. We wish to present the evidences in details which support this assignment and to discuss the configurations of the double bonds in PA.

Ozonolysis of PA diacetate afforded "Acid

III" (IIa) by fissions of the double bonds at C-7~C-8 and C-11~C-12. Since IIa possesses all assymmetric centers originally contained in PA, elucidation of the stereochemistry of IIa was conducted.

The structure IIa previously assigned for "Acid III" was supported by the double resonance experiment as illustrated in Fig. 1. Furthermore, this formula was verified by the following synthesis shown in Scheme I. The

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FIG. 1. NMR Spectra of the Natural and Synthetic I Ib.

(a) The natural I Ib

(b) The synthetic I Ib

○ Signals due to the natural I Ib

* Signals due to diastereomer of the natural I Ib

SCHEME I

Reformatsky reaction between methacrolein and methyl α -bromopropionate and successive acetylation gave methyl 3-acetoxy-2,4-dimethyl-4-pentenoate (IIIb), which was ozono-

lysed to give I Ib. Although I Ib thus synthesized is a racemic mixture of diastereomers at C-2 and C-3, its IR spectrum was essentially identical with that of I Ib prepared from

the natural IIa. However, the NMR spectrum of the synthetic IIb revealed additional signals besides those of the natural one as shown in Fig. 1. Gas-liquid chromatography of the synthetic IIb on a succinate polyester column gave two peaks, while the natural IIb gave only one showing an identical retention time with that of the second peak of the former. This experiment indicates that racemization did not occur in the process of the preparation of IIa from PA. Hydrogenation of the natural IIa in acetic acid over Adams' catalyst gave a 1:1 mixture of a lactone (IV) and a hydroxy acid (V), while hydrogenation in methanol afforded a mixture of hydroxy acids containing only a trace amount of IV. The NMR spectrum of IV and its double resonance experiment (Fig. II) supported the proposed structure. The 50% yield of V on hydrogenation of the natural IIa in acidic medium indicates that V would possess the

configuration opposite to IV at C-4 and could not form a γ -lactone ring due to large steric hindrance by all *cis* substituents. Now three stereochemical expressions for IV, namely, VI, VII and VIII or their enantiomeric structures, are possible. The difficulty of lactonization of V excludes the formula VIII, because the hydroxy acid epimeric to VIII at C-4 is expected to form readily a γ -lactone having all *trans* substituents.

Catalytic hydrogenation of the synthetic IIb in methanol gave a complex mixture, from which another γ -lactone (VII) with a different NMR spectrum from that of IV was isolated by silicic acid chromatography. The remarkable easiness of lactonization of hydroxy ester derived from the synthetic IIb leads to assign the structure VII with all *trans* substituents to the new lactone. The absence of VII in the hydrogenation products from the natural IIa indicates that VII must have been derived

FIG. 2. NMR Spectrum of the Lactone (IV) and its Decoupling Study.

from the diastereomer of the natural IIa. Thus the structure VI was assigned to the lactone from the natural IIa. In the preliminary paper,⁴⁾ the coupling constants, $J_{H-2-H-3}=6$ cps (*cis*) and $J_{H-3-H-4}=3$ cps (*trans*), have been presented to support this stereochemical assignment. However, the NMR spectrum of VII with all *trans* substituents showed $J_{H-2-H-3}=5.5$ cps and $J_{H-3-H-4}=6.5$ cps, suggesting the difficulty in applying the Karplus equation to the five membered ring systems. The evidence above cited allow to assign the relative configuration of C-2 and C-3 in the natural IIa as *S-R* or *R-S*, and consequently C-9 and C-10 in PA as *R-R* or *S-S*.

The remaining problem is to decide the absolute configuration for one of the asymmetric centers by correlating it to the compound with any known absolute configuration. Lithium aluminum hydride reduction in boiling tetrahydrofuran of the natural IIb afforded a triol (IX), which was treated with two moles of methanesulfonyl chloride at 0°C. From the reaction product dimethanesulfonate (X) was isolated in a fairly good yield by silicic acid chromatography. X was reduced with lithium aluminum hydride in boiling ether to give an alcoholic product, which was characterized as a 3,5-dinitrobenzoate, mp 98~99°C, $[\alpha]_D^{16} = +7.67^\circ$. The IR (in solution) and the NMR spectra of the benzoate were identical with those of the authentic 3,5-dinitrobenzoate of ($\pm$)-2-methyl-3-pentanol (XI). This indicates that two methanesulfonyl groups were introduced into C-2 and C-4 hydroxyl groups of IX, and the structure of the dimethanesulfonate was established as X. Since the

(-)-ester or (-)-alcohol have been correlated with L-glyceraldehyde by Büchi,⁵¹ the absolute configuration of C-3 in the (+)-ester from the natural IIa must be *R*. Thus *S*-configurations were assigned to both C-9 and C-10 in PA from the stereochemical correlation of PA to (+)-2-methyl-3-pentanol (XI).

The very strong UV absorption of PA at $239\text{ m}\mu$ (ϵ 40000) gave a useful information for the configuration of the tetrasubstituted diene system. Even if the effect of the end absorptions due to the pyridine ring and two trisubstituted ethylenic double bonds is subtracted, the intensity of this band would be about ϵ 30000~35000, suggesting the presence of *trans-trans* conjugated system. This was further confirmed by the large coupling constant between H-4 and H-5 (16 cps) in its NMR spectrum.

The signal due to four allyl methyl groups of PA are observed in the range δ 1.5~1.8 as illustrated in Fig. 3. Among them, the C-3 methyl attached to the conjugated diene system

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* The optical rotation datum reported previously for "Acid III" is revised.

Synthesis of methyl 3-acetoxy-2-methyl-4-oxopentanoate (IIb)

a) **Methyl 3-acetoxy-2,4-dimethyl-4-pentenoate (IIIb).** In a 200 ml three necked flask, equipped with a mechanical stirrer, a condenser and a dropping funnel were placed 30 ml of dry benzene and 11 g of activated zinc. In the dropping funnel was placed a solution of 30 g of methyl α -bromopropionate and 13 g of methacrolein in 50 ml of dry benzene. Initially a few ml of the above solution and one piece of iodine were added to the flask with stirring. When exothermic reaction set in, the remainder of the solution was gradually added for 30 min, and then flask was heated to maintain gentle boiling for additional 1 hr. The reaction mixture was cooled and treated with 50 ml of 10% aqueous sulfuric acid. The aqueous layer was extracted with three 100 ml portions of benzene. Combined extracts were evaporated under reduced pressure and the residue distilled. The yield of methyl 3-hydroxy-2,4-dimethyl-4-pentenoate (IIIa), bp 71~73°C/1.2~1.5 mm, was 12.5 g.

To a solution of 12 g of IIIa in 20 ml of dry pyridine was added 15 ml of acetic anhydride. On standing at room temperature for 15 hr, the reaction mixture was poured into 100 ml of 10% aqueous sulfuric acid. The aqueous layer was extracted with three 70 ml portions of benzene and combined extracts were washed with 3% aqueous sodium bicarbonate solution. The benzene solution was evaporated under reduced pressure, and the residue distilled. Yield of pure methyl 3-acetoxy-2,4-dimethyl-4-pentenoate (IIIb), bp 87~89°C/1.5 mm, was 11 g. *Anal.* Found: C, 60.20; H, 8.13. *Calcd.* for $C_{10}H_{16}O_5$: C, 59.98; H, 8.05%. $\nu_{\max}^{\text{Film}}$: 3480, 1740, 1725, 1260, 1195, 1170, 1032, 905 cm^{-1} .

b) **Methyl 3-acetoxy-2-methyl-4-oxopentanoate (IIb).** Ozone enriched oxygen stream was passed through a solution of 11 g of IIIb in 70 ml of dry chloroform at -15~-20°C for 2 hr. At the end of the reaction, the solution turned pale blue. Chloroform was distilled under reduced pressure and the residual oil was boiled with 50 ml of water for 30 min. After cooling, the aqueous solution was extracted with three 60 ml portions of benzene, and the combined extracts were subjected to vacuum distillation. Methyl 3-acetoxy-2-methyl-4-oxopentanoate (IIb) was collected at 87~90°C/2 mm in an yield of 9.8 g. *Anal.* Found: C, 54.07; H, 7.27. *Calcd.* for $C_9H_{14}O_5$: C, 53.46; H, 6.98%. $\nu_{\max}^{\text{Film}}$: 1750 (broad), 1250 (broad), 1063, 1050, 1026 cm^{-1} . The NMR spectrum is shown

in Fig. 1. It shows two peaks at retention times 10 and 11.2 min on gas-liquid chromatography.

Catalytic hydrogenation of the natural IIa

a) **In acidic medium.** Three hundred mg of the natural IIa was hydrogenated over 300 mg of Adams' catalyst in acetic acid for 3 hr until 42 ml (1 molar equivalent) of hydrogen was absorbed. After removal of the catalyst, acetic acid was distilled off under reduced pressure. The residual syrup was dissolved in ethyl acetate, which was extracted with 3% aqueous bicarbonate solution. Evaporation of the ethyl acetate gave 110 mg of crude lactone. The product was chromatographed on a silicic acid (10 g) column by the use of benzene-ethyl acetate (100:4) to give pure lactone (VI). $\nu_{\max}^{\text{Film}}$: 1780, 1750, 1230, 1180 cm^{-1} . $[\alpha]_D^{18} = +57^\circ$ ($c=2.1$, methanol). The NMR spectrum is shown in Fig. 2. The sodium bicarbonate layer separated as mentioned above was acidified and the acidic solution was extracted with ethyl acetate. Evaporation of the solvent afforded 100 mg of hydroxy acid (V). $\nu_{\max}^{\text{Film}}$: 3500~2800 (broad), 1750~1720 (broad), 1245, 1050 m^{-1} .

b) **In the neutral medium.** Three hundred mg of the natural IIa was hydrogenated over 300 mg of Adams' catalyst in methanol. It uptook 1 molar equivalent of hydrogen for 5 hr. The product was treated in the usual way to give 10 mg of a neutral fraction together with 210 mg of a mixture of hydroxy acids.

Catalytic hydrogenation of the synthetic IIb

Three hundred and forty mg of the synthetic IIb was subjected to catalytic hydrogenation over 200 mg of Adams' catalyst in methanol. In the usual way, 310 mg of neutral fraction was obtained, which showed absorption bands at 3440, 1776, 1745 (broad), 1230, 1045 cm^{-1} , suggesting the presence of a lactone and hydroxy esters. The product was purified through a silicic acid (20 g) column. Elution with benzene-ethyl acetate (100:4) afforded 35 mg of oily lactone (VII). $\nu_{\max}^{\text{Film}}$: 1782, 1745, 1235, 1180 cm^{-1} . NMR spectrum: 3H, d, δ 1.33, 3H, d, δ 1.47; of 3H, s, δ 2.10; 1H, m, δ 2.72; 1H, m, δ 4.38; 1H, t(d-d), δ 4.82. Further elution with benzene-ethyl acetate (100:10 and 100:20) gave a mixture of hydroxy esters but recovery from the column was low (60 mg).

Preparation of 3,5-dinitrobenzoate of (+)-3-methyl-2-pentanol from the natural IIb

a) **2-Methyl-1,3,4-pentanetriol (IX).** To a solution of 2 g of lithium aluminum hydride in 30 ml of tetrahydrofuran was added a solution of 3 g of the

natural IIB in 10 ml of tetrahydrofuran at 70~80°C and the reaction mixture was heated at 80°C for 7 hr. After cooling, small amounts of ethyl acetate and water were added. The precipitate were removed by filtration and washed successively with tetrahydrofuran and ethyl acetate several times. Combined solutions were evaporated under reduced pressure to give 2.2 g of oily crude triol (IX), which was purified through a silicic acid (40 g) column. Elution with benzene-ethyl acetate (70:25:5) gave 300 mg of 3-acetoxy-2-methyl-1,4-pentanediol. Further elution with benzene-ethyl acetate-acetone-methanol (70:20:5:5) afforded 1.7 g of pure triol (IX). *Anal.* Found: C, 53.39; H, 10.53. Calcd. for $C_6H_{14}O_3$: C, 53.71; H, 10.52%. $\nu_{\text{max}}^{\text{Film}}$: 3400 (broad) 1040, 990 cm^{-1} .

b) Dimethanesulfonate of IX (X). To a solution of 600 mg of IX in 10 ml of pyridine was added 1.24 g of methanesulfonyl chloride at -5°C. After standing at 5°C overnight, the reaction mixture was poured into 30 ml of 10% aqueous sulfuric acid with stirring and aqueous layer was extracted with two 50 ml portions of benzene and successively with two 50 ml portions of ethyl acetate. Evaporation of the benzene solution gave 520 ml of syrup, which was a mixture of di- and trimethanesulfonates. On evaporation of the ethyl acetate fraction, 360 mg of dimethanesulfonate (X) was obtained. The mixture of di- and trimethanesulfonates was chromatographed on a silicic acid (22 g) column. Elution with benzene-ethyl acetate (100:15) gave 130 mg of crystalline trimethanesulfonate melting at 114.5°C. *Anal.* Found: C, 29.72; H, 5.73; S, 2603. Calcd. for $C_6H_{20}O_9S_3$: C, 29.33; H, 5.47; S, 26.11%. $\nu_{\text{max}}^{\text{Nujol}}$: 1340 (broa), 1172, 1032, 980, 930, 860, 770, 721 cm^{-1} . Elution with benzene-ethyl acetate (70:30) afforded 136 mg of X. The total yield of X was 500 mg. *Anal.* Found: C, 33.23; 6.23; S, 22.29. Calcd. for $C_6H_{16}O_7S_2$: C, 33.09; H, 6.24; S, 22.09%. $\nu_{\text{max}}^{\text{Film}}$: 3550, 1350, 1176, 970, 915 cm^{-1} .

c) 3,5-Dinitrobenzoate of (+)-2-methyl-3-pentanol (XI). Five hundred and sixty mg of X was reduced with 700 mg of lithium aluminum hydride in boiling ether for 5 hr. The reaction mixture was treated with a small amount of water and the ether layer separated by centrifuge from the precipitate, which was washed with ether several times. Combined ether solutions were evaporated under atmospheric pressure to give a liquid alcoholic residue. The residue was dissolved in 10 ml of pyridine and 250 mg of 3,5-dinitrobenzoyl chloride was added to the solution at 0°C. On standing at 5°C for 24 hr, the reaction mixture was poured into cold 10% aqueous sulfuric acid. The aqueous layer was extracted with a benzene-ether mixture several times. Evaporation of the solvent gave 400 mg of crude 3,5-dinitrobenzoate, which was purified through a silicic acid (22 g) column. Elution with benzene-hexane (1:1) gave 150 mg of pure ester of XI melting at 98~99°C. $[\alpha]_D^{15} = +7.7^\circ$ (C=2.9, chloroform). *Anal.* Found: C, 53.09; H, 5.52; N, 9.52. Calcd. for $C_{13}H_{16}N_2O_6$: C, 52.70; H, 5.44; N, 9.46%. $\nu_{\text{max}}^{\text{Nujol}}$: 3090, 1730, 1630, 1548, 1360, 1290, 1175, 735, 725 cm^{-1} . NMR spectrum: 3H, t, δ 0.91; 6H, d, δ 1.00; 2H, m, δ 8.1~8.5; 1H, m, δ 7.8~8.1; 1H, m, δ 5.0.

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