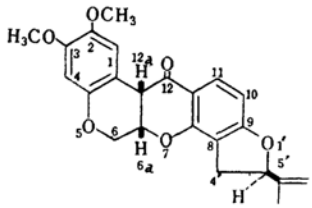
The Absolute Configuration of Rotenone[1]

By Masao NAKAZAKI and Hisao ARAKAWA

(Received February 9, 1961)

Rotenone, one of the most powerful vegetable insecticides, was first isolated by Nagai[2] from *Milletia taiwaniana* Nagai, a native of Formosa, and was found to be identical with tubotoxin[4] obtained from *Derris elliptica* which is now widely cultivated.



I

The accepted formula I[5] was advanced independently by Takei[6], Butenandt[7] and LaForge[8] at about the same time (1932).

To study the interesting relationship between the insecticidal action of rotenone and its stereochemistry, the establishment of the absolute configuration is essential. This contribution is concerned with the determination of the absolute configuration at C-5′ carbon atom of rotenone (I).

Tubaic acid (IIa) obtained by the alkaline degradation of rotenone was catalytically hydrogenated to give (−)-dihydrotubaic acid (IIb)[10], which was dissolved in acetic acid and exhaustively ozonolyzed[11] by passing ozone through it for 25 hr. After further oxidation with hydrogen peroxide, oxalic acid was removed as the calcium salt and the solution was passed through the column of Amberite IR-120 to be freed of the excess of calcium ions. The solvent was removed in vacuo and crude 3-hydroxy-4-methyl-pentanoic acid (III) (or IV in Fischer projection) was esterified with diazomethane to give the methyl ester IVb, b. p. 77~86°C/18 mmHg, $[\alpha]_D$ +19.0°.

To correlate IVb with D-valine, the methyl ester IVb was converted into the hydrazide IVc, m. p. 152~153°C, $[\alpha]_D$ +43°, which was treated with nitrous acid[12] to give (−)-5-isopropyloxazolidone (V), m. p. 113~114°C, $[\alpha]_D$ −44.5°, the structure of which was confirmed by the comparison of the infrared spectrum with the racemic V, m. p. 87~88°C, prepared from the racemic hydroxylic acid IVa[13,14].

(−) Methyl 2-hydroxy-3-methylbutanoate (VIIb), b. p. 62~63°C/15 mmHg, $[\alpha]_D$ −1.4°, which was prepared from D-valine (VI)[15] with retention of configuration[16], was treated with liquid ammonia in a steel bomb to give the (+)-amide VIIc, m. p. 99~101°C, $[\alpha]_D$ +65.10. Lithium aluminumhydride redcution of the amide VIIc afforded the aminoalcohol VIIIa

1) A preliminary note appeared in This Bulletin, **34**, 453 (1961).

2) K. Nagai, *J. Chem. Soc. Japan, Pure. Chem. Sec.* (*Nippon Kagaku Zasshi*), **23**, 744 (1902).

3) T. Kariyone, K. Atsumi and M. Shimada, *J. Pharm. Soc. Japan* (*Yakugaku Zasshi*), **43**, 736 (1923).

4) T. Ishikawa, *Tokyo Igaku Zasshi*, **31**, 187 (1917).

5) In the structure formula I is shown the numbering of carbon atoms employed by Büchi and Crombie (vide infra). The asymmetric carbon atom C-5′, about which this paper is concerned is C-2 according to the numbering used by Miyano and Matsui (vide infra) in their recent synthetic works.

6) S. Takei, S. Miyajima and M. Ono, *Ber.*, **61**, 1041 (1932).

7) A. Butenandt and W. McCartney, *Ann.*, **494**, 17 (1932).

8) F. B. LaForge and H. L. Haller, *J. Am. Chem. Soc.*, **54**, 810 (1932).

9) The configurations of carbon atoms at 6a and 12a seem not to be crucial to the insecticidal action; e. g. (−)-deguelin shows almost the same physiological action as that of (±)-deguelin (S. Takei and S. Miyajima, *Scientific Papers Inst. Phys. Chem. Research* (*Tokyo*), **12**, 946 (1933)). Cahn and coworkers (R. S. Cahn, R. F. Phillipers and J. J. Boam, *J. Chem. Soc.*, **1938**, 513). obtained (+)-isorotenone, the enantiomer of (−)-isorotenone which keeps the original configuration of rotenone at 6a and 12a carbon atoms, from "mutarotenone"; but there seems to have been no study on the comparison of the physiological properties between them. Although Miyano amd Matsui (M. Miyano, A. Kobayashi and M. Matsui, *Chem. Ber.* in press) reported the total synthesis of natural rotenone, the effect of the configuration C-5′ carbon atom has remained unknown, since the enantiomer of natural rotenone could not be prepared.

10) S. Takei and M. Koide, *Ber.*, **62**, 3030 (1929).

11) H. Arakawa and M. Nakazaki, *Chem. & Ind.*, **1959**, 671; **1960**, 173; *Ann.*, **636**, 111 (1960); H. Arakawa, This Bulletin, **33**, 200 (1960).

12) C. Schöpf, G. Dummer and W. Wüst, *Ann.*, **676**, 134 (1959); C. Shcöpf and W. Wüst, ibid., **676**, 150 (1959) employed 5-phenyloxazolidone as a relay intermediate when they ralated the absolute configuration of (−)-sedamine and (−)-allosedamine with (+)-mandelic acid.

13) Methyl ester of racemic IVa, b. p. 92~93°C/20mmHg, was prepared by sodium borohydride reduction of ethyl isobutyroylacetate (X). Racemic hydrazide IVc melted at 148~149°C.

14) G. Büchi, J. S. Kaltenbron, L. Crombie, P. J. Godin and D. A. Whiting (*Proc. Chem. Soc.*, **1960**, 274) obtained the racemic IVa by the Reformatsky reaction of isobutyraldehyde and benzyl bromoacetate followed by hydrogenolysis.

15) We are grateful to Dr. Setsuji Sakurai, Ajinomoto Co. for his generous gift of D-valine.

16) W. Klyne, "Pprogress in Stereochemistry", Vol. I, Butterworth Pub., London (1950), p. 195; E. Fisher and H. Scheibler, *Ber.*, **41**, 2891 (1908); P. D. Bartlett, M. Kuna and P. A. Levene, *J. Biol. Chem.*, **118**, 303 (1937).

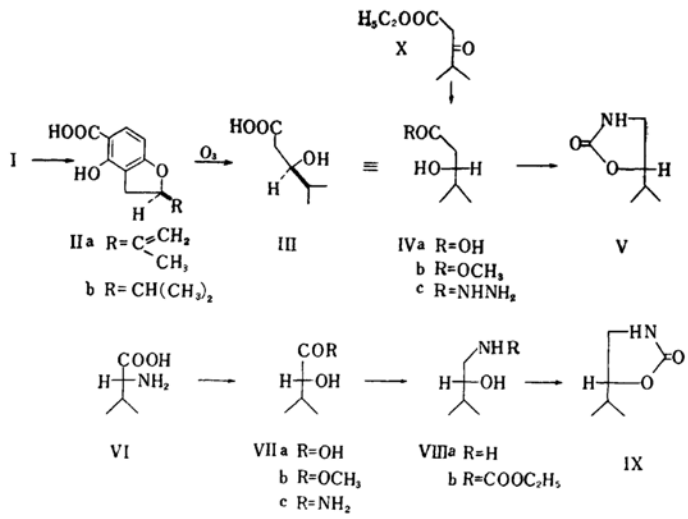


Fig. 1.

which was transformed, via the carbamate VIIIb, into (+)-5-isopropyloxazolidone (IX), m. p. 113~114°C, $[\alpha]_D$ +44.2°.

The fact that 5-isopropyloxazolidone (V and IX) were enatiomorphous was established by comparison of their intrared spectra, which can be superimposed in every detail, moreover, a mixed melting point determination of the 5-isopropyloxazolidone synthesized from the racemic hydroxylic acid IVa with the racemic compound prepared by mixing equal parts of (−)-5-isopropyloxazolidone from rotenone and (+)-5-isopropyloxazolidone from D-valine and recrystallization from benzene showed no depression. These facts clearly show, as can be seen from Fig. 1, that rotenone has (R)-configuration at the carbon atom C-5′ as illustrated in I.

During the course of this investigation, Büchi and Crombie[14] published a short communication in which they reported the same conclusion. They ozonolyzed (−)-dihydrotubaic acid (IIb) to obtain (+)-3-hydroxy-4-methylpentanoic acid (IVa). In order to secure enough starting material, they synthesized (±)-IVa and resolved via the quinine salt obtaining (−)-acid (enantiomer of III from rotenone) which they converted via three steps into (−)-2-methylpenta-3-ol with known configuration.

Thus we could confirm Büchi and Crombie's result[17], although our approach appears more direct and simple, giving well defined crystalline compounds in each step, which will be proved to be useful as key compounds in establishing absolute configuration of other compounds.

17) They also succeeded in establishing (S)-configuration at 6a carbon atom by the exhaustive ozonolysis of acetyldihydrorotenone to obtain D-glyceric acid in the form of its *p*-bromophenacyl ester. See Ref. 14.

Experimental

Dihydrotubaic Acid (IIb).—IIb was prepared according to Takei and Koide's procedure, m. p. 166°C, $[\alpha]_D^{25}$ −103.7° (*c* 1.08, methanol).

Found: C, 64.88; H, 6.25. Calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35%. (literature[10]), m. p. 168°C, $[\alpha]_D$ −82.67°, chloroform).

Exhaustive Ozonolysis of Dihydrotubaic Acid (IIb).—To a solution of 4.4 g. of dihydrotubaic acid (IIb) in 70 cc. of 95% acetic acid, was passed ozone at room temperature for 25 hr. The color of the solution changed from colorless to brown after 5 min., and pale yellow after 30 min. and then colorless again. After 8 cc. of 30% hydrogen peroxide was added, the solution was kept at room temperature for 17 hr, then 50 cc. of water was added with a small amount of palladium-carbon to decompose the excess of hydrogen peroxide. In order to remove oxalic acid, 2 g. of calcium acetate was added and the resulting precipitate was filtered. After the solution was freed from excess calcium ion by passing through a column of Amberite IR-120, the solution was concentrated in vacuo to give a syrup which was dried in a vacuum desiccator overnight. The viscous hydroxylic acid III was dissolved in a small amount of methanol and esterified with excess diazomethane solution in ether. After being allowed to stand overnight and being dried over anhydrous sodium sulfate, the solution was concentrated, giving a pale yellow residue which was distilled in vacuo, b. p. 77~86°C/18 mmHg, 0.95 g. $[\alpha]_D^{30}$ +19.0° (*C* 5.77, ethanol).

(+)-3-Hydroxy-4-methylpentanhydrazide (IVc).—To a solution of 0.95 g. of the methyl ester IVb in 20 cc. of ethanol, 2 g. of 80% hydrazine hydrate solution was added and the mixture was heated on a water bath for 1 hr. After standing in a refrigerator overnight, the solid mass obtained was

recrystallized from ethanol giving crystals, m. p. 151~153°C, $[\alpha]_D^{19}$ +38~−40° (ethanol), 0.32 g. To prepare an analytical sample the product was recrystallized from ethanol, m. p. 152~153°C, $[\alpha]_D^{19}$ +43°) (*c* 1.01, ethanol).

Found: N, 19.14. Calcd. for $C_6H_{14}O_2N_2$: N, 19.17%.

(−)-5-Isopropyloxazolidone (V).—After 10 cc. of benzene was added to a solution of 0.3 g. of the hydrazide IVc in 10 cc. of 1N hydrochloric acid, a solution of 1.5 g. of sodium nitrite in 2 cc. of water was added during 10 min. at 5~6°C with stirring. After being kept at room temperature for 1 hr., the benzene layer was separated and washed with saturated aqueous sodium hydrogen carbonate and dried over calcium chloride for 30 min. When the benzene solution, being freed from the drying reagent, was heated on a water bath at 75°C, generation of nitrogen was observed, and the solution was refluxed for 30 min. Removal of the solvent gave crystals (0.22 g.) which were recrystallized from benzene to afford an analytical sample, m. p. 113~114°C, $[\alpha]_D^{18}$ −44.5° (*c* 1.01, ethanol), 70 mg. infrared spectrum (solvent: chloroform) was found completly superimposable on the one of (−)-isomer IX prepared from D-valine.

Found: C, 55.93; H, 8.55; N, 10.98. Calcd. for $C_6H_{11}O_2N$: C, 55.79; H, 8.58: N, 10.84%.

(−)-Methyl 2-Hydroxy-3-methylbutanoate (VIIb).—According to Fischer's procedure[16], 4.9 g. of D-valine ($[\alpha]_D^{16}$ −27.6° *c* 2.68, 6N hydrochloric acid) was converted into 2-hydroxy-3-methylbutanoic acid (VIIa). The hydroxylic acid was esterified with diazomethane in ether and the methyl ester VIIb was distilled to give an oil, boiling at 62~63°C/15 mmHg, $[\alpha]_D^{17}$ −1.4° (*c* 9.18, ethanol), 2.4 g.

(+)-2-Hydroxy-3-methylbutanamide (VIIc).—A mixture of 2.4 g. of methyl ester VIIb and 8 cc. of liquid ammonia was placed in a steel bomb and kept at 30°C overnight. After heating at 40~45°C for 2 hr., the excess of ammonia was evaporated, giving a residue, which was dissolved in ethanol. Removal of the solvent gave a viscous oil which was caused to solidify by trituration with benzene, m. p. 98~100°C, $[\alpha]_D^{15}$ +62° (*c* 1.0, methanol), 620 mg. The mother liquor was concentrated and the residue was again ammonolyzed with 80 cc. of liquid ammonia by heating at 140°C for 1 hr., and at 100°C for 15 hr. to give 600 mg. of the amide. The crude amides were recrystallized from ethanol-benzene to give crystals, m. p. 95~100°C, $[\alpha]_D^{15}$ +50° (*c* 1.02, methanol), 370 mg. For an analytical sample, the product was recrystallized from benzene, then from methanol, m. p. 99~101°C, $[\alpha]_D^{15}$ +65.1° (*c* 0.76, methanol).

Found: N, 11.82. Calcd. for $C_5H_{11}O_2N$: N, 11.96%.

(+)-5-Isopropyloxazolidone (IX).—In the thimble of a modified Soxhlet extractor inserted between the reflux condenser and the reaction flask which contained a slurry of 900 mg. of lithium aluminum-hydride in 100 cc. of tetrahydrofurane, was placed 940 mg. of the amide VIIc. After refluxing for 6 hr., a small amount of water, just enough to decompose the reaction complex, was added and the precipitated aluminum hydroxide was filtered. The aluminum hydroxide was continuously extracted with tetrahydrofurane for 2 hr., and the extract was combined with the filtrate. After 2 cc. of concentrated hydrochloric acid was added, the solvent was removed in vacuo, water was added to the residue and the solution was washed with ether three times. The acidic solution was made alkaline with 6 cc. of 50% potassium hydroxide and saturated with potassium carbonate, and then extracted with ether. After the ether extract was dried over anhydrous potassium carbonate, the solvent was removed to give the aminoalcohol VIIIa, which has an odor reminiscent of amine. The oil was dissolved in 15 cc. of benzene and was allowed to stand overnight. After the precipitated amide (140 mg.) was recovered, 0.7 cc. of pyridine was added with a solution of 0.65 g. of ethyl chloroformate in 4 cc. of benzene. After stirring for 1 hr., the reaction mixture was allowed to stand at room temperature for 1 hr. Ten cubic centimeters of water was added and the aqueous layer, after separating from the benzene layer, was extracted twice with benzene. The combined benzene extract was washed twice with 1 cc. of 1N hydrochloric acid and then with water. After the solvent was removed in vacuo, 10 cc. of benzene was added and evaporated to remove any trace of water by azeotropic distillation giving the carbamate VIIIb, 450 mg. To a sodium methoxide solution which was prepared from 50 mg. of sodium and 3 cc. of methanol, 10 cc. of toluene was added and the methanol was removed by heating with free flame. A solution of 450 mg. of the carbamate VIIIb in 17 cc. of toluene was added to the sodium methoxide suspension and heated at 100~105°C for 1 hr. with stirring. The reaction mixture was chilled in an ice bath and 10 cc. of water was added with stirring. The aqueous layer which separated from the toluene layer, was extracted twice with toluene. The combined toluene extract was concentrated to 5 cc. in vacuo to precipitate crystals which were recrystallized from benzene-benzine (b. p. 70~80°C), m. p. 111~112°C, 200 mg. Recrystallization from benzene raised the melting point to 113~114°C. $[\alpha]_D^{14}$ +44.2° (*c* 1.04, ethanol).

Found: C, 56.18; H, 8.68; N, 11.13. Calcd. for $C_6H_{11}O_2N$: C, 55.79; H, 8.58; N, 10.84%.

(±)-Ethyl 3-Hydroxy-4-methylpentanoate.—To a chilled solution of 8 g. of ethyl isobutyroylacetate in 40 cc. of methanol, 5 g. of sodium borohydride was added with stirring during 1 hr., and the reaction mixture was allowed to stand at room temperature for 1 hr. After the methanol was evaporated, water was added and the whole was extracted with choloroform. The chloroform extract was washed with diluted sulfuric acid and then with saturated sodium hydrogen carbonate solution. After drying over anhydrous sodium sulfate, the solvent was removed to give an oil, which distilled at 92~93°C/20 mmHg, 2.9 g.

(±)-3-Hydroxy-4-methylpentanhydrazide.—To a solution of 2.6 g. of the above-mentioned ester in 6 cc. of ethanol, was added 6 g. of 80% hydrazine hydrate and the reaction mixture was heated on a water bath for 1 hr. and allowed to stand in a refrigerator overnight. The precipitated crystals

were recrystallized from ethanol yielding crystals, m. p. 148~149°C, 1.72 g.

Found: N, 19.22. Calcd. for $C_6H_{14}O_2N_2$: N, 19.17%.

(±)-5-Isopropyloxazolidone.—Following the procedure described for the preparation of the (−)-isomer V, 1.7 g. of the racemic hydrazide was converted into (±)-5-isopropyloxazolidone, m. p. 87~88°C, 0.65 g.

Found: C, 55.88; H, 8.50; N, 11.12. Calcd. for $C_6H_{11}O_2N$: C, 55.79; H, 8.55; N, 10.84%.

The mixed melting point of the synthetic (±)-5-isopropyloxazolidone, m. p. 87~88°C, with the racemic compound, m. p. 87~88°C, which was prepared by mixing equal amounts of (−)-isomer V, m. p. 113~114°C from rotenone and (+)-isomer IX, m. p. 113~114°C from D-valine and recrystallization from benzene, was 87~88°C.

We are indebted to Mr. Mitsuru Notake for his effort in obtaining *Derris elliptica*, and to Nitto Kasei Co. for financial support.

Department of Chemistry
Institute of Polytechnics
Osaka City University
Kita-ku, Osaka