The Aroma of Cigar Tobacco

Part III. Isolation and Synthesis of R-(+)-3-Isopropyl-5-hydroxypentanoic Acid Lactone†

By Hajime KANEKO and Michi HARADA

*Central Research Institute, Japan Monopoly Corporation,
Nishi-shinagawa, Shinagawa-ku, Tokyo*

Received October 12, 1971

A minor component of cigar tobacco (+)-3-isopropyl-5-hydroxypentanoic acid lactone (I) was isolated. Synthesis of (+)-I from (+)-limonene established the absolute R-configuration. Stereochemistry of (+)-I is also described.

Though several lactones have been added to the number of aroma components of cigar tobacco,^{1~4)} many peaks observed in gas chromatograms of the steam-volatile neutral fraction of cigar tobacco have not been identified. The high polar parts of the steam-volatile neutral fraction particularly consist exclusively of many unidentified minor components, among which some new aroma compounds are thought to exist. In this paper the isolation and synthesis of (+)-3-isopropyl-5-hydroxypentanoic acid lactone (I) is described.

A general isolation procedure for aroma components from cigar leaf tobacco is shown in Fig. 1. To obtain a sufficient amount of each minor component for identification, 500 kg of leaf tobacco was extracted. Of the volatile neutral fractions from steam distillation, Fraction-III had the most fragrant odor (Table I). Silicic acid column chromatography of this fraction is shown in Table II. Fraction-III-14 was rechromatographed with a silicic acid column using benzene-dichloromethane as the eluant. Fraction-III-14-5 to Fraction-III-14-8 contained a liquid with a

fragrant odor as the main component. Preparative gas chromatography of these fractions gave 85 mg of a pure compound (Compound A).

Compound A, $C_8H_{14}O_2$, using a high resolution mass spectrum, showed a $R-C(=O)-O-R$ group in the infrared portion. In the NMR

FIG. 1. Isolation Procedure for Aroma Components in Cigar Leaf Tobacco.

† A part of this work was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, Apr. 2, 1971.

TABLE I. DISTILLATION OF THE NEUTRAL FRACTION IN FIG. 1

Fraction	Boiling point	Yield
I	~32°C / 29 mmHg	194.3 g
II	~30°C / 5 mmHg	8.0
III	~145°C / 2 mmHg	44.6
IV	146~160°C / "	54.3
V	161~165°C / "	17.9
VI	166~175°C / "	8.4
Residue		30.3

spectrum an isopropyl group (τ 9.06, 6H(d)) and an oxymethylene group (τ 5.8, 2H(m)) were indicated. On alkaline permanganate oxidation, Compound A afforded 3-isopropylglutaric acid, whose methylester was identified with an authentic sample. From these data 3-isopropyl-5-hydroxypentanoic acid lactone (I) was suggested as a possible structure for Compound A. I was synthesized from 3-isopropylglutaric acid according to the method of Schumacher *et al.*⁵¹ Infrared and mass spectra of the isolated sample were identical with those of the synthetic one.

In general, aliphatic γ - or δ -lactones, isolated from natural products as aroma components, contain a γ - or δ -alkyl substituents. Therefore, I should be the first δ -lactone having a β -substituent isolated from nature.

Natural I had a specific rotation of $[\alpha]_D^{20} +$

TABLE II. COLUMN CHROMATOGRAPHY OF FRACTION-III IN TABLE I

Column; Silicic Acid (Mallinkrodt 100 mesh) 7 cm ϕ $\times$ 47 cm.

Fraction	Solvent		Yield
	<i>n</i> -Hexane : Ether	Volume	
III- 1	100 : 0	3.0 liter	20.3 g
III- 2	98 : 2	1.5	0.2
III- 3	90 : 10	1.0	4.6
III- 4	"	"	2.6
III- 5	"	"	2.2
III- 6	80 : 20	0.5	1.4
III- 7	"	"	1.6
III- 8	"	"	1.6
III- 9	"	"	1.3
III-10	"	"	1.2
III-11	"	"	1.1
III-12	50 : 50	1.0	4.0
III-13	"	"	2.2
III-14	"	"	2.6
III-15	0 : 100	1.0	3.7
III-16	"	"	1.5
III-17	"	"	0.3
III-18	Methanol	2.0	0.9

16.8° and showed a plus Cotton effect in its ORD curve. To establish the absolute configuration of this compound, (+)-I was synthesized from (+)-limonene using the following procedure (Fig. 2).

FIG. 2. Schema for the Synthesis of (+)-3-Isopropyl-5-hydroxypentanoic Acid Lactone.

(+)-3-Isopropyl-6-ketoheptanoic acid (II) was prepared from (+)-limonene according to the method of Kataoka and Hanada.⁶¹ The methyl ester (III) of the acid was treated with perfluoroacetic acid in dichloromethane. On alkaline hydrolysis followed by an acid treatment, the product afforded (+)-I which

had $[\alpha]_D^{22} + 22.4^\circ$ in ethanol. The infrared spectrum of synthetic (+)-I was identical with that of the natural compound (Fig. 3).

The absolute configuration of (+)-limonene has been established as R-configuration⁷¹; thus, (+)-I must have R-configuration as shown in Fig. 2.

FIG. 3. Infrared Spectra of (+)-3-Isopropyl-5-hydroxypentanoic Acid Lactone. (liquid films)
(a) Isolated sample (b) Synthetic sample

FIG. 4. Mass Spectrum of 3-Isopropyl-5-hydroxypentanoic Acid Lactone.

Based on X-ray analysis of various saturated δ -lactones, the group $\text{C}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{O}-\text{C}$ has been

shown to be planar. Accordingly the δ -lactone ring exists in boat or half-chair conformations which, in turn, are asymmetric.⁸¹ Wolf⁹¹ showed that the sign of the Cotton effect in the ORD curve of δ -lactone was determined by the chirality of the asymmetric boat or half-chair conformation of the δ -lactone ring. Application of Wolf's observation to (+)-I, which has a plus Cotton effect with the first extreme at 235 m μ , indicates that most of the (+)-I has conformations shown as V or VI.

If not oriented otherwise by certain steric factors, the δ -lactones normally assume the boat conformation.⁸¹ Therefore, (+)-I is represented as V. In fact, a comparison of Dreiding models of V and its antipode showed that the isopropyl group in the former was sterically less hindered than in the latter.

Both ($\pm$)-I and (+)-I had herbaceous lactone flavors, but the flavor of the racemate was somewhat weaker than that of the (+)-enantiomer.

EXPERIMENTAL

Infrared measurements were made with a JASCO IR-S spectrophotometer. The NMR spectrum was measured with a JNMC-100 in carbon tetrachloride, and the mass spectra with a Hitachi RMU-6E. Specific rotations were measured with a Yanagimoto OR-10 polarimeter and the ORD with a JASCO ORD/UV-5. Gas chromatography was conducted with a Hitachi GC K-53.

Isolation of compound A. Five hundred kilograms of chopped cigar leaves (Izabela 2A, 1967 crops in Phillipine) were extracted twice with ether. Evaporation of the ether afforded 22.4 kg of dark green extract. The extract was steam-distilled. About 12 liters of the distillate was collected for each 100 g of extract. After saturation with NaCl the distillate was extracted five times with ether. The combined ether solution of volatile components was successively shaken with 5% H_2SO_4 and 5% Na_2CO_3 solutions to remove acidic and basic fractions, then it was washed with water, dried over anhydrous Na_2SO_4 and concentrated using a Widmer column. Thus, 368.3 g of the neutral fraction which still contained fairly large amounts of ether was obtained. Distillation of the neutral fraction was conducted in a Claisen flask under nitrogen and the distillate was separated into six fractions as shown in Table I. Fractions I and II were collected in a dry ice-acetone trap. Gas chromatographic analysis showed that almost all of Fraction-I was ether.

The column chromatography of Fraction-III is shown in Table II. Fraction-III-14 was rechromatographed with a silicic acid column of 3 cm ϕ $\times$ 23 cm high, then separated into 18 portions using benzene-dichloromethane eluants. Gas chromatographic analysis showed Fraction-III-14-5 to Fraction-III-14-8 contained Compound A as a main peak.

Preparative gas chromatography of the above fractions was carried out under the following conditions. Column: 10% silicone SE-30 on Diasolid L, 3 mm $\times$ 3 m, Temperature: 180°C, Carrier gas: 2 kg He/cm². Under these conditions Compound A had a retention time of 5.8 min and was collected in a dry ice-acetone trap to afford 85 mg of a liquid.

Spectral data of compound A. The infrared and mass spectra of Compound A are shown in Figs. 3 and 4. High resolution mass; M^+ : 142.102 (Calcd. for $\text{C}_8\text{H}_{14}\text{O}_2$ 142.100). $[\alpha]_D^{20} +16.8^\circ$ ($c=1.19$, EtOH). ORD ($c=0.030$, EtOH) $[\alpha]^{18}$ (m μ): $+870^\circ$ (235, peak), 0° (220), -960° (210, trough). NMR $\tau_{\text{Me}_4\text{Si}}^{\text{CCl}_4}$: 9.06 (6H, d), 7.38 (1H), 7.48 (1H), 5.8 (2H, m), 8.0~8.5 (4H).

Permanganate oxidation of compound A. To a 0.5 ml of 10% KOH aqueous solution containing 15 mg of Compound A, was dropwise added 5% aqueous KMnO_4 solution until the color of permanganate ion persisted. The reaction mixture was kept for 1 hr at room temperature. After decomposition of excess

KMnO₄ with H₂O₂, the reaction mixture was acidified with dilute H₂SO₄, and MnO₂ was decomposed by NaHSO₃. The aqueous solution was saturated with NaCl and extracted with ether. Evaporation of the ether afforded a solid, mp 92~95°C, IR 1700 cm⁻¹. The solid was esterified with CH₂N₂ then purified by preparative gas chromatography. IR (ν C=O 1740 cm⁻¹) and mass spectra [parent peak m/e 171 ($M^+ - 31$), base peak m/e 129] of the methyl ester were identical with those of methyl 3-isopropylglutarate.⁵⁾

Synthesis of (+)-3-isopropyl-5-hydroxypentanoic acid lactone. (+)-3-Isopropyl-6-ketoheptanoic acid (II) ($[\alpha]_D^{20} + 3.11$, $c = 5.16$, EtOH) was prepared from (+)-limonene ($[\alpha]_D^{20} + 100.0^\circ$, neat) according to the method of Kataoka and Hanada.⁶⁾ Treatment of the acid with CH₂N₂ gave a methyl ester (III). Mass; parent peak m/e 169 ($M^+ - 31$), base peak m/e 43. IR; ν C=O 1735, 1715 cm⁻¹ $[\alpha]_D^{20} + 7.45$ ($c = 2.15$, EtOH).

To a 2.0 g solution of III in 7.5 ml CH₂Cl₂, CF₃CO₃H prepared from 0.41 ml of 90% H₂O₂ in 2.5 ml CH₂Cl₂ and 2.54 ml trifluoroacetic anhydride was added dropwise. After refluxing the solution for 30 min on a water bath, the solvent was distilled from the reaction mixture. Without purification, the resulting methyl 5-acetoxy-3-isopropylpentanoate (IV) was hydrolysed with 20 ml of 10% KOH at 60°C for 1 hr. After washing it with ether, the alkaline solution was acidified with dilute HCl and extracted with ether. After evaporation of the ether, 1 ml of 9% HCl aqueous solution was added to the residue,

which was kept at 70°C for 30 min. The reaction mixture was extracted with ether, then the ether layer was washed successively with 5% NaHCO₃ solution and water and dried over anhydrous Na₂SO₄. Evaporation of the ether afforded 0.5 g of (+)-I (35% yield for III). Pure (+)-I was obtained by preparative gas chromatography under the conditions given above. $[\alpha]_D^{22} + 22.4^\circ$ ($c = 2.37$, EtOH).

Acknowledgements. We wish to express our thanks to Dr. A. Sakurai of the Institute of Physical and Chemical Research for the ORD measurements and to Mr. K. Aizawa of Tokyo University for the NMR measurements.

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