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Plant Constituents biologically Active to Insects. II.[1] Juvabione Analogs from *Abies sachalinensis* MAST. (1).

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(+)-Juvabione (I) and two new analogs (III and IV) were isolated, together with *trans*-4-hydroxycinnamic acid (XIII) and vanillin (XIV), from the wood of *Abies sachalinensis* MAST. (Pinaceae). The structures of III and IV were established to be *cis*-4-[3(*S*)-hydroxy-1(*R*),5-dimethylhexyl]cyclohexane-1-carboxylic acid and *trans*-4-[3(*S*)-hydroxy-1(*R*),5-dimethylhexyl]cyclohexane-1-carboxylic acid, respectively, on the basis of chemical and spectral evidence.

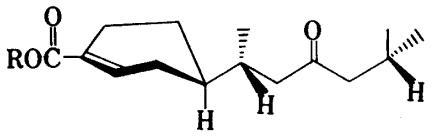
Keywords——*Abies sachalinensis*; Pinaceae; sesquiterpene; (+)-juvabione; tetrahydrotodomatuic acid; hydroxycinnamic acid; vanillin

The chemistry of the wood of fir has been widely investigated[2-13] since Slama and Williams[14] in 1965 discovered that paper towelling contained a compound having insect juvenile hormone activity, which was described as "the paper factor." Bowers *et al.*[2] in 1966 isolatd the active material, (+)-juvabione, from native balsam fir, *Abies balsamea* (L.) MILLER, and identified it as the methyl ester of (+)-todomatuic acid.[15] Afterwards, it was synthesized by several methods,[3] and some other juvenile hormone mimics were found in the wood of balsam and Douglas fir.[4]

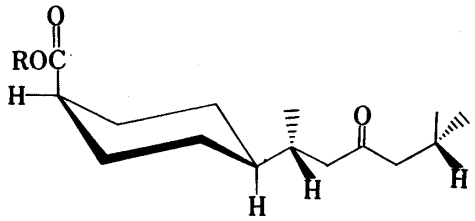
Tsuchihashi and Hanzawa[15a] in 1940 first isolated (+)-todomatuic acid from the hydrolyzed bisulfite-treated pulp oil of the mixed wood of *Abies sachalinensis* MAST. (Japanese name, todomatsu) and *Picea jezoensis* CARR. (Japanese name, ezomatsu). They presumed that the acid occurs as the methyl ester in the wood of *A. sachalinensis*. However, the acid and the methyl ester have never been isolated directly from the wood. This paper describes the isolation and identification of (+)-juvabione (I), two new analogs (III and IV), *trans*-4-hydroxycinnamic acid (XIII), and vanillin (XIV) from the wood of *A. sachalinensis* growing in Hokkaido.

The wood was finely cut and extracted with hot acetone. The extract was fractionated into basic, acidic, phenolic, and neutral fractions. The neutral fraction was purified by repeated silicic acid column chromatography. Elution with benzene gave an oily material (I) which had proton nuclear magnetic resonance (^{1}H NMR), carbon-13 nuclear magnetic resonance (^{13}C NMR), ultraviolet (UV), infrared (IR), mass, optical rotatory dispersion (ORD), and circular dichroism (CD) spectra in accord with those reported for (+)-juvabione.[7,9] The acid (II), mp 57—58 °C, obtained on saponification of this compound, was proved to be identical with (+)-todomatuic acid from bisulfite-treated pulp oil by direct comparison.

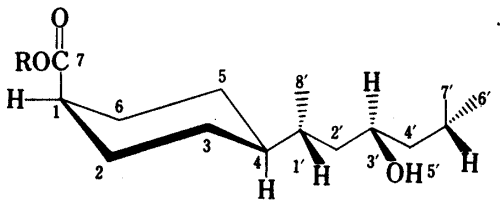
The acidic fraction was passed through a silicic acid column to yield two crystalline materials, III and IV. Compound IV, mp 123—129 °C, was assigned the molecular formula $C_{15}H_{28}O_3$ on the basis of elemental analysis. The IR spectrum showed bands at 2350 to 3520 (br), and 1705 cm^{-1}, characteristic of a carboxyl group. The ^{1}H NMR spectrum exhibited peaks corresponding to two terminal methyls (isopropyl) at δ 0.94 (6H, d, J=7 Hz), one other seconday methyl group at 0.88 (3H, d, J=7 Hz), one methine proton attached to carboxyl at 2.00 (1H, m), another methine proton attached to hydroxyl at 3.77 (1H, m), and hydroxyl and carboxyl groups at 6.11 (2H, br s). The absence of double bonds was inferred from the UV,



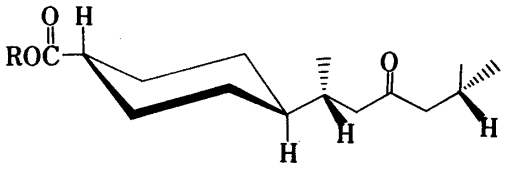
I : R=CH_3
II : R=H



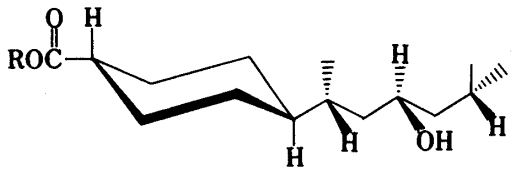
VII : R=H
IX : R=CH_3



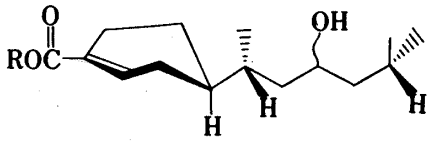
III : R=H
V : R=CH_3



VIII : R=H
X : R=CH_3



IV : R=H
VI : R=CH_3



XI : R=CH_3
XII : R=H

Chart 1

IR, ^1H and ^{13}C NMR spectra.

Methylation of IV with diazomethane gave the monomethyl ester (VI) as an oily substance which showed in the ^1H NMR spectrum a signal corresponding to a methoxyl group at δ 3.65 (3H, s) and in the IR spectrum bands at 3610 and 3480 cm^{-1}, indicative of a hydroxyl group, in addition to an ester carbonyl band at 1730 cm^{-1}. Although the mass spectrum (MS) does not display the molecular ion, the M−18 ion at m/z 252 formed by the elimination of water confirms the elemental composition of VI and, consequently, IV. Fragments at m/z 213, 184, 181, 153, 152, and 135 are associated with α-cleavage of a hydroxy moiety in a side chain. The formation of the ion at m/z 168 by elimination of water and the unsaturated hydrocarbon, C_6H_{12}, also proves the presence and position of a hydroxy moiety in a side chain. The base peak (m/z 109) and the other fragments shown in Chart 2 provide structural information. All the fragments indicated are supported by the molecular formulas determined by high resolution mass spectral measurement.

Based on the above-mentioned spectral data, the structure of IV was assumed to be 4-(3-hydroxy-1,5-dimethylhexyl)cyclohexane-1-carboxylic acid (tetrahydrotodomatuic acid).

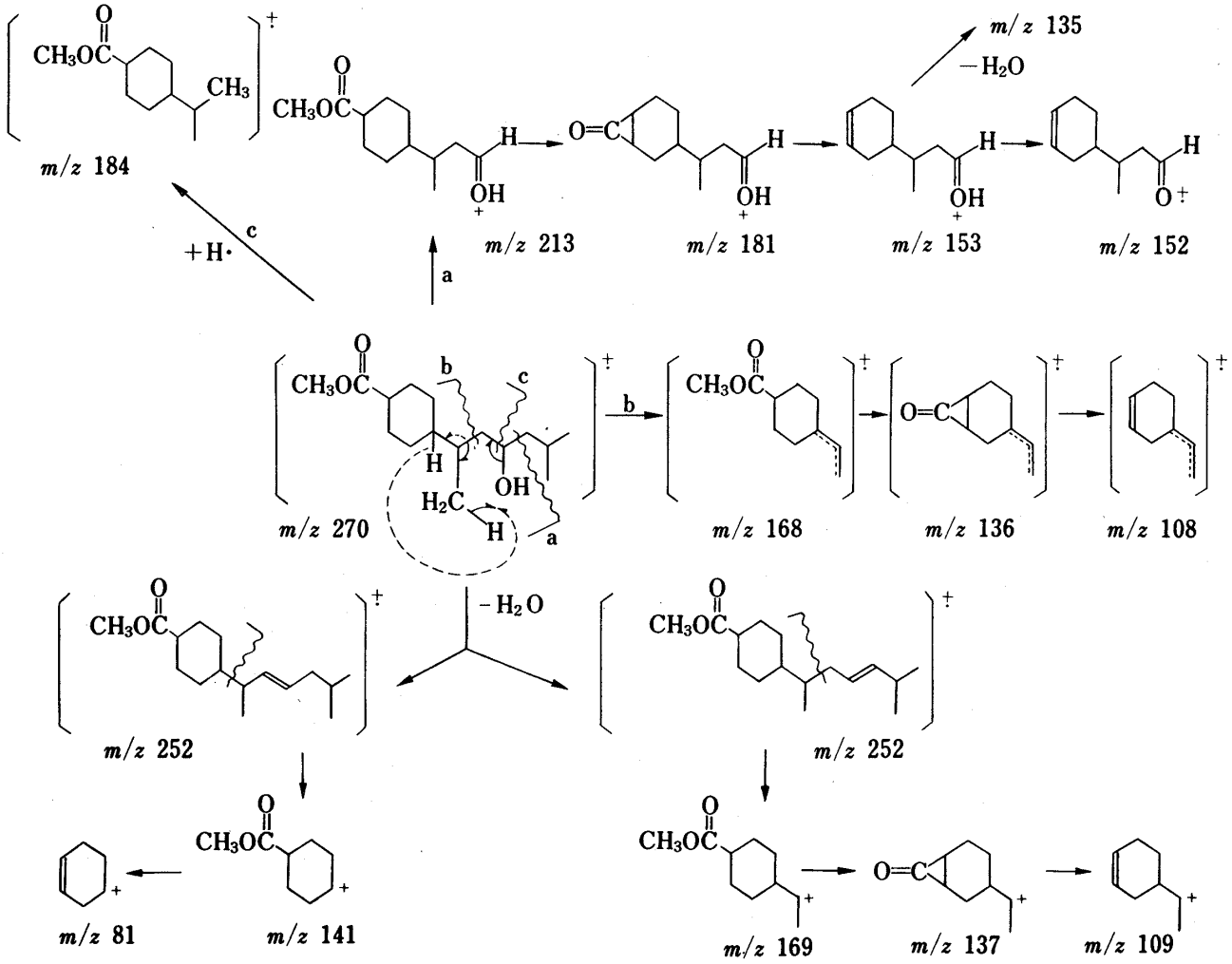


Chart 2

The general features of the IR and ^{1}H NMR spectra of compound III were very similar to those of IV except that the signal of the methine proton attached to a carboxyl group was shifted to δ 2.68 in the ^{1}H NMR spectrum. The ^{13}C NMR spectral data of the side chain moiety of III were in accord with those of IV, while the chemical shifts of the cyclohexane moiety of III relative to those of IV appeared upfield by 3.6 ppm at C-1 and by about 2 ppm at C-2, -3, -5, and -6. Table 1 presents these ^{13}C NMR spectral data, which were assigned on the basis of chemical shift considerations, off-resonance decoupled data, and comparison with

TABLE I. ^{13}C NMR Spectral Data for III and IV, δ(ppm) from TMS in $CDCl_3$[a)]

Position	III	IV	Position	III	IV
1	39.63	43.26	1′	33.08	33.78
2	26.98[x]	29.06[x]	2′	42.63	42.72
3	26.66[y]	29.26[y]	3′	67.84	67.77
4	42.56	42.53	4′	47.85	47.90
5	25.62[y]	27.76[y]	5′	24.71	24.73
6	27.06[x]	29.13[x]	6′	22.25[z]	22.25[z]
7	180.86	181.42	7′	23.35[z]	23.35[z]
			8′	16.12	15.81

a) Mesaured on a Varian XL-200 (50.3 MHz). Assignments with the same superscript for each compound may be interchanged.

published data for juvabiol, dihydrojuvabione, and other analogs.[9,13] The MS of the methyl ester (V) of III was very similar to that of VI except that the *m/z* 213 fragment of V showed about five times the intensity of that of VI. Oxidatin of both III and IV with chromium trioxide–pyridine complex gave two stereoisomeric keto acids, VII and VIII, respectively, which were methylated with diazomethane to yield the corresponding keto esters, IX and X. Base-catalyzed epimerization of IX followed by methylation with diazomethane gave a mixture of the starting material (IX) and X in the ratio 1 to 5. It was therefore considered that IX and X are stereoisomers with the opposite configuration at the carbon attached to the carboxyl group, and that the carboxyl groups of IX and X are axial and equatorial, respectively, since epimerization generally favors formation of the more thermodynamically stable equatorial form. These results show that III is a stereomer of IV at the carbon attached to the carboxyl group, and the carboxyl groups of III and IV are axial and equatorial, respectively, since the signal of the methine proton attached to the carboxyl group of III in the ^{1}H NMR spectrum appears at lower field than that of IV, in addition to the result of epimerization of IX. Also, III was anticipated to have the side chain in the *cis* configuration relative to the carboxyl group, while IV was expected to have the *trans* configuration, since it is most probable that a bulky substituent is in a more thermodynamically stable equatorial orientation.

In order to determine the configuration at C-1′ of the side chain, we attempted to correlate III and IV to juvabione (I). A mixture of diastereomers of hydroxy acids was derived from I *via* XI and XII by reduction with $NaBH_4$, base-catalyzed hydrolysis, and catalytic hydrogenation. It was purified by silicic acid column chromatography and recrystallization to yield III and IV, which were identified by comparison of IR, ORD, and ^{1}H NMR spectra with those of authentic samples. This correlation between I and III or IV shows that the methyl group at C-1′ has *R* configuration.

The ^{1}H NMR spectra of the keto esters (IX and X) described above were measured at 200 MHz. The spectrum of IX was in accord with the data reported by Manville[4b] for dihydrojuvabione, which has juvenile hormone activity. This result confirms the above-mentioned stereochemistry of III except for the configuration of the hydroxyl group.

The absolute configuration of the hydroxyl group of IV was determined by application of Horeau's rule.[16] Since treatment of the methyl ester (VI) of IV with (±)-α-phenylbutyric anhydride gave levorotatory α-phenylbutyric acid, the hydroxyl group of VI and, consequently, IV was proved to have *S* configuration.

The absolute configuration of the hydroxyl group of III was deduced by comparison of its ^{13}C NMR spectrum with that of IV (Table I). Since the signal of C-3′ of III was identical with that of IV, the hydroxyl group of III is considered to have the same configuration as that of IV. Consequently, the absolute configurations of III and IV have been determined as the structures represented by III and IV in Chart 1. The structure presented herein for IV is in accord with the result of X-ray crystallographic analysis carried out by Ishida and Inoue.[17]

Purification of the phenolic fraction by silicic acid column chromatography gave two phenolic components, *trans*-4-hydroxycinnamic acid (XIII) and vanillin (XIV), which were identified by comparison with authentic samples. Tests of juvenile hormone activities of III and IV on freshly molted pupae of *Tenebrio moritor* L. are in progress in our laboratory.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The UV spectra were recorded with a Hitachi 323 spectrophotometer and the IR spectra with a Hitachi EPI-G2 spectrometer. Most ^{1}H NMR spectra were recorded with a Hitachi R 40 spectrometer (90 MHz) and the ^{1}H NMR spectra of IX and X were obtained with a Varian XL-200 (200 MHz). Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as an internal standard. MS were taken on a Hitachi M 80 spectrometer. The ORD curves were measured on a JASCO Model ORD/UV-5 spectropolarimeter. CD measurements were made with a JASCO J-20 spectropolarimeter. Gas liquid chromatography (GLC) was run

on a Hitachi 063 equipped with a flame ionization detector. A stainless steel column (2 m×3 mmϕ) was packed with 20% PEG-20M on 60—80 mesh Chromosorb W and operated at 215 °C with a nitrogen carrier gas flow rate of 30 ml/min. Injector and detector temperaures were 260 and 250 °C, respectively. Retention times are given in minutes.

Extraction and Separation———Finely cut wood of *A. sachalinensis* (1150 g) was extracted three times with hot acetone (12 l each) for 3 h. The combined extracts were evaporated at 60 °C under reduced pressure. The oily residue (153.5 g) was dissolved in ether and the solution was successively extracted with 10% aqueous hydrochloric acid, 10% aqueous sodium hydrogen carbonate, and 5% aqueous sodium hydroxide. The ether layer was dried over anhydrous sodium sulfate and evaporated to give the neutral fraction (23.76 g). The aqueous acidic solution was basified with conc. ammonia and extracted four times with 200 ml portions of ether. The extract was evaporated to yield the basic fraction (0.77 g). The sodium hydrogen carbonate solution and the sodium hydroxide solution were acidified with conc. hydrochloric acid and extracted four times with ether, respectively. The two ether solutions were evaporated to give the acidic (4.49 g) and phenolic fraction (6.24 g), respectively.

Juvabione (I)———Separation of the compounds present in the neutral fraction was achieved by repeated column chromatography on Mallinckrodt silicic acid. Elution was carried out with an *n*-hexane–benzene gradient. The fraction eluted with benzene gave (+)-juvabione (271.3 mg) as a colorless oil, $[\alpha]_D^{21}$ +66.7° (c=1.05, $CHCl_3$), which had UV, IR, ^1H NMR, ^{13}C NMR, ORD, and CD spectra in accord with the published data.[7,9]

Saponification of I with alcoholic potassium hydroxide afforded todomatuic acid (II), mp 57—58 °C, which was identical with an authentic sample as judged by direct comparison.

Compound III———The acidic fraction (4.49 g) was fractionated by silicic acid column chromatography using chloroform as the eluent. The initial fraction eluted with chloroform was further purified by silicic acid column chromatography with an ethyl acetate–benzene gradient as the eluent. The fraction eluted with 10% ethyl acetate in benzene yielded on evaporation of the solvent a crystalline substance, which was crystallized from ethyl acetate to give the hydroxy acid (III) (252.4 mg) as colorless needles, mp 98—102 °C, $[\alpha]_D^{25}$ +14.0° (c=1.2, EtOH). *Anal.* Calcd for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 70.42; H, 10.84. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 3520—2300 (br), 1700 (COOH). ^1H NMR ($CDCl_3$) δ: 0.88 (3H, d, J=7 Hz, C_1'-CH_3), 0.93 (6H, d, J=6 Hz, C_5'-CH_3×2), 2.68 (1H, m, C_1-H), 3.78 (1H, m, C_3'-H), 6.39 (2H, br s, OH, COOH). ORD (c=1.2, EtOH, 25 °C): $[\phi]_{589}$ +35.8, $[\phi]_{500}$ +51.2, $[\phi]_{400}$ +85.3, $[\phi]_{300}$ +196.2, $[\phi]_{250}$ + 358.4.

Methylation of III with diazomethane in ether gave the methyl ester (V) as an oily substance. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3585, 3480 (OH), 1725 ($COOCH_3$). ^1H NMR ($CDCl_3$) δ: 0.88 (3H, d, J=7 Hz, C_1'-CH_3), 0.94 (6H, d, J=6 Hz, C_5'-CH_3×2), 2.63 (1H, m, C_1-H), 3.69 (3H, s, $COOCH_3$), 3.73 (1H, m, C_3'-H). MS m/z (relative intensity %): 252 (M^+-H_2O, 4), 239 (2), 213 (73), 184 (34), 181 (81), 169 (7), 168 (50), 153 (9), 152 (8), 141 (30), 137 (16), 136 (37), 135 (83), 109 (100), 108 (63), 81 (87), 79 (28), 69 (50), 67 (43).

Compound IV———Further elution of the acidic fraction on the above-mentioned column gave on evaporation of the solvent a crystalline material, which was recrystallized from ethyl acetate to yield the hydroxy acid (IV) (241 mg) as colorless needles, mp 123—129 °C, $[\alpha]_D^{25}$ +7.6° (c=1.05, EtOH). *Anal.* Calcd for $C_{15}H_{28}O_3$: C, 70.27; H, 11.01. Found: C, 70.42; H, 10.84. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520—2350 (br), 1705 (COOH). ^1H NMR ($CDCl_3$) δ: 0.88 (3H, d, J=7 Hz, C_1'-CH_3), 0.94 (6H, d, J=7 Hz, C_5'-CH_3×2), 2.00 (1H, m, C_1-H), 3.77 (1H, m, C_3'-H), 6.11 (2H, br s, OH, COOH). ORD (c=1.05, EtOH, 25 °C): $[\phi]_{589}$ +19.5, $[\phi]_{500}$ +29.3, $[\phi]_{400}$ +58.5, $[\phi]_{300}$ +156.0, $[\phi]_{250}$ +302.3.

Methylation of IV with diazomethane in ether gave the methyl ester (VI) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3480 (OH), 1730 ($COOCH_3$). ^1H NMR ($CDCl_3$) δ: 0.88 (3H, d, J=6 Hz, C_1'-CH_3), 0.93 (6H, d, J=6 Hz, C_5'-CH_3×2), 1.32 (1H, s, OH), 3.65 (3H, s, $COOCH_3$), 3.72 (1H, m, C_3'-H). MS m/z (%) (high resolution MS: Calcd; Found): 252 (M^+-H_2O, 5) (252.2087 for $C_{16}H_{28}O_2$; 252.2045), 239 (3) (239.2010 for $C_{15}H_{27}O_2$; 239.2014), 213 (16) (213.1489 for $C_{12}H_{21}O_3$; 213.1519), 184 (40) (184.1462 for $C_{11}H_{20}O_2$; 184.1420), 181 (71) (181.1227 for $C_{11}H_{17}O_2$; 181.1185), 169 (12) (169.1227 for $C_{10}H_{17}O_2$; 169.1205), 168 (72) (168.1150 for $C_{10}H_{16}O_2$; 168.1157), 153 (17) (153.1279 for $C_{10}H_{17}O$; 153.1292), 152 (18) (152.1200 for $C_{10}H_{16}O$; 152.1189), 141 (23) (141.0914 for $C_8H_{13}O_2$; 141.0846), 137 (23) (137.0964 for $C_9H_{13}O$; 137.0926), 136 (37) (136.0887 for $C_9H_{12}O$; 136.0895), 135 (63) (135.1172 for $C_{10}H_{15}$; 135.1139), 109 (100) (109.1026 for C_8H_{13}; 109.1053), 108 (63) (108.0938 for C_8H_{12}; 108.0883), 81 (73) (81.0703 for C_6H_9; 81.0660), 79 (22) (79.0547 for C_6H_7; 79.0502), 69 (60) (69.0703 for C_5H_9; 69.0697), 67 (44) (67.0548 for C_5H_7; 67.0550).

Oxidation of III———A solution of III (65 mg) in pyridine (0.1 ml) was treated with chromium trioxide (40 mg) in pyridine (0.7 ml), and the mixture was kept at room temperature overnight. After usual work-up, purification by silicic acid column chromatography with chloroform as the eluent provided the keto acid (VII) (24 mg) as a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550—2300 (br), 1706 (COOH, $\geq$C=O). ^1H NMR ($CDCl_3$) δ: 0.86 (3H, d, J=7 Hz, C_1'-CH_3), 0.96 (6H, d, J=6 Hz, C_5'-CH_3×2), 2.68 (1H, m, C_1-H), 8.5 (1H, br s, COOH).

The methyl ester (IX), prepared with diazomethane in ether and purified by silicic acid column chromatography with chloroform as the eluent, is a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1727 ($COOCH_3$), 1715 ($\geq$C=O). The ^1H-NMR spectrum and MS were in accord with the publishd data.[4b]

Oxidation of IV———A solution of IV (41 mg) in pyridine (0.1 ml) was treated with chromium trioxide (40 mg) in pyridine (0.7 ml), and the mixture was kept at room temperature overnight. After usual work-up, purification by silicic acid column chromatography with chloroform as the eluent and by crystallization from *n*-hexane provided the keto acid (VIII) (19 mg) as needles, mp 47—54°C. *Anal.* Calcd for $C_{15}H_{26}O_3$: C, 70.83; H, 10.30. Found: C, 70.63; H, 10.05. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550—3350 (br), 1706 (COOH, >C=O). ^1H NMR ($CDCl_3$) δ: 0.86 (3H, d, J=7 Hz, C_1'-CH_3), 0.94 (6H, d, J=6 Hz, C_5'-CH_3×2), 10.5 (1H, br s, COOH).

The methyl ester (X), prepared with diazomethane in ether and purified by silicic acid column chromatography with chloroform as the eluent, is a colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1722 ($COOCH_3$), 1709 (>C=O). ^1H NMR ($CDCl_3$) δ: 0.84 (3H, d, J=6.7 Hz, C_1'-CH_3), 0.91 (3H, d, J=6.4 Hz, C_5'-CH_3), 0.92 (3H, d, J=6.4 Hz, C_5'-CH_3), 1.97 (1H, m, C_1'-H), 2.03 (1H, m, C_1-H), 2.16 (1H, dd, J=15.7 Hz, 3.5 Hz, C_2'-H), 2.26 (2H, d, J=6.2 Hz, C_4'-H), 2.41 (1H, dd, J=15.7 Hz, 4.8 Hz, C_2'-H), 3.65 (3H, s, $COOCH_3$). MS m/z (%) (high resolution MS: Calcd; Found): 268 (M^+, 1) (268.2037 for $C_{16}H_{28}O_3$; 268.2077), 237 (7) (237.1854 for $C_{15}H_{25}O_2$; 237.1895), 211 (16) (211.1333 for $C_{12}H_{19}O_3$; 211.1337), 209 (21) (209.1903 for $C_{14}H_{25}O$; 209.1895), 179 (18) (179.1071 for $C_{11}H_{15}O_2$; 179.1071),169 (69) (169.1227 for $C_{10}H_{17}O_2$; 169.1200), 168 (88) (168.1148 for $C_{10}H_{16}O_2$; 168.1129), 151 (27) (151.1121 for $C_{10}H_{15}O$; 151.1120), 137 (73) (137.0965 for $C_9H_{13}O$; 137.0939), 136 (82) (136.0886 for $C_9H_{12}O$; 136.0878), 133 (52) (133.1016 for $C_{10}H_{13}$; 133.1011), 127 (70) (127.1122 for $C_8H_{15}O$; 127.1129), 123 (37) (123.1172 for C_9H_{15}; 123.1165), 109 (94) (109.1015 for C_8H_{13}; 109.0999), 108 (100) (108.0937 for C_8H_{12}; 108.0930), 107 (41) (107.0859 for C_8H_{11}; 107.0829).

Reduction, Hydrolysis and Hydrogenation of (+)-Juvabione (I)———I (580 mg) and $NaBH_4$ (300 mg) in methanol (50 ml) were kept at room temperature for 8 h, then the mixture was acidified with 10% aqueous hydrochloric acid, diluted with water and extracted with chloroform. Concentration of the chloroform layer gave the hydroxy ester (XI) (552 mg) as a colorless oil. XI and 10% methanolic potassium hydroxide (18 ml) were refluxed for 1 h, then the mixture was concentrated under reduced pressure. The residue was diluted with water, and the solution was washed with ether, acidified with 10% aqueous hydrochloric acid, and extracted with chloroform. Evaporation of the chloroform gave the hydroxy acid (XII) (530 mg) as an oil. XII (530 mg) in ethanol (20 ml) was shaken with Adams catalyst (0.1 g) in hydrogen for 5 h. The filtered solution was evaporated to dryness *in vacuo* and the residue (520 mg) was passed through a silicic acid column. The fraction eluted with chloroform was crystallized from ethyl acetate to give the hydroxy acid (IV) (85 mg) as needles, mp 123—129°C, $[\alpha]_D^{25}$ +7.8° (c=1.66, EtOH). The mother liquor was allowed to stand at room temperature to yield the hydroxy acid (III) (35 mg) as needles, mp 98—102°C, $[\alpha]_D^{25}$ +12.8° (c=0.96, EtOH). III and IV obtained herein were identified by comparison of IR, ORD and ^1H NMR spectra with those of authentic samples.

Epimerizaton of IX———A solution of Na (100 mg) in *n*-amyl alcohol (10 ml) was added to IX (6.3 mg), and the mixture was heated under reflux for 6.5 h. The *n*-amyl alcohol was removed by distillation *in vacuo*. A solution (10 ml) of 10% KOH in methanol was added to the residue and the mixture was heated under reflux for 1 h, then concentrated. The concentrate was diluted with water, washed with chloroform, acidified with 10% aqueous hydrochloric acid, and extracted with chloroform. Concentration of the extract gave an oil (6 mg), which was esterified with excess diazomethane. GLC analysis showed two peaks of t_R 23.7 and 30, corresponding to IX and X, respectively, in the ratio 1 to 5.

Treatment of VI with α-Phenylbutyric Anhydride———A solution of VI (14 mg, 0.05 mmol) and (±)-α-phenylbutyric anhydride (40 mg, 0.13 mmol) in pyridine (0.5 ml) was kept at room temperature overnight and then at 55°C for 1.5 h. After addition of water, the mixture was extracted with benzene and the organic phase was washed with aqueous sodium hydrogen carbonate. The washing was acidified with dilute hydrochloric acid and extracted with chloroform. The extract gave on evaporation of the solvent α-phenylbutyric acid (36.7 mg), $[\alpha]_D^{23}$ −1.1° (c=3.6, MeOH). The ORD spectrum displayed a negative plain curve.

***trans*-4-Hyroxycinnamic Acid (XIII) and Vanillin (XIV)**———The phenolic fraction (6.24 g) was fractionated by silicic acid column chromatography with chloroform as the eluent. The earlier fractions were further purified by silicic acid column chromatography with ethyl acetate–benzene gradient as the eluent. The fraction eluted with 10% ethyl acetate in benzene yielded a crystalline substance, which was recrystallized from ethyl acetate to give *trans*-4-hydroxycinnamic acid (XIII)(252 mg), mp 210—215°C. This product was identical with an authentic sample. Further elution of the phenolic fraction on the above-mentioned column gave a fraction which was further chromatographed on silicic acid to give vanillin (XIV)(17 mg), mp 80—82°C (methanol-water). This product was identical with an authentic sample.

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