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171. **Toshio Miyazaki and Susumu Mihashi** : Studies on the Constituents of *Boenninghausenia albiflora* MEISSNER var. *japonica* S. SUZUKI. I. Structure of Matsukaze-lactone. (1).*1

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On the constituents of *Boenninghausenia albiflora* MEISSNER var. *japonica* S. SUZUKI (Japanese name : Matsukaze-sô, Rutaceae), Ohta and one of the authors (T.M.)1) have reported the presence of dictamnine (0.0006%) and bergapten (0.0015%) in the air-dried leaves and stems. The present article is concerned with the isolation and the structure elucidation of a new constituent of this plant.

The concentrated methanol extract of the air-dried powdered leaves of the plant gave a dark green crystalline precipitates after standing several weeks. Repeated chromatography and recystallization of them led to a homogeneous sample (I) with m.p. 267~268° in 0.30% yield.

This substance (I) was optical inactive and slightly soluble in organic solvents but soluble in chloroform, and showed a blue fluorescence under ultraviolet light either at the crystalline state or in the solution. It was slowly dissolved in cold diluted sodium hydroxide solution but rapidly in hot, and recovered unchanged after acidification. Moreover, it gave no reactions with magnesium-hydrochloric acid, ferric chloride, and bromine in chloroform or glacial acetic acid solution. After treatment with acetic anhydride-sulfuric acid or -pyridine, the starting material (I) was recovered. Semicarbazone and 2,4-dinitrophenyl hydrazone were not obtained.

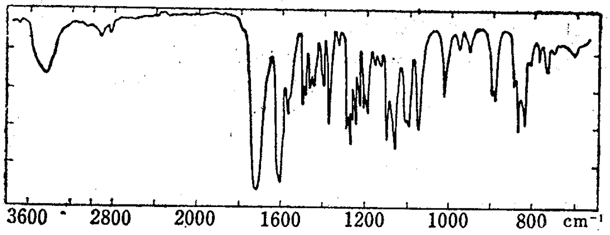

Fig. 1. Infrared Spectrum of Matsukaze-lactone (I) (in KBr)

The results of elemental analysis and determination of molecular weight by Akiya-Barger's method were consistent with the molecular formula $C_{20}H_{14}O_6$, which possessed two methoxyls. Hydrolytic titration indicated the presence of two lactone groups.

The infrared spectrum of I showed only one strong carbonyl band at 1724 cm^{-1}, and it was assumed that two lactones were present in forms of α-pyrone, possibly of coumarin (Fig. 1).

The ultraviolet spectrum of I in ethanol was similar to those of monomethoxycoumarins,2) and the absorption maxima were shifted to a longer wave length in alkaline solution3) (Fig. 2).

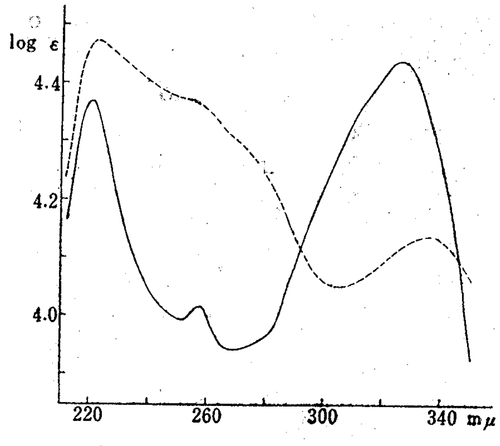

Fig. 2. Ultraviolet Spectra of Matsukaze-lactone (I)

──── in EtOH
------- in 0.01*N* NaOH-EtOH

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*1 A part of this work was presented at the Kanto Branch Meeting of the Pharmaceutical Society of Japan, Nov., 1961, Tokyo.

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1) T. Ohta, T. Miyazaki : Yakugaku Zasshi, 78, 1067(1958).

2) T. Nakabayashi, *et al.* : Yakugaku Zasshi, 73, 669 (1953).

3) A. J. Harle, L. E. Lyons : J. Chem. Soc., 1950, 1577.

Above mentioned evidences suggested that the compound (I) was a new coumarin, probably derived from two molecules of a monomethoxycoumarin by means of carbon-carbon coupling. Therefore, I was named matukaze-lactone. In order to confirm this presumption, the following reactions were carried out.

Methylation of I with dimethyl sulfate and methanolic alkali gave quantitatively an O-dimethyl ether (Ⅱa), $C_{22}H_{22}O_8$, m.p. 217~218°, which decolorized bromine in glacial acetic acid solution. Since the neutral equivalent of Ⅱa showed the presence of two carboxyl groups and its infrared spectrum had a α,β-unsaturated carboxyl band at 1690~1680 cm^{-1}, Ⅱa must correspond to a cinnamic acid derivative. When Ⅱa was methylated with diazomethane in ether, it gave a compound (Ⅱb), m.p. 131~132°, corresponding to $C_{24}H_{26}O_8$ which proved to be a dimethyl ester from the analytical data.

Oxidation of Ⅱa with potassium permanganate in alkaline solution at room temperature yielded two crystalline substances, $C_{18}H_{18}O_8$ (Ⅲa), m.p. 223~224°, and $C_{18}H_{18}O_6$ (Ⅳ), m.p. 136~136.5°.

Ⅲa was a dibasic aromatic acid containing a carboxyl band at 1662 cm^{-1} in the infrared spectrum. Methylation of Ⅲa was performed exactly by the same way as for Ⅱa. The product (Ⅲb), m.p. 161~163.5°, consisted of $C_{20}H_{22}O_8$.

The oxidation product (Ⅳ) was insoluble in sodium bicarbonate solution but easily reacted with *m*-nitrophenylhydrazine. The infrared spectrum of Ⅳ showing a conjugated carbonyl band at 1670 cm^{-1}, it was concluded that Ⅳ was an aromatic dialdehyde corresponding to Ⅲa. In fact, oxidation of Ⅳ with hot alkaline potassium permanganate solution gave Ⅲa.

Decarboxylation of Ⅲa by heating with copper powder in quinoline gave a neutral substance (Ⅴ), $C_{16}H_{18}O_4$, m.p. 107.5~108°, which showed no characteristic absorption in the infrared spectrum except of aromatic rings and methoxyl groups. Therefore, the compound (Ⅴ) was seemed to be one of the tetramethoxybiphenyl isomers. The ultraviolet spectrum of Ⅴ showed absorption maxima at 221.5, 248, and 280 mμ, and was similar to those of 2-methoxybiphenyl which has maxima at 247 and 284 mμ[4] (Fig. 3).

Then, Ⅴ was anticipated 2,2′,4,6′-tetramethoxybiphenyl described by Wachtmeister,[5] which was synthesized and found to be identical with Ⅴ by a mixed fusion and comparison of ultraviolet and infrared spectra.

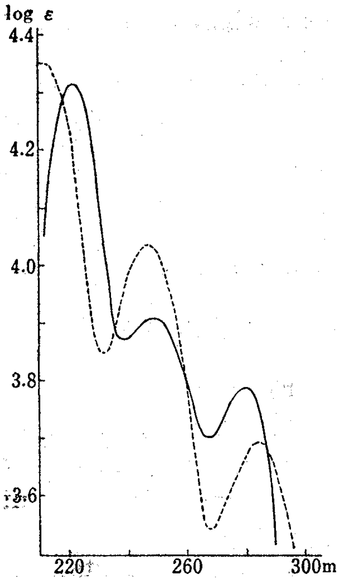
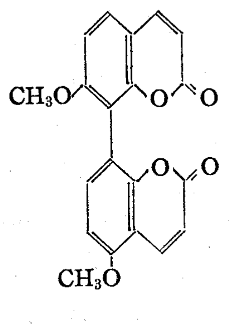
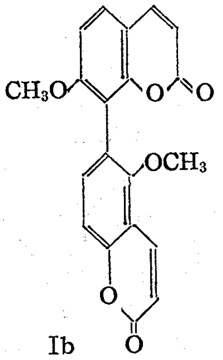


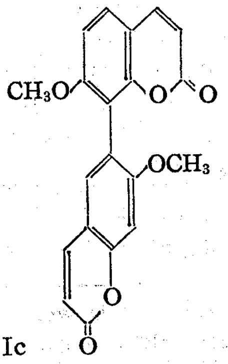
Fig. 3. Ultraviolet Spectra
——— Ⅴ in EtOH
------- 2-methoxybiphenyl in cyclohexane[4]



Ia



Ib



Ic

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4) R. A. Friedel, M. Orchin: "Ultraviolet Spectra of Aromatic Cmpounds," #177 (1951), John Wiley & Sons, Inc., New York.

5) C. A. Wachtmeister: Acta Chem. Scand., 8, 1440 (1954).

Based upon these results, formula (Ia), (Ib), and (Ic) are proposed for the reasonable structures of matsukaze-lactone (I) as above :

Experimental[*3]

Isolation of Matsukaze-lactone (I)——The air-dried pulverized leaves (2.45 kg.) of *B. albiflora* var. *japonica* which were collected at the Yabitsu Pass in Kanagawa Pref. on Sept. 5, 1960, were extracted with MeOH (15 L.) at room temperature for a month, and this process was repeated three times. The combined MeOH extracts were concentrated to a small volume (about 1 L.) and allowed to stand for several weeks. The dark green precipitates separated out were collected by filtration and repeatedly washed with Et_2O, and the greenish yellow crystals were obtained. Concentration of the mother liquor and the Et_2O washings gave a further quantity of the same material. The crystals thus obtained were combined and boiled with a large amount of Me_2CO. The insoluble matter was removed by filtration, and the filtrate was concentrated to a small volume. A pale yellow crystals separated out on standing were collected, dissolved in $CHCl_3$, and filtered. The filtrate was passed through a column of alumina to remove impurities. From the $CHCl_3$ eluates, there was obtained 7.40 g. of colorless crystals (0.302%). Recrystallization from Me_2CO gave matsukaze-lactone (I) as colorless prisms, m.p. 267~268°. $[\alpha]_D^{20}$ ±0° (c=2.2, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{14}O_6$: C, 68.57; H, 4.03; $2OCH_3$, 17.73; mol wt., 350.3. Found: C, 68.72; H, 4.31; OCH_3, 17.62; mol. wt., 329 (Akiya-Barger[*4]). UV λ_{max}^{EtOH} mμ (log ε): 220 (4.37), 257 (4.02), 326 (4.44). UV $\lambda_{max}^{0.01N\ NaOH-EtOH}$: 221 (4.48), 337 (4.13). IR ν_{max}^{KBr} cm^{-1}: 1724 (coumarin carbonyl), 1605, 1566, 1500, 831, 820 (aromatic ring), 1374, 1250, 1100 (methoxyl).

The same procedure was applied to the stems (1.34 kg.) collected at the same time, but I was not obtained.

Hydrolytic Titration of I——I (30.533 mg.) was dissolved in 0.5*N* KOH-EtOH (5.00 ml.) with slight warming. To the solution were added H_2O (10 ml.) and phenolphthalein (1 drop), and it was quickly titrated with 0.1*N* HCl (f=0.952) : 21.20 ml. Blank test with the same condition : 23.50 ml. The difference : 1.85 ml. Calcd. for dilactone : 1.83 ml.

Methylation of Matsukaze-lactone (I)——To a solution of I (2.00 g.) dissolved in Me_2CO (300 ml.) and H_2O (85 ml.) was added a portion (15 ml.) of KOH-MeOH (20 g. of KOH was dissolved in MeOH to make 300 ml.), and the mixture was refluxed for 20 min., during which time the color became pale yellow. $(CH_3)_2SO_4$ (43.3 g.) and the remaining portion of KOH-MeOH (285 ml.) were alternately added under reflux with stirring for 3 hr. After addition of a concentrated aqueous solution of KOH (10 g.), it was further refluxed. The filtered Me_2CO solution was concentrated to a small volume and acidified with HCl under ice cooling. After standing overnight in a refrigerator, the pale yellow crystals separated out were collected, washed with H_2O, and dried. The crude acid (2.40 g.) thus obtained was recrystallized from MeOH to afford Ⅱa as pale yellow prisms, m.p. 217~218° (decomp.). *Anal.* Calcd. for $C_{22}H_{22}O_8$: C, 63.76; H, 5.35; neutralization equivalent, 207.2. Found : C, 63.36; H, 5.14; neut. equiv., 212. UV λ_{max}^{EtOH} mμ (log ε) : 229 (4.44), 263 (4.47). IR ν_{max}^{KBr} cm^{-1} : 1690~1680 (α,β-unsaturated carbonyl).

Methylation of IIa——Ⅱa (100 mg.) was treated with diazomethane by the usual way. The product dissolved in $CHCl_3$ was chromatographed on alumina. The residue from main fractions was recrystallized from 70% EtOH to afford Ⅱb as colorless needles, m.p. 131~132°. *Anal.* Calcd. for $C_{24}H_{26}O_8$: C, 65.15; H, 5.92. Found : C, 66.25; H, 6.32. IR ν_{max}^{KBr} cm^{-1} : 1719 (ester carbonyl).

Permanganate Oxidation of IIa——To a stirred solution of Ⅱa (2.40 g.) in 10% Na_2CO_3 (24 ml.) was added dropwise $KMnO_4$ (5.4 g.) in H_2O (192 ml.) at room temperature for 4 hr. After stirring for further 1 hr., the mixture was filtered, and the residue was washed with H_2O. Acidification of the filtrate and washings with HCl gave a yellow precipitate (0.78 g.), which was recrystallized from MeOH to afford Ⅲa as colorless prisms, m.p. 223~224° (decomp.). *Anal.* Calcd. for $C_{18}H_{18}O_8$: C, 59.66; H, 5.01; neut. equiv., 181.3. Found : C, 59.54; H, 4.51; neut. equiv., 180.2. UV λ_{max}^{EtOH} mμ (log ε) : 226 (4.47), 285 (3.85). IR ν_{max}^{KBr} cm^{-1} : 1662 (carboxyl conjugated with aromatic ring).

The residual MnO_2 was repeatedly extracted with hot Me_2CO and the combined extracts were evaporated to dryness. An oily residue was purified by passing through a column of alumina as a $CHCl_3$ solution. From the $CHCl_3$ eluates, there was obtained a colorless oil, which crystallized by addition of a small amount of MeOH. Recrystallization from EtOH gave colorless prisms (Ⅳ), m.p. 136~136.5°. Yield, 160 mg. It does not dissolve in $NaHCO_3$ solution. *Anal.* Calcd. for $C_{18}H_{18}O_6$: C, 65.44; H, 5.49. Found : C, 65.38; H, 5.55. UV λ_{max}^{EtOH} mμ (log ε) : 240 (4.45), 276 (4.43). IR ν_{max}^{KBr} cm^{-1} : 1670 (conjugated carbonyl).

Ⅳ was easily reacted with *m*-nitrophenylhydrazine by the usual way and gave yellowish red needles, m.p. ca. 195°, but purification of this material was unsuccessful.

[*3] All melting points are uncorrected.

[*4] Rast's method was unsatisfactory.

Permanganate Oxidation of IV——To a suspension of IV (50 mg.) in H_2O (1.5 ml.) was added dropwise an aqueous solution of $KMnO_4$ (32 mg. in 0.65 ml.) with stirring at 70° for 1.5 hr. After addition of 10% NaOH(1 ml.), the reaction mixture was filtered, and the residual MnO_2 was washed three times with hot H_2O. The filtrate and washings were acidified and allowed to stand in a refrigerator. The precipitate was collected, washed with H_2O, and recrystallized from MeOH to afford pale yellow crystals (14.4 mg.), m.p. 222° (decomp.). It was identical with IIIc by a mixed fusion and comparison of IR spectra (in KBr).

Methylation of IIIa——IIIa (100 mg.) was methylated by the same way as for IIa. Recrystallization from 50% EtOH gave IIIa as colorless prisms, m.p. 161~163.5°. Yield, 98 mg. *Anal.* Calcd. for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 63.16; H, 6.28. IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1702 (ester carbonyl).

Decarboxylation of IIIa——A mixture of IIIa (100 mg.), freshly distilled quinoline (3 ml.), and Cu powder (300 mg.) was refluxed for 4 hr. After cooling, the content was diluted with Et_2O, and the Cu powder was removed by filtration. The filtrate was added dropwise into a mixture of conc. HCl (4 ml.) and H_2O (6 ml.) with stirring and while cooling. The aqueous layer separated out was further extracted with Et_2O, and the combined ethereal layer was washed three times with 5% H_2SO_4, dried over anhyd. Na_2SO_4, and evaporated to dryness. The brownish oily residue was purified by passing through a column of alumina as a $CHCl_3$ solution. Recrystallization from EtOH gave V as a colorless needles, m.p. 107.5~108°. Yield, 32 mg. *Anal.* Calcd. for $C_{16}H_{18}O_4$: C, 70.05; H, 6.61. Found: C, 70.12; H, 6.27. UV $\lambda_{\max}^{\text{EtOH}}$ mμ (log ε): 221.5 (4.31), 248 (3.91), 280 (3.79). IR $\nu_{\max}^{\text{KBr}}$ cm^{-1}: 1617, 1594, 1517, 822, 782, 728 (aromatic ring), 1248, 1174. 1110, 1030 (methoxyl). This compound was identified as 2,2′,4,6′-tetramethoxybiphenyl, which was synthesized by condensation of 2-iodo- and 4-iodoresorcinol dimethyl ether according to Wachtmeister,[5] by a mixed fusion and comparison of UV and IR spectra.

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Summary

From the methanolic extract of the leaves of *Boenninghausenia albiflora* Meissner var. *japonica* S. Suzuki, a new coumarin, named matsukaze-lactone (I), $C_{20}H_{14}O_6$, m.p. 267~268°, was isolated. Methylation of I with dimethyl sulfate and methanolic alkali gave an acid (IIa), $C_{22}H_{22}O_8$, which was oxidized to an acid (IIIa), $C_{18}H_{18}O_8$. By decarboxylation of IIIa with copper powder, 2,2′,4,6′-tetramethoxybiphenyl (V) was obtained. From these results, the structural formula (Ia), (Ib) and (Ic) were proposed for matsukaze-lactone.

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