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On the Constituents of the Unripe Fruits of *Osmanthus fragrans* L. I

By

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From the unripe fruits of *Osmanthus fragrans* L. 2-(4-hydroxyphenyl)ethanol, succinic acid, 1-0-β-D-glucosyl-(4-hydroxyphenyl)ethanol, and a caffeic ester were isolated.

Introduction and Results

Several investigations have been reported about the constituents of the leaves[1] and flowers[2] of *Osmanthus fragrans* L. var aurantiacus M. However, there has been no reprot about the same genus, *Osmanthus fragrans* L. (Japanese name: Usugimokusei). And the bitter taste of the unripe fruits of this plant stimulated us to study the chemical constituents of them. This paper describes the isolation and identification of non-bitter constituents from the unripe fruits of the plant. The Fig. 1 showed the procedure of isolation.

A was crystallized as colorless needles, mp 93.5–94°, from chloroform. The molecular formula, $C_8H_{10}O_2$, was assigned on the basis of the elementary analysis and the mass spectrum. The IR spectrum of **A** showed absorption bands at 3400cm^{-1} due to an alcoholic hydroxyl group, 3140 cm^{-1} due to a phenolic hydroxyl group, and 1600, 1518, and 820 cm^{-1} due to a p-substituted phenyl group. The NMR spectrum (CD_3COCD_3) included the signals for an alcoholic hydroxyl group at δ 3.13 (1H, br s), a phenolic hydroxyl group at δ 8.23 (1H, br s), and a -$C\underline{H}_2C\underline{H}_2$- group at δ 2.73 (2H, t, J=7 Hz) and 3.73 (2H, t, J=7 Hz). Furthermore, an A_2' B_2' system at δ 6.77–7.27 suggested the presence of a p-substituted phenyl group. The above data indicated that **A** was 2-(4-hydroxyphenyl)ethanol.[2b].

B was crystallized as colorless prisms, mp 168–169°C, from ethyl acetate. The IR spectrum showed a band at 1700cm^{-1} ascribed to a carboxyl group. **B** was identified as succinic acid by comparing its IR spectrum with that of an authentic sample.

C, colorless prisms, mp 162–163°, has molecular formula, $C_{14}H_{20}O_7$, established by mass spectrum (high resolution). It gave green color with alcoholic ferric chloride. The

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IR spectrum of **C** showed bands attributable to a hydroxyl group at 3200 cm^{-1} and a p-substituted phenyl group at 1620, 1600, 1518 and 820 cm^{-1}. The NMR spectrum (C_5D_5N) showed the presence of a phenolic hydroxyl group at δ 9.20 (1H, s), a -C$\underline{H}_2$-CH_2- group at δ 3.08 (2H, t, J=7 Hz), and four aromatic protons at δ 7.48–7.55.

Hydrolysis of **C** with 4N-hydrochloric acid yielded a crystalline compound, $C_8H_{10}O_2$, mp 93°, and D-glucose which was confirmed by paper chromatography. The IR spectrum of the former compound was identical with that of **A**, 2-(4-hydroxyphenyl) ethanol. These data suggested that **C** was 1-0-D-glucosyl-2-(4-hydroxyphenyl) ethanol. In addition, the coupling constant (J=7 Hz) of a doublet at δ 5.08 due to the anomeric poroton in the NMR spectrum of **C** revealed that D-glucose was linked β-orientation[3].

From these data **C** could be assigned as 1-O-β-D-glucosyl-2-(4-hydroxyphenyl) ethanol.[4]

D was isolated as an amorphous compound. It exhibited a broad hydroxyl band at 3400 cm^{-1}, phenyl bands at 1600 and 1520 cm^{-1}, and a conjugated ester band at 1700 cm^{-1} in the IR spectrum.

Acetylation of **D** with acetic anhydride and pyridine gave an amorphous compound, which seemed to be pure on thin-layer chromatogram, although all attempts to crystallize it failed. The NMR spectrum ($CDCl_3$) of the acetate showed signals due to a C$\underline{H}_3$CH- group at δ 1.07 (3H, d, J=7 Hz), eight acethyl protons at δ 1.92, 2.00, 2.08, 2.15 (3H×5, s) and 2.37 (3H×3, s), aromatic protons at δ 7.35–7.67 (6H), and conjugated trans olefinic protons at δ 6.42 (1H, d, J=18 Hz) and 8.02 (1H, d, J=18 Hz).

Methanolysis of **D** with methanolic hydrogen chloride afforded a methyl ester, mp 162°. The NMR spectrum (CD_3COCD_3) of the latter compound showed a singlet at δ 3.73 (3H) due to carbomethoxy protons, two doblets at δ 6.33 (1H, d, J=18 Hz) and 7.67 (1H, d, J=18 Hz) due to conjugated trans olefinic protons, a multiplet of three protons at δ 6.87–7.25 due to three protons of a 1,2,4-trisubstituted phenyl group, and a broad singlet at δ 8.43 due to two phenolic hydroxyl protons. These chemical and physical properties of the methyl ester were in good agreement with those of methyl caffeate.

On the other hand, the alcoholic part of **D** was obtained as methyl ether by permethylation followed by hydrolysis with alkali. The IR spectrum of methyl ether indicated absorption bands of a hydroxyl group at 3400 cm^{-1}, and a phenyl group at 1590 and 1510 cm^{-1}, while in the NMR spectrum ($CDCl_3$) the signals of C$\underline{H}_3$CH- group at δ 1.29 (3H, d, J=6 Hz), six methoxy protons at δ 3.42 (3H, s), 3.48 (3H, s), 3.53 (3H, s), 3.57 (3H, s), and 3.88 (6H, s), and aromatic protons at δ 6.89 (3H, s) were observed.

These data suggest that **D** is caffeate of an alcohol with six hydroxyl groups and a phenyl group. Further investigation is in progress.

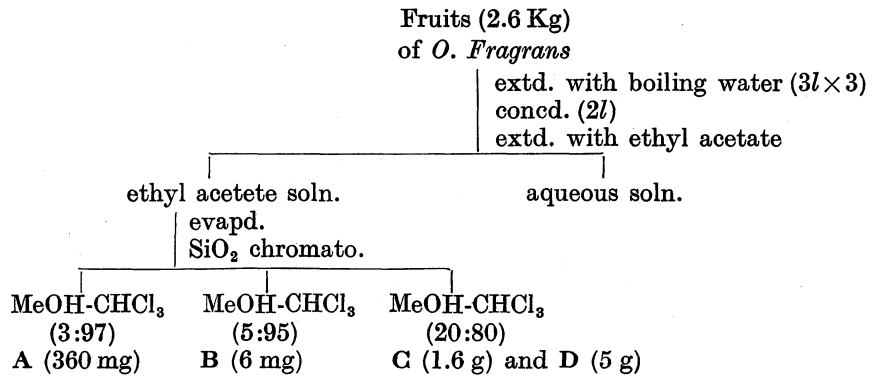

Fig. 1. Isolation of the compounds

Experimental

Melting points were determined on a Yanagimoto Micro Melting Point Apparatus, and uncorrected. IR spectra were measured as Nujol mull with a Schimadzu IR-27 Recording Infrared Spectrometer or as KBr with a Nippon Bunko IR-S Spectrometer. NMR spectra were recorded on a JEOL MH-60 Spectrometer. Chemical shfits are given in δ values with TMS as the internal standard.

Extraction The unripe fruits of *Osmanthus fragrans* (2.6 Kg) were extracted with boiling water (3*l*×3). The combined extracts were evaporated under reduced pressure to 2*l*, and the resulting solution was extracted continuously with ethyl acetate. The extract was dried over sodium sulfate, and the solvent was evaporated to give a brown residue (31.4 g) *in vacuo*.

Isolation of **A, B, C** and **D** *from the residue.* The residue was chromatographed on a column of silicic acid.

Elution with 3% methanol in chloroform and recrystallization from chloroform gave colorless needles (**A**, 360 mg), mp 93.5–94°, IR ν_{max}^{nujol} cm^{-1}: 3400, 1600, 1518, 820; NMR (CD_3COCD_3) δ: 2.73 (2H, t, J=7 Hz), 3.73 (2H, t, J=7 Hz), 6.77–7.27 (4H, $A_2'B_2'$–q). *Anal.* Calcd. for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.46; H, 7.26.

Elution with 5% methanol in chloroform and recrystallization from ethyl acetate gave colorless prisms (**B**, 6 mg), mp 168–169°. IR ν_{max}^{KBr} cm^{-1}: 1700, 1420, 920. The IR spectrum was superimposable on that of succinic acid.

Elution with 20% methanol in chloroform and recrystllization from ethanol gave colorless prisms (**C**, 1.6 g), mp 162–163°, IR ν_{max}^{nujol} cm^{-1}: 3200, 1620, 1600, 1518, 820; NMR (C_5D_5N) δ: 3.08 (2H, d, J=7 Hz), 3.83–4.73 (13H) 5.08 (1H, d, J=7 Hz), 7.48–7.55 (4H), 9.20 (1H, s). Mass Spectrum m/e M^+300.1188 (Calcd. for $C_{14}H_{10}O_7$: 300.1207).

Further elution with 20% methanol in chloroform gave an amorphous substance (**D**, 5 g), IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1600, 1520, 820; NMR (CD_3COCD_3) δ: 1.13 (3H, d, J=6 Hz), 6.52 (1H. d, J=17 Hz), 7.93 (1H, d, J=17 Hz).

Hydrolysis of **C** **C** (102 mg) was dissolved in methanol (6 ml) and 4N- hydrochloric

acid (6 ml) was added. The solution was heated under reflux for 6 hr and was extracted with ether. The extract was washed with water, dried and the solvent was removed to give a crystalline material (28 mg), mp 93°, IR ν_{max}^{KBr} cm^{-1}: 3400, 1600, 1518, 820. *Anal.* Calcd. for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.43; H, 7.28. The IR spectrum was identical with that of 2-(4-hydroxyphenyl) ethanol. The aqueous solution was evaporated to dryness under reduced pressure and the residue was dissolved in a small amount of water. The presence of glucose in the residue was confirmed by paper chromatography.

Acethylation of **D D** (100 mg) was acethylated with acetic anhydride (2 ml) and pyridine (2 ml). The crude product was chromatographed on a coloum of silicic acid (15 g). Elution with 2% methanol in chloroform gave an amorphous material (42 mg), IR ν_{max}^{nujol} cm^{-1}: 1760, 1640, 1500, 1200; NMR ($CDCl_3$) δ: 1.07 (3H, d, J=7 Hz), 1.92, 2.00, 2.08, 2.15 (3H×5, s), 2.37 (3H×3, s), 6.42 (1H, d, J=18 Hz), 7.37–7.67 (6H), 8.02 (1H, d, J=18 Hz).

Methanolysis of **D D** (2.5 g) was dissolved in methanol (15 ml) saturated with hydrogen chloride. The solution was stirred for 18 hr at 10°, and then diluted with water and extracted with ether. The extract was washed with water, dried and the solvent was removed to give an amorphous material. The crude material was chromatographed on a column of silicic acid (75 g). Elution with 5% methanol in chloroform and recrystallization from water gave colorless needles, mp 162°, IR ν_{max}^{KBr} cm^{-1}: 3480, 3080, 1660, 1540; NMR (CD_3COCD_3) δ: 3.73 (3H, s), 6.33 (1H, d, J=18 Hz), 6.87–7.25 (3H, m), 7.67 (1H, d, J=18 Hz), 8.43 (2H, br s). Mass Spectrum m/e M^+194.0558 (Calcd. for $C_{10}H_{10}O_4$: 194.0568).

Permethylation of **D** *followed by alkali hydrolysis* Sodium hydride (500 mg) was heated with dimethyl sulfoxide (10 ml) at 70° under nitrogen. To this solution, **D** (300 mg) in dimethyl sulfoxide (5 ml) was added and the mixture was stirred for 1 hr and added with methyl iodide (3 ml). The reaction mixture was stirred further for 3 hr at 70°, and poured into ice water, and extracted with ether. The extract was washed with water, dried and the solvent was removed to give a crude material. The crude material was chromatographed on a column of silicic acid (10 g). Elution with 2% methanol in chloroform gave an oily material (220 mg). The oily material was dissolved in methanol (5 ml) and 2N-sodium hydroxide (5 ml). The solution was refluxed for 2 hr, and was diluted with water and extracted with ether. The extract was washed with water, dried, and the solvent was removed to give an oily material (66 mg). The oily material was chromatographed on a column of silicic acid (5 g). Elution with chloroform gave an oily material (38 mg), IR ν_{max}^{KBr} cm^{-1}: 3400, 1590, 1510; NMR ($CDCl_3$) δ: 1.29 (3H, d, J=6 Hz), 3.42, 3.48, 3.53, 3.57 (each 3H, s), 3.88 (6H, s), 6.89 (3H, s).

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