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81. Hiroshi Mitsuhashi and Tsuneo Itoh : Studies on the Constituents of Umbelliferae Plants. VI.*1 Studies on the Constituents of *Angelica edulis* MIYABE. (2).

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In the course of a research on anti-cholinergic activity of the component of Umbelliferae plants, a new compound was isolated from the root of *Angelica edulis* MIYABE and named edultin.*1 The present paper deals with the chemical structure of edultin.

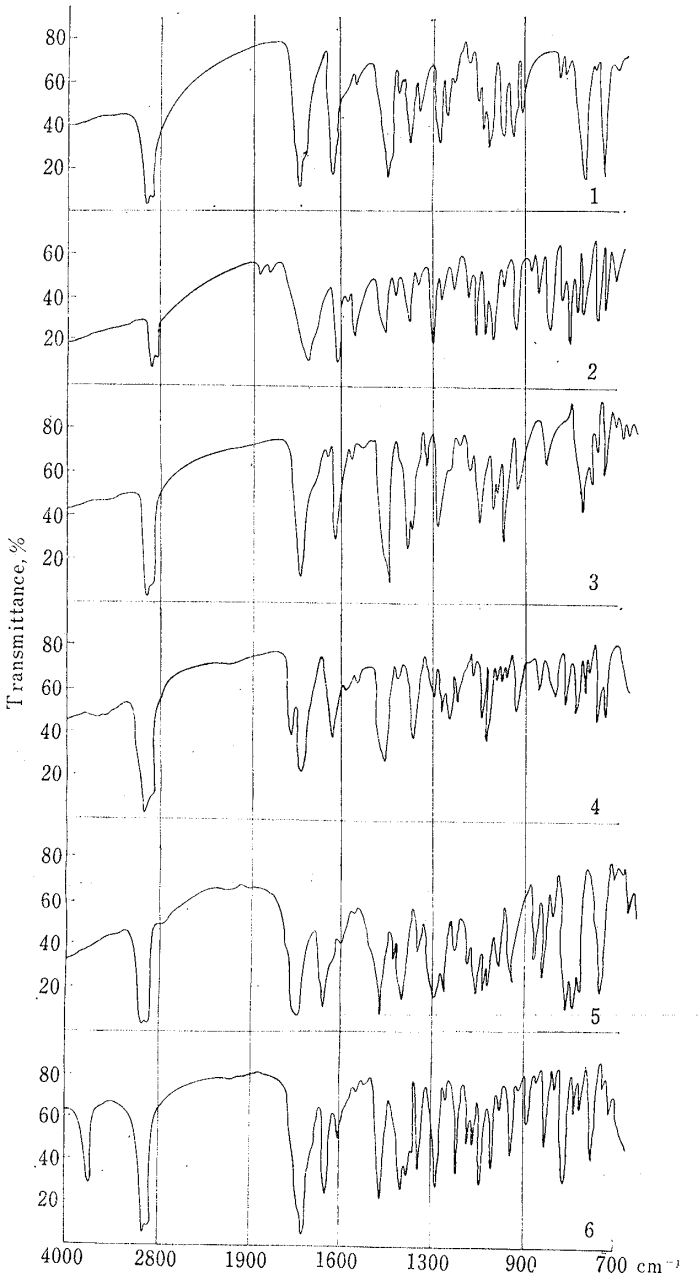

Fig. 1. Infrared Spectra
1 Angelicine
2 Oroselone
3 Oroselol methyl ether
4 Substance, m.p. 204°
5 Oroselol acetate
6 Oroselol

*1 Part V : This Bulletin, **10**, 511 (1962). Preliminary Communication, H. Mitsuhashi, T. Itoh This Bulletin, **9**, 170 (1962). Paper presented at the Hokkaido local meeting of the Pharmaceutical Society of Japan, January 1961.

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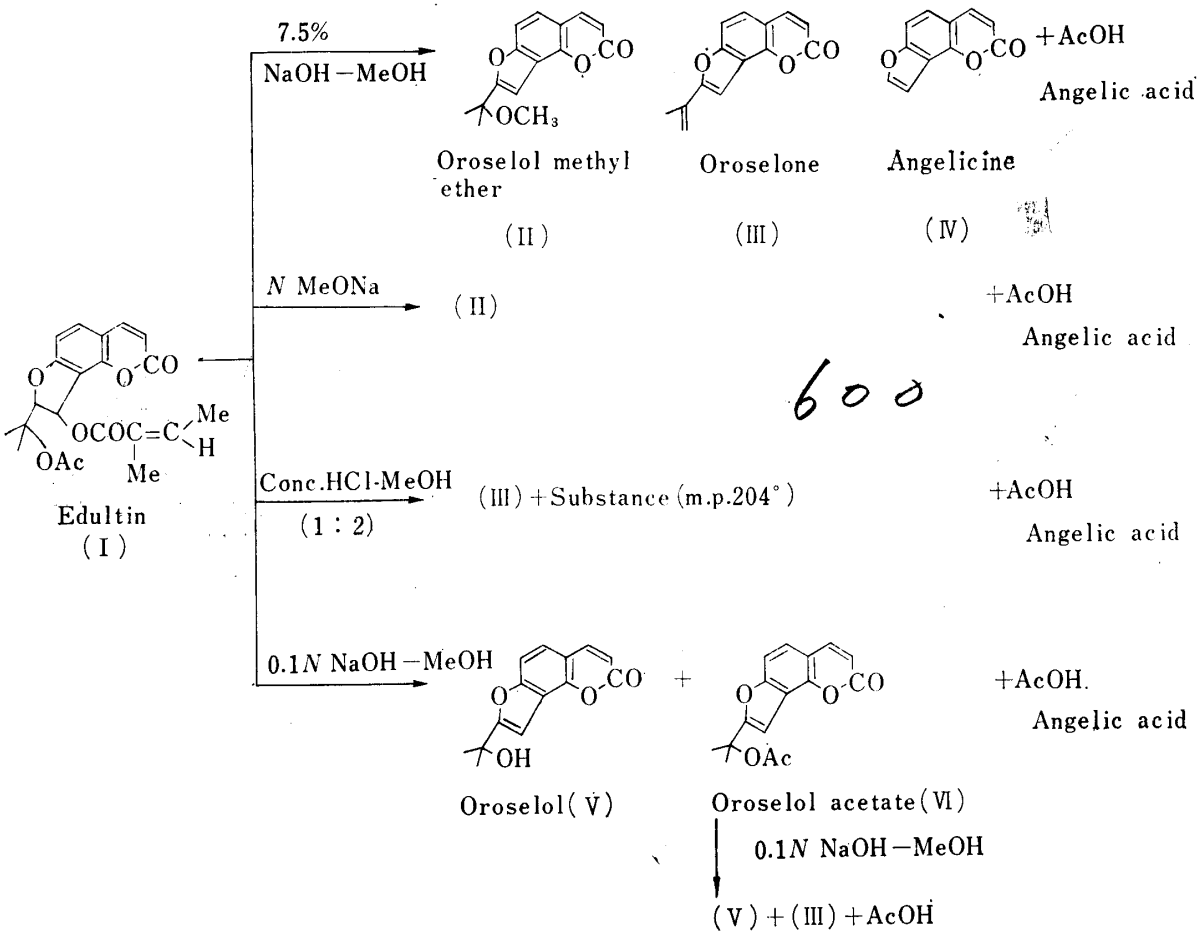


Chart 1.

Analytical values of this compound (I) suggested the molecular formula of $C_{21}H_{22}O_7$ apparently involving ester groupings and a coumarin ring.

(I) was easily hydrolyzed by 7.5% sodium hydroxide in methanol to give acids (A) and amorphous white compounds as the neutral portion (B). The acid fraction (A), when kept in a refrigerater, deposited crystals, m.p. 45°, the infrared spectrum of which showed absorption bands at 2800~2600 (COOH), 1680, 1634 (-C=C-CO-), 930 (COOH) cm^{-1} in Nujol.

The melting point and infrared spectrum suggested that this acid would be angelic acid, and this was confirmed by reduction of this compound to 2-methylbutyric acid, and also by admixture with the authentic angelic acid. The crude acid portion was submitted to paper chromatography and two spots weredetected on the paper chromatogr am, one was found to be identical with acetic acid and the other, angelic acid.[1]

The neutral portion (B) was further purified by column chromatography using silica gel, whereby three compounds, (II) m.p. 118°, (III) m.p. 180.5° and (IV) m.p. 140°, were isolated. From the results of elemental analysis and molecular weight determination, these compounds corresponded to $C_{15}H_{14}O_4$ (II), $C_{14}H_{10}O_3$ (III) and $C_{11}H_6O_3$ (IV), respectively. (II) is insoluble in aqueous alkali but readily dissolves in alcoholic alkali with a stable yellow color. This solution precipitated the starting material on acidification, its behavior towards alkaline solution suggested the presence of a coumarin neucleus in (II). By (II) closely resembles oroselol methyl ether in chemical properties as compared with the degradation products of athamantin isolated from *Athamanta oroselium* L. (*Peucedanum*

1) E. Späth, *et al.*: Ber., **66**, 1150 (1933).

oreoselium MÖNCH).[2~4] The ultraviolet spectrum of (II) exhibits absorption maxima at 251 (ε 26,100) and 299 mμ (ε 9,350), which closely resembles that of oroselol methyl ether The identity was established by mixed melting point determination with authenticoroselol methyl ether kindly provided by professor Dr. H. Schmid.

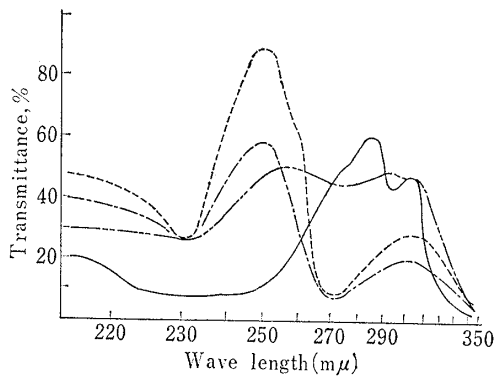


Fig. 2. Ultraviolet Absorption Spectra (in EtOH)
- ····· Oroselol methyl ether
- — — Angelicine
- — · — Substance m.p. 204
- ——— Oroselone

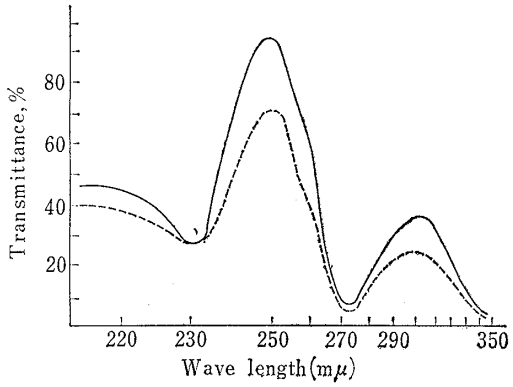


Fig. 3. Ultraviolet Absorption Spectra
- ——— Oroselol
- ····· Oroselol acetale

The compound (III) showed absorptions at 1720, 1620, and 1450 cm^{-1} corresponding to a coumarin ring in the infrared spectrum, and absorption maxima at 286 and 299 mμ in the ultraviolet spectrum. This compound was then shown to be identical with an authentic sample of oroselone.[3]

Infrared spectra of (IV) exhibited the characteristic absorption bands of furocoumarin in the region of 1740, 1620, 1590, 1460 and 885 cm^{-1}. (IV) was proved to be identical with angelicine. On the concentration of the solution from alkaline saponification of (I), a sort of alkali fusion took place and angelicine was obtained.

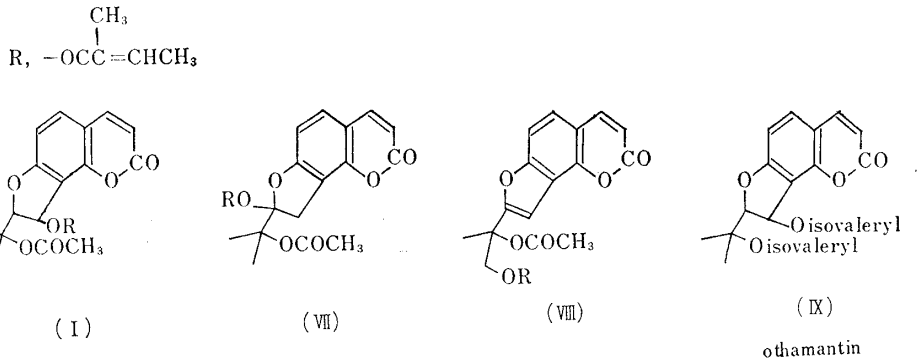
Hydrolysis of edultin (I) with sodium methoxide by the method of H. Schmid[4] gave (II).

Acid hydrolysis of (II) with a mixture of conc. hydrochloric acid-methanol (1:2) for 30 minutes afforded (III) and a substance of m.p. 204°, which is not now identified. Its infrared and ultraviolet spectra are shown in Figs. 1. and 2. Further investigation on the constitution of this substance is now in progress.

The neutral fractions obtained from the hydrolysate of (I) under various conditions, did not contain hydroxyl group. Therfore, partial hydrolysis of (I) with 1/10*N* sodium hydroxide in methanol at 40~50° for 3 hours was carried out, and oroselol (V) and oroselol acetate (VI) were obtained. The infrared spectrum of (V) exhibited the hydroxyl group (Figs. 1 and 3). The analytical value of (VI) indicated that this compound is the acetate of (V). The foregoing results suggested that every possible structure for (I) was reduced to three formula as shown in Chart 2. The structure (VIII) has difficulties in explaining the formation of (II) and (V) as the hydrolysis products.

Next, the fact that angelic acid was obtained as a sole product by the partial hydrolysis of edultin, indicates that C-8 or C-9 position of edultin is substituted with an angeloxy group. The C-8 of edultin, however, lacks the semiketal behavior, so that the formula

2) G. Schnedermann, F. L. Winckler : Ann.., **51**, 315 (1844).
3) E. Späth, N. Platzer, H. Schmid : Ber., **73**, 709 (1940); *Ibid.*, **73**, 1309 (1940).
4) O. Halpern, P. Wasser, H. Schmid : *Ibid.*, **40**, 758 (1957).
5) E. Späth, O. Pesta : *Ibid.*, **67**, 853 (1934).



(I) (VII) (VIII) (IX)

othamantin

Chart 2. Deducing Structural Formula of Edultin

(VIII) is inconceivable for edultin. In comparison with the investigation on the structure of athamantin (IX) by Schmid,[3~4] 8-(1-acetyloxyisopropyl)-9-angeloyloxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one (I) is more preferable than (VII) or (VIII) for edultin.

Experimental*3

Hydrolysis of Edultin (I)——A solution of 4.16 g. of (I) in 80 cc. of 7.5% NaOH-MeOH was refluxed on a boiling water bath for 45 min. After cool, 30 cc. of H_2O was added, and the MeOH was evaporated *in vacuo*. The alkaline aqueous reaction mixture was acidified with dil. HCl, saturated with NaCl and extracted five times with Et_2O. The combined Et_2O extracts were washed with 5% $NaHCO_3$, H_2O, dried over Na_2SO_4, and the solvent was evaporated to leave a crystalline mass (2.057 g.). The 5% $NaHCO_3$ aqueous layer left after Et_2O extraction was acidified with dil. HCl, and extracted with Et_2O. Upon removal of the solvent a pale yellow oily substance was obtained; yield, 0.609 g.

Acid Fraction (A)——The acids, obtained on hydrolysis of (I), was allowed to stand in a refrigerator, and crude crystals were obtained. Further purification of the crystals was performed by microsublimation *in vacuo* (80~120°, 3~5 mm. Hg); yield, 170 mg, m.p. 45.3°. *Anal.* Calcd. for $C_5H_8O_2$; C, 59.98; H, 8.05. Found: C, 59.72; H, 8.14. No depression was observed when this acid was admixed with authentic angelic acid.

A mixture of 0.411 g. of angelic acid from (I) and Pd-C (20 mg. of $PdCl_2$ and 200 mg. of Norit) in 10 cc. of H_2O was shaken in H_2 atomosphere, under ordinary pressure absorbing 118.9 cc. of H_2 in 18 hr. (theoretical amount 99 cc.) After filtration, the solution were saturated with NaCl and evaporated to leave an oily substance (0.205 g.). The amide showed no depression of melting point on admixture with the corresponding synthetic 2-methylbutyramide.

Paper Chromatography——Using the ascending technique, Toyo Roshi No. 50 paper, and the solvent system of BuOH saturated with 1.5*N* NH_4OH were used. The dried chromatograms were detected with BTP, and the Rf values obtained were as follows: natural acids, 0.085 and 0.292; authentic acetic acid 0.084; angelic acid 0.293.

Column Chromatography of Neutral Portion (B)——i) 2.0 g. of the neutral portion (B) was chromatographed over 130 g. of silicagel (Mallincrodt, 100 mesh. for chromatography). Benzene-AcOEt (85:15) was used for elution, and each eluante was 15 cc. The results are: fraction No. 5, 20 mg. of crystals (III), m.p. 178~180°, fraction Nos. 7 and 8, 80 mg. of the crude (IV), m.p. 114~125°, and fraction Nos. 11~12, 250 mg. of (II), m.p. 113~114°.

ii) Detection of oroselol methyl ether (II): The crystals (II) were recrystallized from petr. benzine to give white needles, m.p. 118°, which showed no depression of melting point on mixed fusion with an authentic specimen. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.47; mol. wt. 258. Found: C, 69.77; H, 5.44; mol wt. 252. IR ν_{max}^{Nujol} cm^{-1}: 1730, 1720 (δ-lactone), 1620 (C=C), 1580, 1450 (benzene ring).

iii) Oroselone (III): The product (III) was recrystallized from benzene to give white needles, m.p. 180.5°. *Anal.* Calcd. for $C_{14}H_{10}O_3$: C, 74.33; H, 4.46. Found: C, 74.34; H, 4.39. On admixture with an authentic specimen of oroselone, no melting point depression was observed.

*3 All melting points are uncorrected. Ultraviolet spectra were run in EtOH using Shimazu RS-27-type self-recording spectrophotometer. Infrared spectra were measured with Koken DS-301-type infrared spectrophotometer.

iv) angelicine (IV): White needles, m.p. 140°, upon recrystallization from a mixture of benzene and petr. benzine, were obtained. *Anal.* Calcd. for $C_{11}H_6O_3$: C, 65.27; H, 5.74; mol. wt. 186.16. Found: C, 65.47; H, 5.75; mol. wt. 198. UV λ_{max}^{EtOH} mμ (ε): 228 (4,650), 249 (13,490), 267 (1,160), 300 (4,650).

Hot Hydrolysis with Sodium Methoxide——A mixture of 600 mg. of (I) with *N* MeONa (915 cc.) was refluxed for 3 hr. on a water bath, and then acidified with dil. HCl. After further treatments according to the hot hydrolysis of athamantin, only oroselol methyl ether (m.p. 113°) was obtained as white needles after recrystallization from petr. benzine. Yield, 294 mg. No depression of melting point was observed when admixed with authentic oroselol methyl ether.

Hydrolysis of (I) with Hydrochloric Acid in Methanol——A solution of 3.0 g. of (I) dissolved in 110 cc. of MeOH-conc. HCl (2:1) was heated for 2 hr. on a water bath, cooled, and then H_2O was added. The reaction mixture was concentrated *in vacuo* at room temperature, precipitating white crystals. The solution was washed with H_2O; yield, 1.9 g. The crystalline products were separated by means of column chromatography using silica gel as an adsorbent and $CHCl_3$-AcOEt (9:1) as the developing solvent. Fraction Nos. 10～13 gave 600 mg. of crystals m.p. 184～208°. The crystals from fraction Nos. was recrystallized several times from $CHCl_3$ to white needles, m.p. 204° (decomp. moist. at 194°).

Partial Hydrolysis of (I)——A mixture of 10 g. of (I) in 27.5 cc. of 1/10*N* NaOH in MeOH was kept for 3 hr. at 40～50° with stirring. The solution turned gradually pale yellow and the reaction mixture was treated as in the case of hydrolysis of edultin mentioned above. From the acid fraction AcOH and angelic acid were detected. By column chromatography of a neutral substance (450 mg.) using silica gel (22.5 g.) and benzene-Me_2CO (9:1), oroselol acetate (IV) and oroselol (V) were obtained.

i) Oroselol (V): white needles from benzene, m.p. 149～151°(157～158°)).[4] *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95. Found: C, 69.17; H, 4.83. UV λ_{max}^{EtOH} mμ (ε): 231 (9,450), 252 (27,700), 273 (4,000), 301 (10,400). IR ν_{max}^{Nujol} cm^{-1}: 1730, 1720, 1615, 1600, 1460 (coumarinring).

ii) Oroselol acetate (VI): white needles from MeOH, m.p. 149～151°. *Anal.* Calcd. for $C_{16}H_{14}O_5$: C, 67.12; H, 4.93. Found: C, 66.98; H, 5.03. UV λ_{max}^{EtOH} mμ (ε): 231 (7,500), 252.5 (24,700), 247 (3,200), 301 (8,600). IR ν_{max}^{Nujol} cm^{-1}: 1730, 1720, 1615, 1600, 1455 (coumarin ring), 1280 (ester).

iii) Hydrolysis of oroselol acetate (VI): (VI) was hydrolysed by heating with 3% NaOH in MeOH for 75 minutes on a steam bath. From the reaction mixture, oroselol (V), oroselone (III) and AcOH (by paper chromatography) were detected.

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Summary

The structure of edultin (I), which was isolated from the root of *Angelica edulis* MIYABE was investigated. The acidic fraction obtained by hydrolysis of (I), was found to be a mixture of acetic acid and angelic acid. By hydrolysis of (I) under various conditions, (II)～(V), and (VI) were identified as the neutral fraction. The complete structure of edultin was established as 8-(1-acetyloxyisopropyl)-9-angeloyloxy-2*H*-[2,3-*h*]-1-benzopyran-2-one.

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