[Chem. Pharm. Bull. 21(3) 518—522 (1973)]

UDC 547.587.51.02 : 581.192

New Ester Coumarins, Angeladin and Isoedultin from *Angelica longeradiata* (Maxim.) Kitagawa

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(Received August 22, 1972)

The roots of *Angelica longeradiata* (Maxim.) Kitagawa afforded two new ester coumarins, angeladin (III) and isoedultin (IV) together with known coumarins, archangelicin (I) and umbelliferone (II). The structures of III and IV were established as 2′(*S*),3′(*R*)-O-angeloyl-3′-*p*-coumaroyloxy-2′,3′-dihydrooroselol and 2′(*S*),3′(*R*)-O-angeloyl-3′-acetoxy-2′,3′-dihydrooroselol, respectively.

Angelica longeradiata (Maxim.) Kitagawa (Umbelliferae) (Japanese name, tsukushi-zeri) is a perennial herb growing in mountainous region of Kyushu, especially in Mt. Kuju, and its chemical investigation has so far not been made. As a part of the continuing studies of the coumarins from the Umbelliferous plants, the authors have made the investigation of the roots of this plant, and have been able to isolate two new ester coumarins, named as angeladin (III) and isoedultin (IV), together with known coumarins, archangelicin (I) and umbelliferone (II). This paper describes the details of the structural elucidation of the new compounds.

The compound III is crystallized from a mixture of hexane and ethyl acetate in colorless needles, mp 156—158°, $[\alpha]_D^{20}$+82.9°, and was assigned to the molecular formula $C_{28}H_{26}O_8$ on the basis of analytical and mass spectral data. The infrared (IR) spectrum of III exhibits absorption bands due to hydroxyl, carbonyl groups of lactone and ester and aromatic ring. The nuclear magnetic resonance (NMR) spectrum (Fig. 1) shows the signals of two singlets at τ 8.39 and 8.23 (3H×2), three pairs of doublets at τ 4.68 and 2.84 (1H×2, J=7 cps), at τ 3.76 and 2.33 (1H×2, J=9.5 cps) and at τ 3.11 and 2.74 (1H×2, J=8.5 cps) which are in accord with the structure of 3′-hydroxy-2′,3′-dihydrooroselol skeleton. The signals of a singlet at τ 8.15(3H), a doublet at τ 8.11(3H, J=10 cps, each peaks split) and a multiplet at τ 3.95 (1H) can be assigned to the protons of angeloyl group, and a pair of doublets at τ 3.90 and 2.45(1H×2, J=16 cps) is due to a group of CH=CH (*trans*). Further pair of doublets at τ 3.20 and 2.53(2H×2, J=8.5 cps) indicate the presence of a *para*-disubstituted phenyl group. Upon the treatment with heavy water the total intensity of the signals in aromatic region is diminished by the amount of the intensity corresponding to one proton without marked change of the pattern of signals, suggesting the presence of a signal due to a proton of hydroxyl in this region.

The treatment of III with acetic anhydride in pyridine afforded a product of mp 167.5—168.5° (V). The NMR spectrum of V shows a singlet due to acetyl protons at τ 7.72, and no absorption band corresponding to hydroxyl is observed in IR spectrum of V. The chemical shift of the signal due to the acetyl protons of V suggests that single hydroxyl in III, which is acetylated in V, might be phenolic, nevertheless III is negative towards usual phenol reagents.

The treatment of III with 0.1 N ethanolic sodium hydroxide led to the formation of oroselone (VI) and oroselol (VII), as well as angelic acid (VIII) and *p*-coumaric acid (IX). This results indicate clearly that III must be angelic and *p*-coumaric acids ester of 3′-hydroxy-

1) Location: *Kawai-cho, Matsubara, Osaka.*

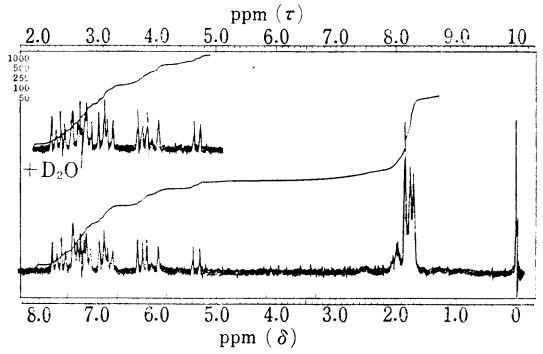


Fig. 1. NMR Spectrum of Angeladin (III)

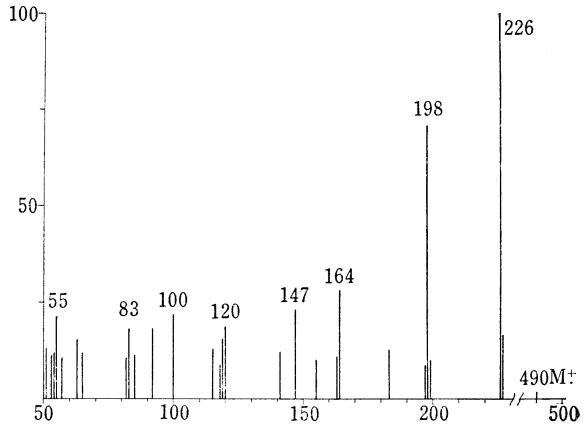


Fig. 2. Mass Spectrum of Angeladin (III)

2′,3′-dihydrooroselol, and this is also supported by the mass spectrum of III (Fig. 2), which shows a base peak at *m/e* 226 (M−264) corresponding to an ion of VI formed by loss of VIII and IX from a parent ion. A peak at *m/e* 198 corresponds to a fragment ion resulted by loss of carbon monoxide from VI. Furthermore, peaks due to ions of IX, VIII, *p*-coumaroyl and angeloyl groups are observed at mass numbers of 164, 100, 147 and 83, respectively.

Hydrogenation of III using paladium catalyst gave a product of viscid oil together with an acid. The oily product after refining by chromatography was proved to be a single substance on thin layer chromatography and was identified as O-(2-methylbutyryl)tetrahydrooroselol(tetrahydrocolumbianadin) (X) from the fact that its NMR and IR spectra are identical with those of authentic sample of X prepared by hydrogenation of columbianadin (XI), and that the oily product upon saponification afforded a compound of mp 112—113° which was identified as dihydrocolumbianetin (XII). On the other hand, the acid formed upon hydrogenation of III was identified as *p*-hydroxydihydrocinnamic acid. From these results III was elucidated to be O-angeloyl-3′-*p*-coumaroyloxy-2′,3′-dihydrooroselol.

The configurational problem was resolved as follows. Firstly, the absolute configuration of III at 2′ position was proved to be *S* from the fact that the hydrogenolysis product (X) is also identical in the optical rotation with authentic sample of X formed from XI which has been confirmed to have *S*-configuration at the same position.[2] On the other hand, it has been shown that in the NMR spectra of the coumarins of this type 2′ and 3′ protons in *cis*-configuration exhibit a pair of doublets with coupling constant of 6.5—7.0 cps, while the protons in *trans*-configuration reveal the signals with that of 3.0 cps.[3] On the basis of this evidence, *cis*-configuration should be assigned to III which shows these signals with coupling constant of 7.0 cps, establishing the structure of III as 2′(*S*),3′(*R*)-O-angeloyl-3′-*p*-coumaroyloxy-2′,3′-dihydrooroselol.

The compound IV is crystallized from ethanol in colorless needles, mp 89—90°, $[\alpha]_D^{20}$ −27.3°, and molecular weight by the mass spectrum is in accord with the formula $C_{21}H_{22}O_7$. The IR spectrum of IV is indicative of the presence of carbonyl groups of lactone and ester and aromatic ring. The NMR spectrum (Fig. 3) shows signals of two signlets at τ 8.31 and 8.24(3H×2), three pairs of doublets at τ 4.70 and 2.99(1H×2, J=7 cps), at τ 3.76 and 2.36 (1H×2, J=9.5 cps) and at τ 3.14 and 2.56(1H×2, J=8.5 cps), which suggest the presence of the same skeleton as III. Further signals of a singlet at τ 8.12(3H), a doublet at 8.08(3H,

2) B.E. Nielsen and J. Lemmich, *Acta Chem. Scand.*, **18**, 2111 (1964).
3) F. Bohlmann and M. Grenz, *Chem. Ber.*, **102**, 1673 (1969); E. Lemmich, J. Lemmich, and B.E. Nielsen, *Acta Chem. Scand.*, **24**, 2893 (1970).

J=10 cps, each peaks split) and a multiplet at τ 3.94(1H) are assigned to the protons of angeloyl group, and a singlet at τ 7.96(3H) to acetyl group. The mass spectrum of IV shows fragment peaks at mass numbers of 244, 226, 83 and 43 corresponding to ions of VII, VI,angeloyl and acetyl groups, respectively. These spectral data suggest that IV is diester of 3′-hydroxy-2′,3′-dihydrooroselol having angeloyl and acetyl groups.

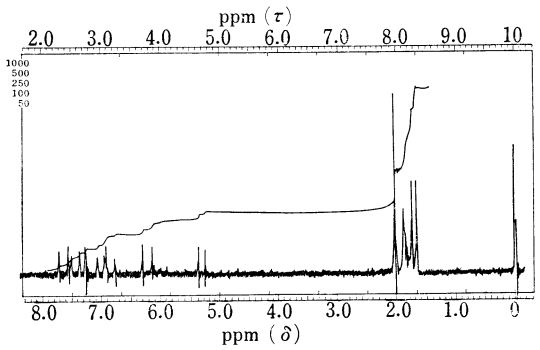
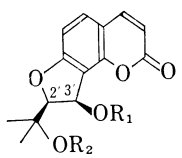
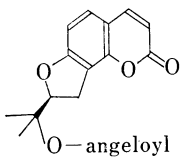


Fig. 3. NMR Spectrum of Isoedultin (IV)

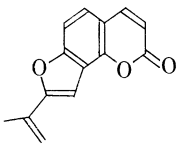
As a kind of this diester coumarin, edultin (O-acetyl-3′-angeloyloxy-2′,3′-dihydrooroselol)[4] (XIII) has been known, but its NMR[5] and IR spectra are different from those of IV, indicating that IV cannot be identical with XIII. The compound IV also cannot be considered to be *trans*-isomer of XIII which has been shown to have *cis*-configuration,[5] since in the NMR spectrum of IV signals due to 2′ and 3′ protons show the coupling constant (7 cps) being equal to that of XIII, indicating that IV has the same relative configuration as XIII. These findings suggest that IV must be a structural isomer of XIII, O-angeloyl-3′-acetoxy-2′,3′-dihydrooroselol, and this was proved by hydrogenation of IV using paladium catalyst to afford a product which was identified as X. The absolute configuration was resolved by the same way as III, and the structure, 2′(*S*),3′(*R*)-O-angeloyl-3′-acetoxy-2′,3′-dihydrooroselol was established for IV.



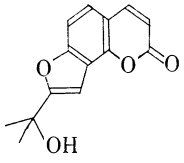
I : R_1=R_2=angeloyl
III : R_1= *p*-coumaroyl, R_2=angeloyl
IV : R_1=acetyl, R_2=angeloyl
V : R_1=O-acetyl-*p*-coumaroyl, R_2=angeloyl
XIII : R_1=angeloyl, R_2=acetyl



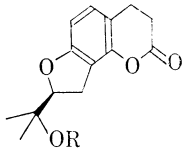
XI



VI



VII



X : R = 2-methylbutyryl
XII : R = H

Chart 1

Experimental

Isolation of the Compounds——The dried and crushed roots (830 g) of the plant collected in Oita Pref. was extracted with four 3 liters portions of ether at room temperature for 10 days. The ether solution was concentrated to brown oil (99 g) with peculiar odor. The oil was treated with hexane and was divided into soluble (78 g) and insoluble (21 g) fractions.

The insoluble fraction was chromatographed on a column of silica gel (700 g), eluted with hexane: EtOAc, and following fractions (25 ml each) were taken: No. 1—36 (4: 1), No. 37—48 (3: 1), No. 49—90 (2: 1),

4) H. Mitsuhashi and T. Itoh, *Chem. Pharm. Bull.* (Tokyo), **10**, 511; 514 (1962).
5) K. Hata, M. Kozawa, K. Baba, and *Yakugaku Zasshi*, **92**, 1289 (1972).

No. 91—112 (1: 1) [( ) shows ratio of the solvents in v/v]. The fractions of No. 10—18 and those of No. 45—59 yielded I and II, respectively. Viscid oily substance (5 g) obtained from the fractions of No.61—72 was rechromatographed on a column of polyamide (150 g) and eluted with EtOAc: EtOH (2: 1) to give II and III. Viscid oil (6 g) obtained from the above fractions of No. 21—44 was chromatographed on a column of silica gel (200 g), eluted with $CHCl_3$ and thirty 200 ml fractions were taken. The substance obtained from this fractions of No. 2—19 was rechromatographed on a column of silica gel (150 g), eluted with hexane: EtOAc (4: 1) and fourty three 150 ml fractions were taken, No. 17—23 of which yielded IV.

The hexane soluble fraction, above mentioned, was chromatographed on a column of silica gel (600 g), eluted with hexane: EtOAc and following fractions (200 ml each) were taken: No. 1—24 (5: 1), No. 25—54 (4: 1), No. 55—80 (3: 1), No. 81—100 (2: 1) [( ) shows ratio of the solvents in v/v]. The fractions of No. 33—42, No. 55—61 and No. 85—90 yielded I, IV, and II, respectively.

Archangelicin (I)——Colorless needles from hexane: EtOAc, mp 107—108°, $[\alpha]_D^{30}$ +196.7° (c=0.86, MeOH) [lit. mp 100.5—102°, $[\alpha]_D^{26}$ +112.7° (c=4.5, MeOH)].[6] The melting point showed no depression on admixture with the sample of archangelicin, mp 103—105°, isolated from Chinese crude drug "She Huangzi."[5] IR and NMR spectra are identical with those of this sample. Yield 0.13%.

Umbelliferone (II)——Colorless crystalline powder from EtOAc, mp 233—234°. The melting point showed no depression on admixture with authentic sample of umbelliferone. yield 0.18%.

Angeladin (III)——Fine colorless needles from hexane: EtOAc, mp 156—158°, $[\alpha]_D^{20}$ +82.9° (c=0.84, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{26}O_8$: C, 68.56; H, 5.34; mol. wt., 490.49. Found: C, 68.61; H, 5.64; mol. wt. (Mass Spectrum), 490. IR ν_{max}^{Nujol} cm^{-1}: 3450 (OH); 1700, 1720 (C=O); 1600, 1580, 1510 (aromatic ring). yield 0.55%.

Isoedultin (IV)——Colorless needles from EtOH, mp 89—90°, $[\alpha]_D^{20}$ −27.3° (c=0.48, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1710, 1700 (C=O); 1620, 1580 (aromatic ring). Yield 0.16%.

Acetylation of III, Formation of V——One hundred milligram of III was dissolved in a mixture of 2 ml of pyridine and 1 ml of acetic anhydride and allowed to stand at room temperature for 12 hr. The reaction mixture was poured into ice water and was stirred for 6 hr. A substance precipitated was collected and was recrystallized from EtOH to colorless needles, yield 70 mg. mp 167.5—168.5°, IR ν_{max}^{Nujol} cm^{-1}: 1760, 1740, 1720 (C=O); 1620, 1600, 1580, 1500 (aromatic ring). Mass Spectrum m/e: 532 (M^+); 226 ($C_{14}H_{10}O_3$); 198 ($C_{13}H_{10}O_2$); 164 ($C_9H_8O_3$); 147 ($C_9H_7O_2$); 55 (C_4H_7); 43 (C_2H_3O). NMR (in $CDCl_3$) τ:[7] 8.28, 8.23 (3H×2, singlets, C<u>H</u>$_3$–C–C<u>H</u>$_3$), 8.13 (3H, broad singlet, HC=C–C<u>H</u>$_3$), 8.09 (3H, doublet, J=10 cps, each peaks split, C=CH–C<u>H</u>$_3$), 7.72 (3H, singlet, COC<u>H</u>$_3$), 4.67, 2.86 (1H×2, doublets, J=7 cps, O–C<u>H</u>–C<u>H</u>–O), 3.96 (1H, multiplet, CH$_3$–C<u>H</u>=CH$_3$), 3.80, 2.37 (1H×2, doublets, J=9.5 cps, C<u>H</u>=C<u>H</u>), 3.70, 2.31 (1H×2, doublets, J=16 cps, C<u>H</u>=C<u>H</u>), 3.12, 2.55 (1H×2, doublets, J=8.5 cps, aromatic H×2), 2.90, 2.50 (2H×2, doublets, J=8.5 cps, aromatic H×4).

Degradation of III with Ethanolic Alkali, Formation of VI, VII, VIII, and IX——To the solution of 1 g of III in 10 ml of EtOH, 10 ml of 0.2N KOH (EtOH) was added at room temperature under stirring, and was allowed to stand for 30 min. The reaction mixture was diluted with 20 ml of water and concentrated to a half volume by means of a rotary evaporator, acidified with 20% H_2SO_4 and extracted with ether. The ether solution was washed with 5% $NaHCO_3$ to remove acid fraction.

(i) Neutral Fraction: The ether solution was evaporated and the residue was chromatographed on a column of silica gel (10 g). Elution with $CHCl_3$ gave, after recrystallization from hexane: EtOAc, VI and VII. VI: Colorless needles, mp 179—180° [lit. mp 180°].[4] Yield 25 mg. IR and NMR spectra are identical with those of the sample of oroselone,[5] and the melting point showed no depression on admixture with this sample. VII: Colorless needles ,mp 150—151° [lit. mp 149—150°].[4] Yield 50 mg. IR ν_{max}^{Nujol} cm^{-1}: 3450 (OH); 1720 (C=O); 1610, 1580 (aromatic ring). IR and NMR spectra are identical with those of authentic sample of oroselol prepared from edultin,[5] and the melting point showed no depression on admixture with this sample.

(ii) Acid Fraction: The 5% $NaHCO_3$ washing was acidified with 20% H_2SO_4 and extracted with ether. The ether solution was evaporated and the residue was sublimated under reduced pressure to give colorless crystalline sublimate (VIII). The residue upon recrystallization from hexane: EtOAc gave IX. VIII: Colorless prisms by sublimation (80°, 15 mmHg), mp 45—46°, yield 50 mg. The melting point showed no depression on admixture with authentic sample of angelic acid. IX: colorless needles, mp 208° (decomp.), yield 160 mg. IR and NMR specrta are identical with those of authentic sample of *p*-coumaric acid.

Hydrogenation of III, Formation of X and *p*-Hydroxydihydrocinnamic Acid——To prereduced 700 mg of paladium catalyst in 30 ml of AcOH, 500 mg of III was added and the mixture was stirred in the presence of hydrogen for 10 hr (4.5 moles of H_2 were taken up). The catalyst was filtered off, and AcOH was removed by means of a rotary evaporator. The residue was dissolved in 50 ml of ether and the solution was washed with 5% $NaHCO_3$ to remove acid fraction.

6) B.E. Nielsen and J. Lemmich, *Acta Chem. Scand.*, **18**, 932 (1964).
7) NMR spectra were measured by means of NEVA Model A-60D Analytical NMR Spectrometer using TMS as internal standard.

(i) Neutral Fraction: The ether solution was evaporated and the residue was chromatographed two times on a column of silica gel (15 g) and eluted with $CHCl_3$ to give oily substance (X), which gave single spot on thin-layer chromatography. X: pale yellow viscid oil, $[\alpha]_D^{20}$ +69.3° (c=0.56, $CHCl_3$). Yield 110 mg. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1720 (C=O); 1630, 1610 (aromatic ring). NMR (in $CDCl_3$) τ: 9.12 (3H, triplet, J=7 cps, CH_2–C$\underline{H}_3$), 8.94 (3H, doublet, J=7 cps, CH–C$\underline{H}_3$), 8.48, 8.42 (3H×2, singlets, C$\underline{H}_3$–C–C$\underline{H}_3$), *ca.* 8.5 (2H, multiplet, CH–C$\underline{H}_2$–CH_3), 7.74 (1H, multiplet, CH_2–C$\underline{H}$–CH_3), 7.14 (4H, multiplet, ph–C$\underline{H}_2$–C$\underline{H}_2$–CO), 6.80 (2H, doublet, J=9 cps, ph–C$\underline{H}_2$–CH), 5.02 (1H, triplet, J=9 cps, O–C$\underline{H}$–CH_2), 3.50, 3.07 (1H×2, doublets, J=8.5 cps, aromatic H×2). IR and NMR spectra are identical with those of authentic sample of tetrahydrocolumbianadin, $[\alpha]_D^{20}$ +71.1° (c=0.97, $CHCl_3$).

(ii) Acid Fraction: The 5% $NaHCO_3$ washing was acidified with 20% H_2SO_4 and extracted with ether. The ether solution was evaporated and the residue upon sublimation (150°, 0.5mmHg) gave colorless crystalline sublimate. *p*-Hydroxydihydrocinnamic acid: colorless needels from hexane: EtOAc, mp 119—121°. yield 93 mg. The melting point showed no depression on admixture with authentic sample of this compound. IR spectrum is identical with that of this sample.

Degradation of X with Ethanolic Alkali, Formation of XII——The solution of 100 mg of X in 30 ml of 1N NaOH (EtOH) was refluxed for 2 hr. After cooling the solution was diluted with 30 ml of water and concentrated to a half volume by means of rotary evaporator, acidified with 20% H_2SO_4, and extracted with ether. The ether solution was evaporated and the residue upon sublimation (150°, 0.5 mmHg) gave colorless crystalline sublimate (XII). XII: colorless needles from hexane, mp 112—113°. Yield 18 mg. *Anal.* Calcd. for $C_{14}H_{16}O_4$ (dihydrocolumbianetin): C, 67.73; H, 6.50. Found: C, 67.52; H, 6.57. The melting point showed no depression on admixture with authentic sample of dihydrocolubianetin.

Hydrogenation of IV——To prereduced 400 mg of paladium catalyst in 25 ml of AcOH, 500 mg of IV was added and the mixture was stirred in the presence of hydrogen for 10 hr (3.5 moles of H_2 were taken up). The catalyst was filtered off, and AcOH was removed by means of a rotary evaporator. The residue was dissolved in 50 ml of ether and the solution was washed with 5% $NaHCO_3$. The neutral fraction was chromatographed two times on a column of silica gel (15 g) and eluted with $CHCl_3$ to give viscid oil (X) which gave single spot on thin-layer chromatography. Yield 100 mg. $[\alpha]_D^{16}$ +65.0° (c=0.8, $CHCl_3$). IR and NMR spectra are identical with those of authentic sample of tetrahydrocolumbianadin.

Acknowledgement The authors are gratefull to Prof. I. Nishioka and the members of the Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kyushu University for affording the conveniences for collecting the plant materials, and to the members of the Institute of Elemental Analysis of Kyoto University for microanalysis. They are also indebted to Dr. A. Numata, Osaka College of Pharmacy, for measuring the NMR spectra and to Dr. S. Matsunaga of this College for the Mass spectra.