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Coumarin Constituents from the Roos of *Angelica koreana* Max.

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강활의 쿠마린 성분

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From the ethereal extract of the roots of *Angelica koreana* Max. (Umbelliferae) two furanocoumarins such as isooxypeucedanin and oxypeucedanin methanolate were newly isolated together with isoimperatorin, osthol, imperatorin, bergapten, oxypeucedanin and β-sitosterol.

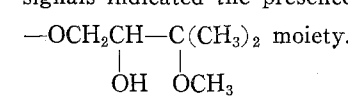
The roots of *Angelica koreana* Max. (Umbelliferae) have long been used in the oriental medicine for the treatment of common cold, headache, neuralgia and arthralgia. Isolation of several coumarins from the plant part such as isoimperatorin, oxypeucedanin, bergapten and osthol were previously reported by Ryu, *et al.*[1], Hata, *et al.*[2] and Chi.[3] The present report deals with the isolation of two minor coumarin constituents, compounds I and II, which were identified as isooxypeucedanin and oxypeucedanin methanolate. This is the first report of their occurrence in the plant part.

Compound I, $C_{16}H_{14}O_5$, mp 143-4°, showed in its NMR spectrum in $CDCl_3$, a pair of ortho-coupled doublets of one proton each at δ6.25 (J=10 Hz, H-3) and 8.25 (J=10, H-4), two doublets of one-proton each at δ7.55 (J=2, H-b) and 7.12 (J=1, H-8) and a one-proton double doublet at δ6.78 (J=2 and 1, H-a), which indicated that this compound was a 5-oxygenated linear furancoumarin. In the high field region, appearence of a singlet at δ5.07 (2H, H-1′), a septet at δ2.79 (1H, J=7, H-3′) and a doublet at δ1.18 (6H, J=7, 2×CH_3) indicated the presence of -OCH_2-CO-CH(CH_3)$_2$ moiety. This observation suggested that this compound was isooxypeucedanin, which was confirmed by direct comparison with the authentic sample synthesized from oxypeucedanin.

Compound II, $C_{17}H_{18}O_6$, mp 124-6°, also showed a characteristic data for 5-oxygenated linear furanocoumarin structure in its NMR spectrum. The pyrone doublets (J=10) appeared at δ6.25 (H-3) and 8.22 (H-4), and furan proton doublets (J=2.5) fell at δ7.58 (H-b) and 7.05 (H-a) which was further coupled (J=1) to C-8 proton at δ7.10. Appearence of a six-proton singlet at δ1.33 and a three-proton singlet at δ3.33 indicated the presence of two equivalent methyl groups attached to insulating quarternary carbon to a deshielding group and one methoxyl group. A clear two-proton octat centered at δ4.50 in which proton appeared as a doublet of doulbets (J_{gem}=10, J_{vic}=3.5 and 7.5), a multiplet of one-proton at δ4.03 and a

doublet of one proton at δ2.97(J=4) were indicative of three protons α to oxygen substituents and an alcoholic proton.

On addition of D_2O, the signal at δ2.97 disappeared and the multiplet at δ4.03 was resolved into a double doublet(J=3.5 and 7.5). The position and multiplicity of these signals indicated the presence of



—$OCH_2CH(OH)C(CH_3)_2(OCH_3)$ moiety. Therefore, compound II must be oxypeucedanin methanolate, which was identified by direct comparison with authentic sample synthesized from oxypeucedanin.

Experimentals

The NMR spectra were determined in $CDCl_3$ using a Varian EM-360 spectrometer with tetramethylsilane as an internal standard. TLC was carried out using toluene-ethyl formate-formic acid (5:4:1) as a developing solvent. Gas-liquid chromatography was carried out with a Pye Unicam 104 Chromatograph apparatus equipped with a hydrogen flame ionization detector. The chromatographic column was 150cm×4mm tube contained Chromosorb W(60~80 mesh) coated with 2% OV-101. The temperatures were column, 190°; detector, 250°. The nitrogen flow was 40ml/min. Mps were uncorrected.

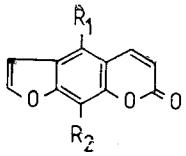
Isolation of coumarins: The dried roots of *Angelica koreana* (5kg) were reduced to a coarse powder and refluxed with 95% methanol for five hours for three times. The methanolic extract was then concentrated under reduced pressure to obtain 305g of dark oily residue. The residue was partitioned with diethyl ether and water to yield 213g of ethereal extract. The ethereal extract was applied to column chromatography using *n*-hexane-ethyl acetate as a gradient elution solvent. The fractions obtained by monitoring with characteristic fluorescence under UV ray(365nm) were 15 in all.

β-Sitosterol: Fr. 3 was concentrated to give β-sitosterol, mp 140-2°, identical with the authentic sample.

Isoimperatorin: From the mother liquor after separation of β-sitosterol white needle-like crystal(5.3g) was obtained. Mp 108~10°, yellowish fluorescence, TLC Rf 0.76, GLC t_R 11.0 min. NMR δ: 8.08(1H, d, J=10, H-4); 7.57 (1H, d, J=2, H-b); 7.05(1H, d, J=1, H-8); 6.92(1H, dd, J=2 and 1, H-a); 6.20(1H, d, J=10, H-3); 5.50(1H, tq, J=7 and 1, side chain —OCH_2—C<u>H</u>=); 4.88(2H, d, J=7, side chain —$OC\underline{H}_2CH$=); 1.82(3H, s, geminal methyl) and 1.72(3H, d, J=1, geminal methyl), identital with the authentic sample of isoimperatorin.

Osthol: The mother liquor from Fr. 3 and Fr. 4 were concentrated and dissolved in methanol and stood for several days in room temperature to yield white rhombic crystal(2.8g), mp 83~4°, pale bluish fluorescence, TLC Rf 0.70, GLC t_R 4.6min. NMR δ: 7.58(1H, d, J=10, H-4); 7.28(1H, d, J=8.5, H-5); 6.82 (1H, d, J=8.5, H-6); 6.19(1H, d, J=10, H-3); 5.23(1H, tq, J=7 and 1, side chain —CH_2—C<u>H</u>=); 3.90(3H, s, —OCH_3); 3.52(2H, d, J=7, side chain —$C\underline{H}_2CH$=); 1.83(3H, s, geminal methyl) and 1.67(3H, d, J=1, geminal methyl), identical with authentic sample of osthol.

Imperatorin: Fr. 5 gave white amorphous crystal(5.8g), mp 104-5°, yellow fluorescence, TLC Rf 0.66, GLC t_R 9.3 min. NMR δ: 7.73 (1H, d, J=10, H-4); 7.67(1H, d, J=2, H-b); 7.33(1H, s, H-5); 6.78(1H, d, J=2, H-a); 6.32(1H, d, J=10, H-3); 5.60(1H, tq, J=7 and 1, side chain —OCH_2—C<u>H</u>=); 4.99 (2H, d, J=7, side chain —$OC\underline{H}_2$—CH=); 1.78(6H, d, J=1, gemdimethyl), identical with the



Isoimperatorin R_1=OCH_2CH=$C(CH_3)_2$; R_2=H

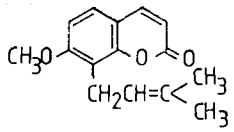
Imperatorin R_1=H ; R_2=OCH_2CH=$C(CH_3)_2$

Oxypeucedanin R_1=OCH_2-CH(O)-$C(CH_3)_2$; R_2=H

Bergapten R_1=OCH_3; R_2=H

Isooxypeucedanin(I) R_1=OCH_2-C(=O)-$CH(CH_3)_2$; R_2=H

Oxypeucedanin methanolate(II) R_1=$OCH_2CH(OH)C(OCH_3)(CH_3)_2$; R_2=H



Osthol

authentic sample of imperatorin.

Bergapten: Fr. 6 was applied to column chromatography again using *n*-hexane-ethyl acetate (10 : 3) to give pale yellowish powder (1.2g), mp 185-7°, yellow fluorescence, TLC Rf 0.61, GLC t_R 2.5 min. NMR δ: 8.12 (1H, d, J=9.5, H-4); 7.58(1H, d, J=2.5, H-b); 7.10 (1H, d, J=1, H-8); 7.00(1H, dd, J=2.5 and 1, H-a); 6.22(1H, d, J=9.5, H-3) and 4.28 (3H, s, methoxyl), identical with the authentic sample of bergapten.

Oxypeucedanin: Fr. 8 was concentrated to yield crude crystal which was recrystallized from ethyl acetate containing small amount of *n*-hexane to yield white amorphous crystal (4.3g), mp 139-40°, racemic, yellow fluorescence, TLC Rf 0.56, GLC t_R 15.5 and 27.0 mins. NMR δ:8.23(1H, d, J=10, H-4); 7.63(1H, d, J=2, H-b); 7.19(1H, d, J=1, H-8); 6.96 (1H, dd, J=2 and 1, H-a); 6.30(1H, d, J=10, H-3); 4.63 and 4.36 (2H, dd dd J_{gem}=11, J_{vic}=4 and 6, side chain $-OC\underline{H}_2-CH(O)-C=$); 3.20(1H, dd, J=4 and 6, side chain $-OCH_2-C\underline{H}(O)-C=$); 1.43 and 1.36 (6H, both s, gemdimethyl), identical with the authentic sample of oxypeucedain.

Compound I: To the mother liquor from Fr. 8 was added excess amount of *n*-hexane and stood for several hours in refrigerator to yield pale yellow powder, compund I(0.7g), mp 143-4°, pale yellowish fluorescence, TLC Rf 0.56, GLC t_R 15.5 min.

Compound II: Fr. 13 was concentrated to yield white amorphous crystal, compound II, recrystallized from *n*-hexane-ethyl acetate(1 : 1) (3.7g), mp125-7°, pale greenish yellow fluorescence, TLC Rf 0.12, GLC t_R 14.5 min, $[\alpha]_D$ +10.7(ethanol).

Synthesis of compound I: To a solution of oxypeucedanin (0.5g) in water-free toluene (25ml) was added 2g of phosphorous pentoxide, refluxed for 30 min and poured into excess amount of water. The toluene layer was washed with diluted alkaline water and concentrated to dryness. The resulted white crystal was recrystallized from ether(0.15g). Mp143-4° (Lit.[4]: 146°).

Synthesis of compound II: Methanolic solution of oxypeucedanin(0.5g) containing two drops of BF_3 etherate was stood for 24 hours in room temperature and poured into excess amount of 10% HCl. The $CHCl_3$ extraction of the resulted solution and subsequent application to column chromatography using *n*-hexane-ethyl acetate(1 : 1) as an elution solvent produced white amorphous crystal (0.17g). Mp 124-6° (Lit.[5]: 123-5°).

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