Fraction 8—11 (420 mg) was rechromatographed on alumina and then separated preparative TLC (benzene:ether=95:5) to give acoronene (VI, 75 mg), colourless prisms, mp 69° (from *n*-hexane), *Rf* 0.31, M+ 234, $[\alpha]_D^{23.5}$ +66.8° (±1.0°) (*c*=1.039 in dioxan), λ_{max} 238 mμ (ε 7650), $\nu_{max}^{CCl_4}$ 1740, 1680, and 928 cm^{-1}, NMR δ 0.92 and 1.00 (isopropyl), 1.08 (CH_3), 1.77 (CH_3), and 6.70 ppm (1H). Found: C, 77.07; H, 9.45. $C_{15}H_{22}O_2$ requires C, 76.88; H, 9.46%.

Hydrogenation of Acoronene (VI)——A solution of VI (30 mg) in methanol (3 ml) was hydrogenated with 5% palladium-charocoal (30 mg) at room temperature. The product showed two spots, *Rf* 0.49 and 0.31 on TLC (benzene:ether=95:5) and then separated by preparative TLC into isoacorone (VII, 20 mg), colourless needles, mp 99—99.5° (from ether), *Rf* 0.49, M+ 236, $[\alpha]_D^{22}$ −80.3° (±5.3°) (*c*=0.228 in ethanol), $\nu_{max}^{CHCl_3}$ 1741 and 1718 cm^{-1} (Found: C, 76.37; H, 10.17. $C_{15}H_{24}O_2$ requires C, 76.22; H, 10.24%), which was identical with the data of isoacorone[8] [IR ($CHCl_3$ and CS_2), mp, and $[\alpha]_D$] and acorone (VIII, 10 mg), colourless needles, mp 93—95° (from ether), *Rf* 0.31, M+ 236, $[\alpha]_D^{22}$ +106.2° (±6.1°) (*c*=0.241 in ethanol), $\nu_{max}^{CHCl_3}$ 1741 and 1718 cm^{-1} (Found: C, 75.71; H, 10.76%), which was identical with the data of acorone[8] [IR ($CHCl_3$ and CS_2), mp, and $[\alpha]_D$].

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Coumarins from the Roots of *Angelica laxiflora* Diels

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In a previous communication,[2] it was reported that the ether extract of the Chinese crude drug "Chuan Duhuo (川独活)," the source of which had been unknown but to be dried roots of a species of *Angelica genus*, upon vacuum distillation afforded 6-formyl-7-methoxy-coumarin, named angelical which was first isolated from the ether extract of the roots of *Angelica pubescens* Max. upon vacuum distillation[3] and was later proved to be an artefact from angelol,[4] and a crystalline compound, mp 115—116°, analytical data of which was in accord with the molecular formula $C_{15}H_{16}O_4$.

As a part of our continuing study of the coumarins from the *Umbelliferous* plants, we have reinvestigated the constituents of "Chuan Duhuo" recently obtained from the Xiang-gang (香港) market, which is assigned to the dried roots of *Angelica laxiflora* Diels. according to the ref. 5).

The ether extract of the crude drug upon chromatography over silica gel followed by elution with a mixture of *n*-hexane and ethyl acetate afforded bergapten, umbelliferone and angelol, identified by the mixed melting point examination with the authentic samples, as well as three compounds, $C_{14}H_{14}O_4$ (I), mp 162—163°, $C_{16}H_{16}O_5$ (II), mp 130—131°, and $C_{19}H_{20}O_5$ (III), mp 116—117°, which were identified as columbianetin,[6] columbianetin acetate and columbianadin,[6] respectively, on the basis of the evidences described in the sequel.

I was suggested to be identical with columbianetin from the analytical data, the melting point and the optical rotation, and this was confirmed from the nuclear magnetic resonance

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2) K. Kimura, K. Hata, K. .Yen and S. Chen, *Yakugaku Zasshi*, **78**, 442 (1958).
3) K. Hata and Y. Tanaka, *Yakugaku Zasshi*, **77**, 937 (1957).
4) K. Hata and M. Kozswa, *Yakugaku Zasshi*, **87**, 210 (1967); *idem*, *ibid.*, **88**, 283, 293 (1968).
5) ("中葯志 I," 中国医学科学院葯物研究所等編人民衛生出版社北京) 1959, p. 345.
6) R. E. Willette and T. O. Soine, *J. Pharm. Sci.*, **53**, 275 (1964).

(NMR) spectrum being superimposable to that of the compound which was established to have the same structure as columbianetin.[7]

The NMR spectrum of II, which differed essentially from that of I only in showing of the signal due to acetyl protons instead of a hydroxyl proton, indicated that II is identical with I-acetate, and this was confirmed by the mixed melting point examination.

Saponification of III afforded I together with angelic acid, identified as its *p*-phenylphenacyl ester, indicating that III is identical with I-angelate, named columbianadin. The NMR data of III is also accomodated to this structure. Further evidence for this identification was given from the comparison of the infrared (IR) spectrum of III with that of columbianadin.[6]

Since the sample of the compound of mp 115—116°, reported in the previous communication[2] mentioned above, is not available for re-examination, identification of it is impossible. It seems, however, that the compound might be identical with III on the basis of its melting point and of the fact that the analytical data which was previously assigned to the molecular formula $C_{15}H_{16}O_4$ is also in agreement with the calculated values for the composition of III, $C_{19}H_{20}O_5$, within a few error. This presumption can be supported from the fact that both the plant materials, previously examined and reported here, were found to contain angelol as a common constituent, suggesting that the both can be considered to be closely related chemotaxonomically to each other.

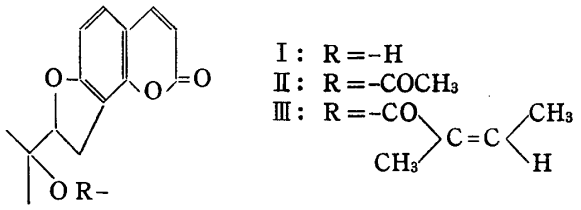

Fig. 1

The Ether Extract of the Roots of *Angelica laxiflora* DIELS

chromatographed over silica gel, eluted with *n*-hexane–ethyl acetate (4:1)

columbianadin (III)

column

eluted with *n*-hexane–ethyl acetate (3:1)

bergapten
columbianetin acetate (II)

column

eluted with *n*-hexane–ethyl acetate (2:1)

umbelliferone
columbianetin (I)

column

eluted with *n*-hexane–ethyl acetate (1:1)

angelol

column

Chart 1

Experimental

Extraction and Isolation of the Compounds——The crushed crude drug (10 kg) was extracted with three 20 liters portions of ether at a room temperature for 3 weeks. The ether solution was concentrated to yield dark brown colored viscid oil (280 g). The oil was chromatographed over silica gel to afford the compounds as shown in Chart 1.

7) B. E. Nielsen and J. Lemmich, *Acta Chem. Scand.*, **18**, 1379 (1964).

Bergapten——Recrystallized from EtOH to colorless fine needles, mp 190—191°, yield 1 g. *Anal.* Calcd. for $C_{12}H_8O_4$: C, 66.67; H, 3.73. Found: C, 66.77; H, 3.75. The melting point was not depressed on admixture with the authentic sample of bergapten.

Umbelliferone——Sublimated in vacuum to colorless crystalline powder, mp 224—227°, yield 0.5 g. *Anal.* Calcd. for $C_9H_6O_3$: C, 66.67; H, 3.73. Found: C, 66.63; H, 3.55. The melting point was not depressed on admixture with the authentic sample of umbelliferone.

Angelol——Recrystallized from ether to colorless needles, mp 101—103°, yield 10 g. The analytical data were taken as acetonide. IR spectrum was superimposable to that of angelol.

Angelol Acetonide——To the solution of 5 g of crude angelol in 200 ml of anhydrous acetone, 2 g of *p*-toluenesulfonic acid was added and the mixture was stirred for 3 hr under reflux. After cool, the acetone solutin was removed by decantation, which was neutralized with Na_2CO_3 solution and filtered. The filtrate was diluted with 50 ml of H_2O, concentrated to 50 ml in vacuum and extracted with ether. The ether solution was evaporated to give a crystalline residue which was recrystallized from EtOH to needles. This was chromatographed over silica gel (500 g) and eluted with $CHCl_3$. The crystalline eluate was recrystallized from EtOH to colorless neeles, mp 191—193°, yield 3 g. *Anal.* Calcd. for $C_{23}H_{28}O_7$: C, 66.23; H, 6.78. Found: C, 66.04; H, 6.85. The melting point was not depressed on admixture with the authentic sample of angelol acetonide.

Columbianetin (I)——Recrystallized from EtOAc to colorless needles, mp 162—163° [lit. mp 164.5—165°,[6] 162.8—163.3°[7]], $[\alpha]_D^{29}$ +166° (c=1.0, $CHCl_3$) [lit. $[\alpha]_D^{27}$ +20° (dioxane),[6] $[\alpha]_D^{24}$ +250° (c=0.5, MeOH],[7] yield 0.5 g. *Anal.* Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.02; H, 5.55. NMR (τ)[8]: 8.75 3H, singlet, CH_3), 8.65 (3H, singlet, CH_3), 7.89 (1H, singlet, OH), 6.69 (2H, doublet, J=9cps, CH–C<u>H</u>$_2$), 5.20 (1H, triplet, J=9 cps, C<u>H</u>–CH_2), 3.83, 2.38 (2H, doublet, J=9.5 cps, C<u>H</u>=C<u>H</u>), 3.27, 2.75 (2H, doublet, J=8.5 cps, 2×aromatic H).

Columbianetin Acetate (II)——Recrystallized from *n*-hexane–EtOAc (2 : 1) to colorless needles, mp 130—131°, $[\alpha]_D^{29}$ +194° (c=1.0, $CHCl_3$), yield 1 g, *Anal.* Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.59; H, 5.36. NMR (τ)[8]: 8.48 (3H, singlet, CH_3), 8.42 (3H, singlet, CH_3), 8.02 (3H, singlet, $COCH_3$), 6.68 (2H, doublet, J=9 cps, CH–C<u>H</u>$_2$), 4.83 (1H, triplet, J=9 cps, C<u>H</u>–CH_2), 3.82, 2.72 (2H, doublet, J=9.5 cps, C<u>H</u>=C<u>H</u>), 3.27, 2.72 (2H, doublet, J=8.5 cps, 2×aromatic H). The melting point was not depressed on admixture with the sample of I-acetate.

Columbianadin (III)——Recrystallized from *n*-hexane–EtOAc (4 : 1) to colorless needles, mp 116—117° [lit. mp 121—122°,[6] 118.4—119°[7]], $[\alpha]_D^{29}$ +288° (c=1.0, $CHCl_3$) [lit. $[\alpha]_D^{27}$ +26.7° (dioxane),[6] $[\alpha]_D^{26}$ +227° ($CHCl_3$)[7]], yield 20 g. *Anal.* Calcd. for $C_{19}H_{20}O_5$: C, 69.50; H, 6.14. Found: C, 69.50; H, 6.24. NMR (τ)[8]: 8.41 (3H, singlet, CH_3), 8.37 (3H, singlet, CH_3), 8.32 (3H, doublet, J=nearly 1 cps, CH=C–C<u>H</u>$_3$), 8.12 (3H, doublet, J=7 cps, =CH–C<u>H</u>$_3$), 6.65 (2H, doublet, J=9 cps, CH–C<u>H</u>$_2$), 4.87 (1H, triplet, J=9 cps, C<u>H</u>–CH_2), 6.00 (1H, broad quartet, J=7 cps, CH_3–C=C<u>H</u>–CH_3), 3.82, 2.40 (2H, doublet, J=9.5 cps, C<u>H</u>=C<u>H</u>), 3.28, 2.75 (2H, doublet, J=8.5 cps, 2×aromatic H). IR spectrum was superimposable to that of columbianadin.[6]

Saponification of III, Isolation of I——5 g of III was refluxed with 200 ml of 0.5N ethanolic NaOH for 3 hr. The cooled reaction mixture was diluted with 50 ml of H_2O, concentrated to 100 ml in vacuum and acidified with H_2SO_4. The crystalline precipitate which formed on standing was removed by filtration and recrystallized from 50% EtOH to give 3 g of I, mp 162—163°. *Anal.* Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.19; H, 5.71. The melting point was not depressed on admixture with the sample of I.

Identification of Angelic Acid——The acidic aqueous filtrate from above was extracted with four 50 ml portions of ether, which were combined and washed with five 30 ml portions of 5% Na_2CO_3. The carbonate wash was combined and acidified with H_2SO_4, and extracted with five 30 ml portions of ether, which were combined and evaporated. The residue was dissolved in 20 ml of *n*-hexane, and the solution was removed from a small amount of insoluble matter by decantation and evaporated.

The *p*-phenylphenacyl ester was prepared from the residue by the usual way and chromatographed over silica gel and eluted with *n*-hexane–EtOAc (9 : 1). The first fraction afforded colorless crystalline compound which was recrystallized from EtOH to platelets, mp 86—87°. The melting point was not depressed on admixture with the authentic sample of *p*-phenylphenacyl angelate. The second fraction afforded colorless crystalline compound which was recryrstallized from EtOH to needles, mp 102—103°. The melting point was not depressed on admixture with the authentic sample of *p*-phenylphenacyl tiglate.

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8) Measured in $CDCl_3$ solution by Varian Associates Recording Spectrometer A 60 D, TMS was used as inner standard.