[Chem. Pharm. Bull.]
[19(9)1963—1967(1971)]

UDC 547.597.02 : 581.192 : 582.893

Coumarins and a Sesquiterpene from the Crude Drug "Korean Qianghuo (韩国羌活)," the Roots of *Angelica* spp.KIYOSHI HATA, MITSUGI KOZAWA, KIMIYE BABA,^{1a)}HYUNG-JOON CHI and MASAO KONOSHIMA^{1b)}*Osaka College of Pharmacy^{1a)} and Faculty of Pharmaceutical Science, Kyoto University^{1b)}*

(Received April 12, 1971)

The chemical investigation of the crude drug "Korean Qianghuo (韩国羌活)," which has been assigned to the dried roots of *Angelica koreana* MAX. (Umbelliferae), was made previously by Ryu, *et al.*,²⁾ who reported the presence of several furocoumarins, *e.g.* isoimperatorin, oxypeucedanin, prangolarine, imperatorin and a new product named koreanin.³⁾ As a part of a series of the studies on the coumarins from the Umbelliferous plants, the re-examination of the chemical constituents of two kinds of this crude drug has now been made, and it was revealed that the plant materials were different entirely from each other in the chemical constituents as described subsequently.

The ether extract of a kind of the material upon the treatment described in the experimental afforded no furocoumarin but a sesquiterpene of mp 157—158°, C₁₅H₂₀O₃ (I) together with ferulic acid. The structure of I was elucidated as formula I, which is identical with that of bisabolangelone, a product from the seeds of *Angelica sylvestris* L., established by Novotný, *et al.*⁴⁾ The sesquiterpene (I) was published as the name angelikoreanol in the previous communication,⁵⁾ but it should be canceled because of the fact that I has recently been proved to be identical with bisabolangelone by the direct comparison of the spectral data and by the mixed melting point examination with the authentic sample of the latter. On the other hand, six known coumarins were isolated from the extract of another kind of the crude drug, which resembled to the former in the external appearance. Out of these coumarins, four were identified as isoimperatorin, oxypeucedanin, prangolarine and imperatorin, coinciding with the report by Ryu, *et al.*,²⁾ and the remainder as osthol and oxypeucedanin hydrate. Elaborate column chromatographic analysis did not succeed, however, to isolate the sesquiterpene (I) and ferulic acid as well as any new coumarin from the material. Thin-layer chromatography did not also show the presence of the sesquiterpene (I).

These findings have suggested that botanical re-examination of these materials should be made to make sure of their original plants. This will be discussed later, and in this paper the authors wish to describe the process of the structure elucidation of I which has been performed independently, resulting in the production of further evidence to the novel structure of bisabolangelone.

The sesquiterpene (I) was characterized as an α,β -unsaturated ketone with a hydroxyl from the properties and the spectral data. Acetylation of I by the usual way afforded a yellow

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5) K. Hata, M. Kozawa, K. Baba, M. Konoshima and H.J. Chi, *Tetrahedron Letters*, **1970**, 4379. The sesquiterpene (I) was earlier isolated together with the same furocoumarins as have been isolated from *Angelica koreana* MAX. by Ryu, *et al.*,²⁾ therefore the material reported in this communication was regarded to be assigned to the plant above mentioned, from which I was isolated as a new product. It has now been, however, revealed that it must be a mixture of two kinds of the material described in this paper.

resinous product under an exhibition of intense fluorescence continuing for nearly half an hour. The product was, however, characterized as not an acetate but possibly a dehydrate from the spectral data, suggesting that the hydroxyl of I is easily removed upon the treatment with dehydrating agents forming an unsaturated bond.

When I was treated with *p*-toluene sulfonic acid in anhydrous benzene at room temperature until the fluorescence almost disappeared, a product of mp 107—107.5°, $C_{15}H_{18}O_2$ (II) was formed in fairly good yield. The product (II) was characterized as a phenol from its properties and was proved to contain 5-methylresorcinol skeleton from the fact that orcinol was identified from the alkali fusion product of II.

On the other hand, II shows no ketone character towards carbonyl reagents and exhibits no absorption band corresponding to carbonyl group in the infrared (IR) spectrum. These findings indicate that II was formed from I by dehydration and by following aromatization which gave rise to the phenolic hydroxyl from the ketone group being in a six membered ring system.

The nuclear magnetic resonance (NMR) spectrum of II shows a series of signals which can be assigned to the protons of isopentenyl group, $-CH_2-CH=C(CH_3)_2$. The compound (II) took up 1 mole of hydrogen in ethanol over Adams catalyst to afford dihydro derivative of mp 95—96°, $C_{15}H_{20}O_2$ (III), which upon ozonolysis yielded *p*-oracetophenone (IV), identified by the comparison with the authentic sample, together with 4-methyl-*n*-valeric acid identified as its *p*-phenylphenacyl ester. On the basis of this evidence the structure of III was established as formula III, 2-(3-methylbutyl)-3,6-dimethyl-4-hydroxybenzofuran, and then taking into consideration the fact that the NMR spectrum of II is indicative of the presence of isopentenyl group, the structure of II is represented as formula II.

Moreover, when the treatment of I with *p*-toluene sulfonic acid was stopped while the intense fluorescence was observed in the reaction mixture, an orange colored product of mp 97—98° was formed. The NMR spectrum of this product agrees with the structure of formula V. Thus, the only one possible structure for I which is in accord with the NMR data and can give rise to II *via* V upon dehydration followed by aromatization is given as formula I.

Fig. 1

Experimental

Isolation of I and Ferulic Acid—The dried and crushed plant material (8.8 kg) was extracted with three 30 liters portions of ether at room temperature for 2 weeks. The ether solution was concentrated to dark brown oil (260 g). The oil deposited on standing in an icebox a crystalline substance, which was re-

moved by filtration, recrystallized from EtOH to yield 9 g of I. The oily filtrate was treated with three 2 liters portions of *n*-hexane and the solution was removed from insoluble matter by decantation. The insoluble portion was chromatographed over silica gel (2 kg) and eluted with *n*-hexane-EtOAc (3:1). The eluate was fractionated into one hundred and thirty 300 ml fractions. Combined fractions of No. 53-69 yielded 3.5 g of I, and that of No. 90-95 yielded 3.4 g of ferulic acid.

The *n*-hexane solution was evaporated and the residue was chromatographed over silica gel (2 kg) and eluted with *n*-hexane-EtOAc (4:1). The eluate was fractionated into eighty 300 ml fractions. Combined fractions of No. 58-80 yielded 1.7 g of I. The column was further eluted with *n*-hexane-EtOAc (3:1) to take fractions of No. 81-95, which yielded no product. Elution was continued with *n*-hexane-EtOAc (2:1) to take fractions of No. 96-118. Combined fractions of No. 108-114 yielded 0.1 g of ferulic acid.

I (Bisabolangelone)—Recrystallized from EtOH to colorless or pale yellow platelets, mp 157-158°, $[\alpha]_D^{25} + 132.2^\circ$ ($c = 1.2$ in CHCl_3), yield 14.2 g. It gradually transforms into yellow resinous substance during storage. This transformation occurs quickly under exposure to sun light. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{20}\text{O}_3$: C, 72.55; H, 8.12; mol. wt. 248.3. Found: C, 72.59; H, 8.23; mol. wt. (Mass) 248. IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1}$: 3300 (OH), 1640 (C=O). NMR (in CDCl_3) τ : 8.38 (3H, singlet, O-C-CH₃), 8.28, 8.22 (3H $\times$ 2, singlets, exhibiting slight splits, CH=C(CH₃)₂), 8.00 (3H, singlet, exhibiting slight splits, CH=C-CH₃), 7.35 (1H, doublet, $J = 7$ Hz, CH-CH-CO), 7.28 (2H, doublet, exhibiting slight splits, $J = 7$ Hz, CH=C-CH₂-CH), 6.52 (1H, singlet, disappears with D₂O, OH), 5.15 (1H, quartet, $J = 7$ Hz, CH₂-CH-CH), 4.67 (1H, doublet, $J = 11.5$ Hz, =CH-CH=), 4.05 (1H, multiplet, $J = \text{nearly } 1$ Hz, CH=C-CH₃), 4.00 (1H, doublet $\times$ multiplet, $J = 11.5$ Hz $\times$ nearly 1 Hz, =CH-CH=C(CH₃)₂). 2,4-Dinitrophenylhydrazine test solution gave orange-red colored precipitation in EtOH. The melting point was not depressed on admixture with the authentic sample of bisabolangelone.

Ferulic Acid—Recrystallized from EtOH-H₂O (1:1) in needles, mp 167-168°, yield 3.5 g. *Anal.* Calcd. for $\text{C}_{10}\text{H}_{10}\text{O}_4$: C, 61.85; H, 5.19. Found: C, 61.79; H, 5.26. The melting point was not depressed on admixture with the authentic sample of ferulic acid.

Catalytic Hydrogenation of I—Two g of I was added to 200 mg of prerduced Adams catalyst in 50 ml of EtOH, and was stirred in the presence of hydrogen until nearly 3 moles of hydrogen was taken up. The catalyst was filtered off, and EtOH was removed by means of a rotary evaporator. The residue was chromatographed over silica gel (60 g) and eluted with *n*-hexane-EtOAc (4:1). The eluate was fractionated into thirty 100 ml fractions. Combined fractions of No. 5-10 and No. 15-23 yielded colorless crystalline products of mp 143-143.5° (I-Ha) and mp 128-129° (I-Hb), respectively. I-Ha: yield 0.4 g. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{26}\text{O}_3$: C, 70.83; H, 10.30. Found: C, 70.95; H, 10.58. IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1}$: 3350 (OH), 1680 (C=O). I-Hb: yield 0.6 g. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{26}\text{O}_3$: C, 70.83; H, 10.30. Found: C, 70.57; H, 10.23. IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1}$: 3350 (OH), 1680 (C=O).

Treatment of I with *p*-Toluensulfonic Acid—To the solution of 2 g of I in 100 ml of anhydrous benzene 800 mg of *p*-toluene sulfonic acid was added and stirred at room temperature until the fluorescence almost disappeared (nearly 1.5 hr). The benzene solution was removed from *p*-toluene sulfonic acid by decantation, shaken with Na₂CO₃ powder, filtered, and benzene was removed by means of rotary evaporator. The residue was chromatographed over silica gel (50 g) and eluted with *n*-hexane-EtOAc (3:1). The eluate was fractionated into five 30 ml fractions. Combined fractions of No. 1-3 yielded crystalline product, which was recrystallized from *n*-hexane to colorless needles, mp 107-107.5°, yield 0.65 g. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{18}\text{O}_2$ (II): C, 78.23; H, 7.88. Found: C, 78.49; H, 7.82. IR $\nu_{\text{max}}^{\text{Nujol}} \text{ cm}^{-1}$: 3250 (OH), 1600 (aromatic ring). NMR (in CDCl_3) τ : 8.25 (6H, singlet, exhibiting slight splits, CH=C(CH₃)₂), 7.67, 7.63 (3H $\times$ 2, singlets, CH₃ on benzene and furan ring), 6.62 (2H, doublet, $J = 7$ Hz, =CH-CH₂), 5.13 (1H, broad singlet, phenolic OH), 4.67 (1H, triplet $\times$ multiplet, CH₂-CH=C(CH₃)₂), 3.67, 3.20 (1H $\times$ 2, doublet $\times$ multiplet, $J = 2$ Hz $\times$ nearly 1 Hz, aromatic protons). Phenol test with phosphomolybdic acid + NH₄OH and Gibbs reagent + NH₄OH gave blue color. Carbonyl test with 2,4-dinitrophenylhydrazine was negative.

Alkali Fusion of II—One g of II was heated with 4 g of KOH and 2 ml of H₂O in nickel crucible at 320-330° for 30 min. After cooling the reaction mixture was dissolved in 100 ml of H₂O, acidified with conc. HCl, and extracted with ether. The ether solution was shaken with 5% aqueous NaHCO₃ and evaporated to dryness. The residue was chromatographed over silica gel (20 g) and eluted with CHCl₃-acetone (10:1). The eluate was fractionated into one hundred and twenty 5 ml fractions. Combined fractions of No. 45-105 yielded pale yellow crystalline product, which was sublimated in vacuum to yield colorless crystalline powder, mp 106-107°, yield 25 mg. The melting point was not depressed on admixture with the authentic sample of orcinol.

Hydrogenation of II—Two g of II was added to 200 mg of prerduced Adams catalyst in 50 ml of EtOH and was stirred in the presence of hydrogen to take up 1.2 moles of H₂. The catalyst was filtered off, and EtOH was evaporated. The crystalline residue was recrystallized from *n*-hexane to colorless needles, mp 95-96°, yield 2 g. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{20}\text{O}_2$ (III): C, 77.55; H, 8.68. Found: C, 77.28; H, 8.44. NMR (in CDCl_3) τ : 9.12 (6H, doublet, $J = 6$ Hz, CH(CH₃)₂), nearly 8.5 (3H, multiplet, CH₂-CH₂-CH (CH₃)₂), 7.73, 7.70 (3H $\times$ 2, singlet, CH₃ on benzene and furan ring), 7.40 (2H, triplet, $J = 7$ Hz, CH₂-CH₂-C=), 5.1 (1H, broad singlet, phenolic OH), 3.73, 3.25 (1H $\times$ 2, doublet $\times$ multiplet, $J = 2$ Hz $\times$ nearly 1 Hz, aromatic protons).

Ozonolysis of III, Identification of *p*-Oracetophenone and 4-Methyl-1-*n*-valeric Acid—Two g of III was ozonized in 50 ml of EtOAc under cooling with ice and the solvent was removed by means of rotary

evaporator. To the dark brown oily residue 50 ml of 2% aqueous NaHSO_3 was added under cooling with ice and stirred for 20 min, gradually warmed on a water bath to 50° for 30 min, and extracted with ether. The ether solution was evaporated to yield dark brown viscid oil, which was heated with 20 ml of 4% ethanolic NaOH on a water bath for 1 hr. After cooling 10 ml of H_2O was added, concentrated by means of rotary evaporator to half volumes, acidified with H_2SO_4 and extracted with ether. The ether extract was washed with small amount of H_2O and extracted with saturated aqueous solution of NaHCO_3 . The ether layer was evaporated and the residue (0.9 g) was chromatographed over silica gel (50 g) and eluted with *n*-hexane-EtOAc (4:1). The elute was fractionated into fifteen 30 ml fractions. Combined fractions of No. 5—10 yielded crystalline product which was recrystallized from *n*-hexane-EtOAc (3:1) to pale yellow needles. Sublimation in vacuum gave colorless fine needles, mp $147\text{--}147.5^\circ$, yield 0.15 g. *Anal.* Calcd. for $\text{C}_9\text{H}_{10}\text{O}_3$ (IV): C, 65.05; H, 6.07. Found: C, 64.96; H, 6.07. NMR (in DMSO) τ : 7.79 (3H, singlet, CH_3 on benzene ring), 7.35 (3H, singlet, COCH_3), 3.75 (2H, singlet, aromatic protons), -1.92 (2H, singlet, phenolic OH). The melting point was not depressed on admixture with the authentic sample of *p*-oracetophenone.

The aqueous bicarbonate extract was acidified with H_2SO_4 and distilled to take nearly 40 ml of distillate. The distillate was exactly neutralized with 0.1N NaOH and evaporated to dryness. The residue was treated to prepare the *p*-phenylphenacyl ester by the usual way and the product was recrystallized from EtOH to colorless platelets, mp $68\text{--}68.5^\circ$, yield 0.7 g. *Anal.* Calcd. for $\text{C}_{20}\text{H}_{22}\text{O}_3$: C, 77.39; H, 7.14. Found: C, 77.17; H, 7.02. The melting point was not depressed on admixture with the authentic sample of *p*-phenylphenacyl 4-methyl-*n*-valerate.

Controlled Reaction of I with *p*-Toluene Sulfonic Acid, Formation of V—To the solution of 1 g of I in 80 ml of anhydrous benzene 1 g of *p*-toluene sulfonic acid was added and stirred at room temperature for 30 min. The benzene solution was removed by decantation, shaken with Na_2CO_3 powder, filtered, and benzene was removed by means of rotary evaporator. The residue was chromatographed over silica gel (50 g) and eluted with *n*-hexane-EtOAc (3:1). The eluate was fractionated into five 50 ml fractions. The fraction of No. 3 yielded orange colored crystalline substance which was recrystallized from EtOH and sublimated in vacuum to fine needles, mp $97\text{--}98^\circ$. Ethanolic solution exhibits intense yellowish green fluorescence under ordinary light. Carbonyl test with 2,4-dinitrophenylhydrazine gave red color in EtOH. NMR (in CDCl_3) τ : 8.20, 8.15 (3H $\times$ 2, singlet, exhibiting slight splits, $\text{CH}=\text{C}(\text{CH}_3)_2$), 7.98 (3H, singlet, exhibiting slight splits, $\text{CH}=\text{C}-\text{CH}_3$), 7.83 (3H, singlet, $=\text{C}-\text{CH}_3$), 7.56, 7.22 (2H, AB portion of ABX pattern, $J_{\text{AB}}=16.5$ Hz, $J_{\text{AX}}=6.5$ Hz, $J_{\text{BX}}=8$ Hz), 4.63 (1H, X portion of ABX pattern), 4.45 (1H, doublet, $J=11.5$ Hz, $=\text{CH}-\text{CH}=\text{C}$), 3.97 (1H, multiplet, $J=\text{nearly } 1$ Hz, $\text{CH}=\text{C}-\text{CH}_3$), 3.73 (1H, doublet $\times$ multiplet, $J=11.5$ Hz $\times$ nearly 1 Hz, $=\text{CH}-\text{CH}=\text{C}(\text{CH}_3)_2$).

Isolation of Coumarins—The dried and crushed plant material (10 kg) was extracted with three 30 liters portions of ether at room temperature for 2 weeks. The ether solution was concentrated to dark brown oil (230 g). The oil deposited on standing in an icebox a crystalline substance, which was removed by filtration, recrystallized with EtOH to yield oxypeucedanin (9.6 g).

The oily filtrate was treated with three 2 liters portions of *n*-hexane, and the solution was removed from insoluble matter by decantation, evaporated, and the residue (132.3 g) was chromatographed over silica gel (2 kg) and eluted with a mixture of *n*-hexane and EtOAc. The eluate was fractionated into one hundred and thirty 300 ml fractions. Combined fractions of No. 15—26 eluted with the mixture of ratio 4:1 yielded isoimperatorin (0.6 g), those of No. 27—37, No. 48—62 and No. 111—117 eluted with the mixture of ratio 3:1 yielded osthol (2.6 g), imperatorin (1.5 g) and prangolarine (0.5 g), respectively.

The insoluble portion (90 g) was chromatographed over silica gel (2 kg) and eluted with a mixture of *n*-hexane and EtOAc. The eluate was fractionated into one hundred and forty 200 ml fractions. Combined fractions of No. 10—13 eluted with the mixture of ratio 4:1 yielded isoimperatorin (0.9 g), those of No. 16—24 and No. 25—32 eluted with the mixture of ratio 3:1 yielded osthol (0.9 g) and imperatorin (3.9 g), that of No. 56—66 eluted with the mixture of ratio 2:1 yielded prangolarine (2.7 g), and that of No. 121—128 eluted with the mixture of ratio 1:2 yielded oxypeucedanin hydrate (0.1 g).

Oxypeucedanin—Recrystallized from EtOH to colorless platelets, mp $141\text{--}142^\circ$, yield 9.6 g. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_5$: C, 67.12; H, 4.93. Found: C, 66.99; H, 4.83. The melting point was not depressed on admixture with the authentic sample of oxypeucedanin.

Isoimperatorin—Recrystallized from EtOH to colorless needles, mp $108\text{--}109^\circ$, yield 1.5 g. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_5$: C, 71.10; H, 5.22. Found: C, 71.13; H, 5.29. The melting point was not depressed on admixture of the authentic sample of isoimperatorin.

Osthol—Recrystallized from EtOH to colorless prisms, mp $82\text{--}84^\circ$, yield 3.5 g. *Anal.* Calcd. for $\text{C}_{15}\text{H}_{16}\text{O}_3$: C, 73.75; H, 6.60. Found: C, 73.72; H, 6.77. The melting point was not depressed on admixture with the authentic sample of osthol.

Imperatorin—Recrystallized from EtOH to colorless needles, mp $100\text{--}101^\circ$, yield 5.4 g. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_5$: C, 71.10; H, 5.22. Found: C, 71.19; H, 5.51. The melting point was not depressed on admixture with the authentic sample of imperatorin.

Prangolarine—Recrystallized from EtOH to colorless platelets, mp $103\text{--}105^\circ$, $[\alpha]_D^{25} +19.3^\circ$ ($c=1.3$

in CHCl_3) [ref. $[\alpha]_D^{20} + 20.1^\circ (\text{CHCl}_3)$],⁶⁾ yield 3.2 g. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{14}\text{O}_5$: C, 67.12; H, 4.93. Found: C, 67.23; H, 5.22. The IR and NMR spectra are superimposable to those of oxypeucedanin.

Oxypeucedanin Hydrate—Recrystallized from $\text{EtOH-H}_2\text{O}$ to colorless needles, mp $136\text{--}137^\circ$, yield 0.1 g. *Anal.* Calcd. for $\text{C}_{16}\text{H}_{16}\text{O}_6$: C, 63.15; H, 5.30. Found: C, 63.29; H, 5.37. The melting point was not depressed on admixture with the authentic sample of oxypeucedanin hydrate.

Acknowledgement The authors are grateful to Dr. L. Novotny, Czechoslovak Academy of Science, Institute of Organic Chemistry and Biochemistry, for supplying the sample of bisabolangelone. They are also indebted to Dr. A. Numata, Osaka College of Pharmacy, for the NMR spectra, to Miss S. Fujimoto of this College for the microanalysis, and to Mr. A. Kato, Faculty of Pharmaceutical Science, Kyoto University, for the mass spectra.

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[Chem. Pharm. Bull.
19(9)1967—1970(1971)]

UDC 547.654.1.09 : 547.495.2.08.09

Quantitative Determination of 2-Amino-1-naphthyl Glucosiduronic Acid in Urine of the Dog dosed with 2-Naphthylamine

SHIGERU TAKANASHI, YOSHIKAZU HINOHARA,^{1a)} YASUYO WATANABE
and MASASHI OKADA^{1b)}

*Research Laboratories, Chugai Pharmaceutical Co., Ltd.^{1a)} and
Tokyo Biochemical Research Institute^{1b)}*

(Received April 14, 1971)

2-Naphthylamine (I) is a potent bladder carcinogen in the dog and man, the metabolism of which has been studied extensively in several species.^{2,3)} Among many metabolites so far isolated or detected, 2-amino-1-naphthol (IIa) and N-hydroxy-2-naphthylamine (IIIa) are regarded as the proximal carcinogen directly responsible for the production of bladder cancer, since they have been shown to be much more carcinogenic in the mouse⁴⁾ and rat⁵⁾ than the parent amine I. These carcinogenic metabolites of I are said to be excreted into urine mainly in the form of conjugates; *i.e.* O-glucuronide, O-sulfate, *etc.*^{2a)} While the O-glucuronide of IIa, 2-amino-1-naphthyl glucosiduronic acid (IIb), was unequivocally identified in urine of the dog, rat, or rabbit dosed with I, no definite evidence has been found^{2d)} for the presence of the O-glucuronide of IIIa, N-hydroxy-2-naphthylamine-O-glucosiduronic acid (IIIb), as a metabolite of I or IIIa in urine of the dog, guinea pig, hamster, rabbit, or rat. It was reasonably postulated^{2a,6)} concerning the mechanism of bladder carcinogenesis by I in the dog

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