Moreover, although sulfanilamide exists mostly as the unionized form in both buffer solutions at pH 6.0 and 7.5 and is most likely absorbed as the unionized form in these buffer solutions, the absorption of the drug is significantly reduced in the presence of K^+. This phenomenon may illustrate that the unionized form of sulfanilamide is not sufficiently lipid soluble (see the partition coefficient in Table IV), and hence that significant amounts of unionized form of the drug can penetrate through the intercellular channels. In order to examine the effect of various cations on the gastrointestinal absorption of drugs, further studies are now in progress.

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Coumarins from the Roots of *Angelica morii* HAYATA

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Angelica morii HAYATA (Japanese name "mori-zengo") (Umbelliferae) is a perenial herb growing in Taiwan, and is called "shan-duhuo (山独活)" which is used as a drug of folk remedy in Taiwan.[2] There is no record of chemical investigation on this plant in literatures. This paper presents the results from an investigation of the coumarins in the plant.

The ether extract of the dried root of this plant upon fractionation over the column of silica gel afforded, in addition to small amount of psoralen, bergapten, umbelliferone and *p*-coumaric acid identified by the comparison with the authentic samples, two crystalline compounds of mp 82—84° (I) and mp 119—120° (II). On the basis of the evidences described below the compounds I and II were identified as pteryxin[3] (3′(*R*), 4′(*R*)-3′-acetoxy-4′-angeloyloxy-3′,4′-dihydroseselin)[4] (Ia) and 3′(*R*),4′(*R*)-3′-acetoxy-4′-senecioyoxy-3′,4′-dihydroseselin[4] (IIa), respectively. Furthermore, the presence of the analogous diester (III) of acetic acid and isovaleric acid was suggested by the nuclear magnetic resonance (NMR) spectrum, but the isolation of III has not so far been successful because III can be hardly freed from the contamination by I. The phenolic fraction of the ether extract afforded a crystaline compound of mp 329—330° (decomp.) (IV), which seems to be a bis-coumarin derivative formed of 2 moles of umbelliferone moieties by the spectral data. It is under investigation on the details.

The compound I was suggested to be O-acetyl-O-angeloylkhellactone by the NMR spectrum, and this was confirmed by the treatment of I with ethanolic sodium hydroxide to give (+)-*cis*-ethylkhellactone (Va), (—)-*trans*-ethylkhellactone (Vb), acetic acid and angelic acid. As the compound of this structure two isomeric diesters, Ia (mp 81.5—82.5°)[3] and isopteryxin

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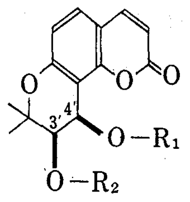
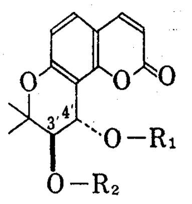
1) Location: *a) Kawai-cho, Matsubara, Osaka*; *b) No. 250, Wu-Shing Street, Taipei, Taiwan, China.*
2) Woei-Song Gan, "Manual of Medicinal Plants in Taiwan," Vol. 3, National Research Institute of Chinese Medicine, Taipei, 1965, p. 646.
3) R.E. Willette and T.O. Soine, *J. Pharm. Sci.*, **51**, 149 (1962).
4) *a)* J. Lemmich, E. Lemmich, and B.E. Nielsen, *Acta Chem. Scand.*, **20**, 2497 (1966); *b)* J. Lemmich, P.A. Pederson, and B.E. Nielsen, *Tetrahedron Letters*, **1969**, 3365.

(mp 135—136°)[5)] (Ib) have been known and the former can be considered to be identical with I from the melting points. The comparison of the NMR spectra of I and Ib[5b)] also provides support for the assignment of I to Ia rather than Ib, *i.e.*, in the NMR spectrum of I the signal arising from the 3′-proton at τ 3.78 shows an upper chemical shift by *ca.* 5 cps compared to the corresponding signal of Ib, while the 4′-proton is visible as the signal at τ 2.38 which is in *ca.* 2 cps lower field than that of Ib, indicating that acetoxy group, having smaller paramagnetic effect than angeloyloxy group, is located in the 3′ position of I. On the other hand, Ia was reported to be *dextro*-rotatory ($[\alpha]_D^{25}$ +9.8° (ethanol)),[4a)] whereas I was found to be slightly *levo*-rotatory ($[\alpha]_D^{22}$ −3.1° (ethanol)). This finding suggests that the investigation of the configurations at C-3′ and 4′ of I should be made in oder to examine the identity of I and Ia.

Firstly, I was proved to have 3′(*R*)-configuration from the fact that solvolysis of I with ethanolic alkali afforded Va and Vb, whose configurations at C-3′ and 4′ have been established as 3′(*R*), 4′(*R*) and 3′(*R*), 4′(*S*), respectively.[4b,6)] Therefore, if I is not identical with Ia in the configurations, it must be 4′-epimer of Ia and, incidentally, its relative configurations must be *trans*. Thus the problem appears to be solved by observation of the coupling constant of 3′ and 4′ protons ($J_{3',4'}$) in the NMR spectrum. It has, however, been shown that several *trans*-khellactone derivatives exhibit considerably fluctuating values of $J_{3',4'}$ dependent on the 3′ and 4′ substituents, *e.g.*, *trans*-khellactone 6.9 cps, *trans*-diacetylkhellactone 4.4 cps, *trans*-disenecioylkhellactone 3.5 cps, *trans*-methylkhellactone 3.0 cps, and that the corresponding *cis*-epimers as well as Ia show $J_{3',4'}$ of 5.0 cps constantly.[4a)] Moreover, the value of $J_{3',4'}$ of the *trans*-epimer of Ia cannot be found in literatures. Therefore, although I is



Ia: R_1=angeloyl, R_2=acetyl
Ib: R_1=acetyl, R_2=angeloyl
IIa: R_1=senecioyl, R_2=acetyl
IIb: R_1=acetyl, R_2=senecioyl
IIIa: R_1=isovaleroyl, R_2=acetyl
IIIb: R_1=acetyl, R_2=isovaleroyl
Va: R_1=C_2H_5, R_2=H
VIa: R_1=R_2=acetyl



Vb: R_1=C_2H_5, R_2=H
VIb: R_1=R_2=acetyl

Chart 1

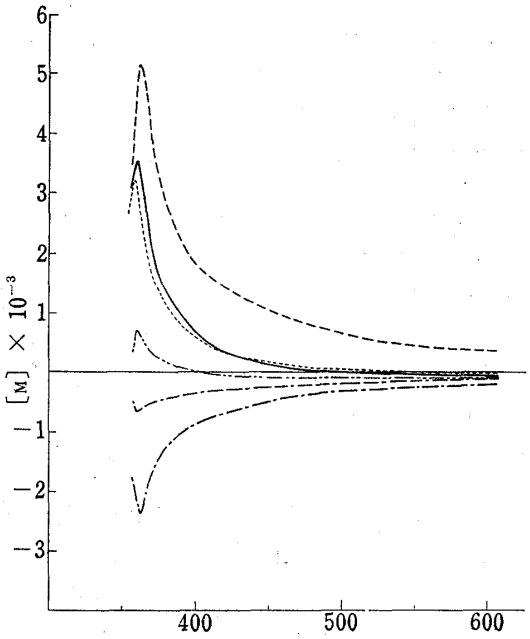


Fig. 1. ORD Curves of the Khellactone Derivatives

-------: I ———: II
– – – –: *cis*-ethylkhellactone (Va)
—·—·—: *trans*-ethylkhellactone (Vb)
-----——: *cis*-diacetylkhellactone (VIa)
—–·—–: *trans*-diacetylkhellactone (VIb)

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5) *a)* B.E. Nielsen and T.O. Soine, *J. Pharm. Sci.*, **56**, 184 (1967); *b)* K. Hata, M. Kozawa, K. Baba, and M. Mitsui, *Yakugaku Zasshi*, **93**, 248 (1973).
6) H.B-Wulf, A. Niggli, L. Ulrich, and H. Schmid, *Helv. Chim. Acta* **52**, 1165 (1965).

suggested to have the *cis*-configuration from its $J_{3',4'}$ found to be 5.0 cps, it should be re-examined by another method.

In order to find the answer to this problem, the optical rotatory dispersion (ORD) of the khellactone derivatives were examined and the results shown in Fig. 1 were obtained. From this observation it was revealed that the compounds with the configurations of 3'(*R*), 4'(*R*), *i.e.* Va and (−)-*cis*-diacetylkhellactone (VIa) show a positive Cotton effect at wave length of *ca.* 360 mμ and that the corresponding 4'-epimers, *i.e.* Vb and (−)-*trans*-diacetylkhellactone (VIb) show a negative one in the same region, indicating that the configuration of 4' can be determined by the direction of the Cotton effect. Thus I was established to be identical with Ia. Therefore, the discrepancy observed between the optical rotation of I and Ia seems to be caused by difference of the condition of the measurement, and this will be investigated later.

The NMR spectrum of II is suggestive of the presence of the khellactone moiety, acetyl and senecioyl groups. The evidence for the assignment of II to O-acetyl-O-senecioylkhellactone was afforded by the solvolysis of II with ethanolic sodium hydroxide resulting in the formation of Va, Vb, acetic acid and senecioic acid. The configurations at C-3' and 4' of II was determined to be 3'(*R*), 4'(*R*) by the same way as I. Thus the melting point of II indicates that II must be identical with IIa (mp 120.5—121°),4a) while the isomeric compound, samidin (mp 135—137°)7) (IIb) differs from II in the melting point.

The catalytic hydrogenation of II using Adams catalyst resulted with the facile uptake of one mole of hydrogen in the formation of a dihydro derivative of mp 139—140° (III), which was shown to be O-acetyl-O-isovaleroylkhellactone by its NMR spectrum. The melting point of III is identical with that of suksdorfin (mp 140.5—141°)3) (IIIa), the dihydro derivative of IIa, and obviously differs from that of the isomer, dihydrosamidin (mp 117—119°)7) (IIIb), providing further evidence for the assignment of II to IIa.

Experimental

Isolation of the Compounds——The dried and crushed roots (8.7 kg) of the plant collected in Kun-yan (昆陽), Nan-tou Country (南投県) of Taiwan in Aug., 1971 was extracted with three 20 liter portions of ether at room temperature for 2 weeks. The ether solution was concentrated to brown oil (320 g). The oil was treated with hexane and was divided into soluble (184 g) and insoluble (136 g) fractions.

The hexane soluble fraction was divided into three equal parts and each of them was chromatographed over the column of silica gel (2 kg) with hexane: EtOAc and the eluate was divided into following fractions: No. 1—38 (5: 1), No. 39—76 (4: 1), No. 77—130 (3: 1), No. 131—155 (2: 1), No. 156—167 (1: 1) [400 ml/fraction; numbers in parentheses show ratio of the solvents in v/v].

The fractions of No. 86—92 and those of No. 131—155 gave resinous substance and umbelliferone (0.07 g), respectively, and the former upon rechromatography over silica gel (80 g) with $CHCl_3$ gave psoralen (0.05 g), bergapten (0.005 g) and Ia contaminated by III (1.45 g). The substance from the fractions of No. 93—130 was chromatographed over the column of silica gel (600 g) with $CHCl_3$ and the eluate was devided into 39 fractions (150 ml/fraction). The fractions of No. 1—7 and those of No. 36—39 gave psoralen (0.03 g) and IIa (3.4 g), respectively. The substance from the fractions of No. 8—24 upon rechromatography over silica gel (150 g) with $CHCl_3$ gave Ia (2.6 g) and IIa contaminated by Ia (3.9 g). The substance from the fractions of No. 25—35 upon rechromatography over silica gel (350 g) with $CHCl_3$ gave Ia (0.7 g), IIa contaminated by Ia (7.9 g) and IIa (5.5 g) (The yields show those from the total hexane soluble fraction).

The hexane insoluble fraction (57 g) was chromatographed over the column of silica gel (1.8 kg) with hexane: EtOAc and the eluate was devided into following fractions: No. 1—23 (4: 1), No. 24—71 (3: 1), No. 72—92 (2: 1), No. 93—114 (1: 1) (500 ml/fraction). The fractions of No. 36—50 gave IIa contaminated by Ia (30 g), those of No. 51—64 upon rechromatography over silica gel (220 g) with $CHCl_3$ gave psoralen (0.05 g) and IIa (7.4 g), and those of No. 72—92 and those of No. 93—109 gave umbelliferone (0.9 g) and *p*-coumaric acid (0.19 g), respectively.

The hexane insoluble fraction (79 g) was dissolved in ether and the ether solution was extracted with four 50 ml portions of cold 5%NaOH, the alkaline extract was acidified and extracted with ether, the ether

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7) E. Smith, N. Hosansky, W.G. Bywater and E.E.v. Tamelen, *J. Am. Chem. Soc.*, 79, 3534 (1957).

solution was concentrated and the residue was chromatographed over the column of silica gel (600 g) with hexane: EtOAc and the eluate was divided into following fractions: No. 1—10 (3: 1), No. 11—20 (2: 1), No. 21—40 (1: 1), No. 41—42 (EtOAc) (500 ml/fraction). The fractions of No. 17—18, those of No. 21—24 and those of No. 34—39 gave umbelliferone (1 g), *p*-coumaric acid (0.26 g) and IV (0.08 g), respectively.

Psoralen——Colorless needles from hexane: EtOAc, mp 162—163°. The melting point showed no depression on admixture with authentic sample of psoralen. Yield 0.002%.

Bergapten——Fine colorless needles from hexane: EtOAc, mp 191.5—193°. The melting point showed no depression on admixture with authentic sample of bergapten. Yield *ca.* 0.00006%.

Umbelliferone——Colorless crystalline powder from hexane: EtOAc, mp 220—224°. The melting point showed no depression on admixture with authentic sample of umbelliferone. Yield 0.022%.

***p*-Coumaric Acid**——Colorless crystalline powder by sublimation in vacuum, mp 208.5—209.5° (decomp.). The infrared (IR) and NMR spectra are identical with those of authentic sample of *p*-coumaric acid. The melting point with decomposition showed no depression on admixture with the authentic sample. Yield 0.005%.

Pteryxin (Ia)——Colorless needles from petr. ether: ether, mp 82—84°, $[\alpha]_D^{32}$ $-3.1°$ (c=1.28, EtOH). IR ν_{max}^{Nujol} cm^{-1}: 1740 broad (C=O), 1640 (C=C), 1605 (aromatic ring). NMR (in $CDCl_3$) τ[8]: 8.53 (6H, singlet, $\mathrm{C\underline{H}_3}$-C-$\mathrm{C\underline{H}_3}$), 8.12 (3H, broad singlet, $\mathrm{C\underline{H}_3}$-C=CH), 8.02 (3H, broad doublet, J=10 cps, $\mathrm{C\underline{H}_3}$-CH=C), 7.89 (3H, singlet, $\mathrm{C\underline{H}_3CO}$), *ca.* 3.9 (1H, broad quartet, $\mathrm{CH_3}$-C=C$\underline{\mathrm{H}}$-$\mathrm{CH_3}$), 4.64, 3.36 (1H×2, doublets, J=5.0 cps, O-C$\underline{\mathrm{H}}$-C$\underline{\mathrm{H}}$-O), 3.78, 2.38 (1H×2, doublets, J=9.5 cps, C$\underline{\mathrm{H}}$=C$\underline{\mathrm{H}}$), 3.22, 2.60 (1H×2, doublets, J=8.5 cps, aromatic H×2). Yield 0.038%.[9]

3′(*R*),4′(*R*)-3′-Acetoxy-4′-senecioyloxy-3′,4′-dihydroseselin (IIa)——Colorless needles from hexane: EtOAc, mp 119—120°, $[\alpha]_D^{23}$ $-15.2°$ (c=1.04, EtOH). IR ν_{max}^{Nujol} cm^{-1}: 1740 broad (C=O), 1650 (C=C), 1600 (aromatic ring). NMR (in $CDCl_3$) τ: 8.57, 8.53 (3H×2, singlets, $\mathrm{C\underline{H}_3}$-C-$\mathrm{C\underline{H}_3}$), 8.11, 7.78 (3H×2, broad singlets, $\mathrm{(C\underline{H}_3)_2C}$=CH), 7.91 (3H, singlet, $\mathrm{C\underline{H}_3CO}$), 4.70, 3.41 (1H×2, doublets, J=5.0 cps, O-C$\underline{\mathrm{H}}$-C$\underline{\mathrm{H}}$-O), 4.35 (1H, broad singlet, $\mathrm{(CH_3)_2C}$=C$\underline{\mathrm{H}}$), 3.80, 2.41 (1H×2, doublets, J=9.5 cps, C$\underline{\mathrm{H}}$=C$\underline{\mathrm{H}}$), 3.21, 2.63 (1H×2, doublets, J=8.5 cps, aromatic H×2). Yield 0.3%.[9]

Compound IV——Colorless crystalline powder from EtOH, mp 329—330° (decomp.). Slightly soluble in ordinary organic solvents, insoluble in saturated $NaHCO_3$, soluble in DMSO, pyridine and 5%NaOH. Phenolic color tests gave no reaction. UV $\lambda_{max}^{ethanol}$ mμ ($E_{1cm}^{1\%}$) 223 sh. (640), 248 (200), 259 (195), 327 (635). IR ν_{max}^{Nujol} cm^{-1}: 3250 (OH), 1720—1680 (C=O), 1600 (aromatic ring). NMR (in DMSO-d_6) τ: 8.75, 8.63 (3H×2, singlets, $\mathrm{(C\underline{H}_3)_2C}$), 6.05, 5.59 (1H×2, doublets, J=8.5 cps, C$\underline{\mathrm{H}}$-C$\underline{\mathrm{H}}$), 3.87, 3.83, 2.08 (1H×2 and 2H, doublets, J=9.5 cps, C$\underline{\mathrm{H}}$=C$\underline{\mathrm{H}}$×2), 3.20, 2.70 (1H×2, singlets, aromatic H×2), 3.09, 2.46 (1H×2, doublets, J=8.5 cps, aromatic H×2). Yield 0.0016%.

Saponification of Ia with NaOH (EtOH), Formation of Va, Vb, Acetic Acid and Angelic Acid——To the solution of 500 mg of Ia in 30 ml of EtOH, 10 ml of 1N NaOH (EtOH) was added and the mixture was allowed to stand at room temperature. After 15 min the solution was diluted with 200 ml of water, acidified with 20%H_2SO_4 and extracted with ether. The ether solution was washed with 5% Na_2CO_3 and evaporated. The residue upon chromatography over silica gel (15 g) with hexane: EtOAc (4: 1) gave two crystalline substances (i) and (ii). (i): Colorless needles from hexane: EtOAc, mp 126—127°. The melting point showed no change on admixture with authentic sample of Va.[10] Yield 38 mg. (ii): Colorless needles from hexane: EtOAc, mp 159—160°. The melting point showed no change on admixture with authentic sample of Vb.[10] Yield 39 mg.

The 5% Na_2CO_3 washing was acidified, extracted with ether and the ether solution was evaporated. The residue was led to *p*-phenylphenacyl ester in the usual manner. The crude product upon chromatography over silica gel (10 g) with $CHCl_3$ gave two crystalline substances (iii) and (iv). (iii): Colorless platelets from hexane: EtOAc, mp 86—87°. The melting point showed no depression on admixture with authentic sample of *p*-phenylphenacyl angelate. Yield 70 mg. (iv): Colorless platelets from hexane: EtOAc, mp 111—112°. The melting point showed no depression on admixture with authentic sample of *p*-phenylphenacyl acetate. Yield 53 mg.

Saponification of IIa with NaOH (EtOH), Formation of Va, Vb, Acetic Acid and Senecioic Acid——To the solution of 500 mg of IIa in 30 ml of EtOH, 10 ml of 1N NaOH (EtOH) was added and the solution was treated in the same way as Ia. The neutral fraction upon chromatography over silica gel (15 g) with hexane: EtOAc (4: 1) gave two crystalline substances (i) and (ii). (i): Colorless Needles from Hexane: EtOAc, mp 126—127°. The melting point showed no change on admixture with authentic sample of Va. Yield 44 mg.

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[8] NMR spectra were measured by means of NEVA Model A-60D Analytical NMR Spectrometer using TMS as internal standard.

[9] The yield was shown as that of the substance obtained purely. Besides the pure substances, Ia contaminated by III, and IIa by Ia were obtained in the yields of 0.016% and 0.95%, respectively.

[10] The melting point was known to show a fair rise on admixture with the optical antipode. [K. Hata, M. Kozawa, Y. Ikeshiro, and K.-Y. Yen, *Yakugaku Zasshi*, **88**, 513 (1968)].

(ii): Colorless Needles from Hexane: EtOAc, mp 159—160°. The melting point showed no change on admixture with authentic sample of Vb. Yield 48 mg.

The acid fraction was led to *p*-phenylphenacyl ester in the usual manner. The crude product upon chromatography over silica gel (10 g) with $CHCl_3$ gave two crystalline substances (iii) and (iv). (iii): Colorless platelets from hexane: EtOAc, mp 141—142°. The melting point showed no depression on admixture with authentic sample of *p*-phenylphenacyl senecioate. Yield 67 mg. (iv): Colorless platelets from hexane: EtOAc, mp 111—112°. The melting point showed no depression on admixture with authentic sample of *p*-phenylphenacyl acetate. Yield 51 mg.

Catalytic Hydrogenation of IIa, into IIIa——To 300 mg of prereduced Adams catalyst in 25 ml of EtOH, the solution of 800 mg of IIa in 20 ml of EtOH was added and the mixture was stirred in the presence of hydrogen for 20 min (*ca.* 1 mole of H_2 was taken up). The catalyst was filtered off, and EtOH was removed *in vacuo*. The residue upon chromatography over silica gel (40 g) with $CHCl_3$ gave a crystalline substance. Colorless needles from hexane: EtOAc, mp 139—140°. NMR (in $CDCl_3$) τ: 9.00 (6H, doublet, $J=6.5$ cps, $(C\underline{H}_3)_2CH$), 8.57, 8.53 (3H × 2, singlets, $(C\underline{H}_3)_2C$), 7.88 (3H, singlet, $C\underline{H}_3CO$), *ca.* 7.8 (1H, multiplet, $CH_2C\underline{H}(CH_3)_2$), 7.73 (2H, doublet, $J=ca.$ 3 cps, $C\underline{H}_2$-CH), 4.69, 3.45 (1H × 2, doublets, $J=5.0$ cps, O-C$\underline{H}$-C$\underline{H}$-O), 3.78, 2.39 (1H × 2, doublets, $J=9.5$ cps, C$\underline{H}$=C$\underline{H}$), 3.21, 2.63 (1H × 2, doublets, $J=8.5$ cps, aromatic H × 2). Yield 500 mg.

ORD Data——

		λ (mμ)					
		589	550	500	450	400	380
$[M]^{23\,\circ}$ (EtOH)	I :	− 12.6	0	+ 42.0	+165	+587	+1143
	II :	− 58.8	− 44.1	0	+118	+662	+1323
	Va :	+404	+454	+656	+1009	+1816	+2774
	Vb :	−221	−276	−331	−497	−884	−1271
	VIa:	− 95.7	− 95.7	− 95.7	− 95.7	0	+172
	IVb:	−119	−138	−178	−257	−356	−455

		λ (mμ)				
		370	362	360	358	356
$[M]^{23\,\circ}$ (EtOH)	I :	+1625			+3196	+2640
	II :	+1985			+3529	+3087
	Va :	+3682	+5144			+3043
	Vb :	−1657	−2375			−1491
	VIa:	+325		+689		+306
	VIb:	−514		−692		−573

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