Des-[Ala^1-Gly^2]-Somatostatin——According to Sakakibara, *et al.*,[12] the above protected dodecapeptide (0.15 g) was treated with HF (approximately 5 ml) in the presence of anisole (0.5 ml) at 0° for 45 min. The excess HF was removed by evaporation and the residue was treated with dry ether. The resulting fine powder was then dissolved in H_2O (1200 ml) and the solution, after adjusting the pH to 6.5 with 10% NH_4OH, was kept on standing at room temperature for 72 hr. The solution was applied to a column of Amberlite IRC-50 (3×5.6 cm), which was washed with H_2O (100 ml). The product retained in the column was eluted with the solvent system of pyridine–AcOH–H_2O (30: 4: 66) as stated above. Fractions (tube No. 7—17) positive to ninhydrin and Ehrich tests (main spot Rf_4 0.44) were combined and the solvent was evaporated and the residue was lyophilized; yield 52 mg (43%). Amino acid ratios in a 3N Tos–OH hydrolysate: 1/2 Cys 1.34, Lys 1.90, Asp 0.93, Phe 3.37, Trp 0.79, Thr 2.27; Ser 1.00 (average recovery 89%).

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Isolation of a New Isoflavone from Chinese *Pueraria* Flowers

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A new isoflavone, 6,4′-dihydroxy-7-methoxy-isoflavone, was isolated from Chinese *Pueraria* flowers.

Pueraria flowers have been used for the treatment of crapulence as a folk medicine in China, Korea, Formosa and Japan. In our previous paper,[2] it has been reported that irisolidone-7-O-glucoside from Japanese *Pueraria* flowers (*Pueraria lobata* (Will.)Ohwi)and tectoridin from Formosan *Pueraria* flowers (*P. montana*(Lour.)Merril)were isolated. Recently, isolation of irisolidone, genistein, daizein and biochanin A as the isoflavonoids and of quercetin as the flavonoid in addition to the essential oily components from the fresh flowers of *Pueraria thunbergiana* Benth.(=*P. lobata* (Will.) Ohwi) was reported by Kurihara and Kikuchi.[3] The present paper is concerned with the isolation and the structure of 6,4′-dihydroxy-7-methoxy-isoflavone from Chinese *Pueraria* flowers[4] (Chinese crude drug, "Gehua, 葛花").

Thin-layer chromatogram (TLC) of 70% methanol extract of the flowers on silica gel plate revealed the presence of several components. Isolation of the components was carried out as shown in Chart 1. The mixture of the isoflavones was subjected to silica gel column chromatography to give an isoflavone named kakkatin (I), mp over 290°, showing one spot on TLC. Final purification was effected by recrystallization from methanol. I was analyzed for $C_{16}H_{12}O_5$. The ultraviolet (UV) spectrum of I exhibited the charactristics of the isoflavone.[5] Additionally, color tests also indicated the isoflavone character of I; a yellow color appeared when the compound was added to aqueous sodium hydroxide, to concentrated

1) Location: *a) 3-4, Kowakae, Higashiosaka, Osaka; b) 33-94, Enoki-cho, Suita, Osaka.*
2) M. Kubo, K. Fujita, H. Nishimura, S. Naruto, and K. Namba, *Phytochemistry*, **12**, 2547 (1973).
3) T. Kurihara and M. Kikuchi, *Yakugaku Zasshi*, **93**, 1201 (1973).
4) Original plant is unidentified.
5) W.D. Ollis, "The Chemistry of Flavonoid Compounds," ed. by T.A. Geissman, Pergamon Press, New York, 1962, p. 353.

Isolation Procedure of Kakkatin

Pueraria flowers

↓ extd. with 70% MeOH

aqueous concentrate

↓ stirrd. with ether

ether layer | ppt | H_2O layer

ppt → chromatographed on silica gel

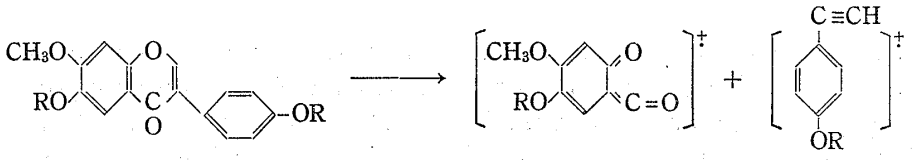
first eluate

↓ recrystallized from MeOH

isoflavone (I), mp over 290°

Chart 1

sulfuric acid, or to magnesium-hydrochloric acid.[5] I showed the absorption bands due to hydroxyl groups at 3400 cm^{-1} and a conjugated carbonyl group at 1620 cm^{-1} in the infrared (IR) spectrum and the absorption maxima at 263 (log ε=4.45) and 322 nm (log ε=4.07) in its UV spectrum. In the mass spectrum of I, the molecular ion peak appeared at *m/e* 284. Scince two oxygens are incorporated within the isoflavone skeleton, it is assumed that I must possess three oxygenated substituents. Its nuclear magnetic resonance (NMR) spectrum exhibited a methoxyl signal at δ 3.90 as singlet and an broad signal at δ 10.0 corresponding to two protons accompanied with the loss on D_2O exchange. On acetylation with acetic anhydride and pyridine, I afforded a diacetate (II), mp 235—238°, exhibiting two acetoxyl signals at δ 2.32 and 2.46 in the NMR spectrum and an ester carbonyl band at 1760 cm^{-1} in the IR spectrum. These data reveal the existence of two hydroxyl and one methoxyl groups in I. The mass spectrum of I exhibited intense peaks at *m/e* 166 and 118 due to a retro-Diels-Alder fragmentation on γ-pyron ring (Chart 2) indicating that one methoxyl and one hydroxyl groups attached on ring A, and that another hydroxyl group on ring B. On methylation with dimethyl sulfate,



I : R=H
II : R=$COCH_3$
III : R=CH_3

m/e 166 *m/e* 118

Chart 2. Mass Spectra of Kakkatin (I) and Its Methylether (III)

I gave a dimethyl ether (III) as colorless needles, mp 176—178°. The compound was identical with 6,7,4′-trimethoxy isoflavone which was synthesized by methylation of afromosin.[6] It follows that I must be an isoflavone having a 6,7,4′-oxygenation pattern with a methoxyl group at either C-6 or C-7 and a hydroxyl group at the C-4′ position. The position of the methoxyl group was deduced from UV spectrum of I. In the UV spectrum of I, the absorption maximum at 263 nm in ethanol did not show any shift upon the addition of anhydrous sodium acetate. This fact suggests that the methylether linkage is located at C-7 position. Based on these mentioned above, the structure of kakkatin (I) has been established to be 6,4′-dihydroxy-7-methoxy-isoflavone.

Experimental

Melting points were measured on a Yanagimoto micro melting points apparatus and uncorrected, NMR spectra were recorded on a Varian HA-100D with tetramethylsilane as an internal standard and abbreviation

6) S. Shibata, T. Murata, and M. Fujita, *Chem. Pharm. Bull.* (Tokyo), **11**, 382 (1963); T.B.H. McMurry and C.Y. Theng, *J. Chem. Soc.*, **1960**, 1491.

used are s=singlet and d=doublet. Mass, IR, and UV spectra were taken on Hitachi RMU-6, Hitachi EPI-S2, and Hitachi ESP-2U spectrometers, respectively. TLC was performed on silica gel (Kiesel gel GF_{254}, Merck). Column chromatography was run on silica gel (100 mesh), Mallinckrodt).

Isolation of Kakkatin (I)——The dried powdered Chinese *Pueraria* flowers (500 g) purchased on Hong Kong market were extracted with 70% MeOH (5 liters). After removal of MeOH under reduced pressure, aqueous concentrate was obtained, To the aqueous concentrate was added ether with stirring, A crude isoflavone mixture (1 g) was deposited at the H_2O–ether interface and collected by filtration. The mixture was submitted to column chromatography on silica gel, using $CHCl_3$–MeOH (17: 3) as an eluent. The residue from the first eluate was recrystallized from MeOH several times to give colorless needles (I) (300 mg), mp over 290°. *Anal.* Calcd. for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26. Found: C, 67.41; H, 4.20.

Acetylation of I——A mixture of I (20 mg), acetic anhydride (0.5 ml) and pyridine (0.5 ml) was allowed to stand over night at room temperature. After the usual work-up, recrystallization from acetone gave colorless needles (II) (20 mg), mp 235—238°. *Anal.* Calcd. for $C_{20}H_{16}O_7$: C, 65.21; H, 4.38. Found: C, 64.90; H, 4.25. UV λ_{max}^{EtOH} nm (log ε): 254 (4.54), 325 (3.93). IR ν_{max}^{KBr} cm^{-1}: 1760, 1610, 1570, 1480. NMR δ (in $CDCl_3$): 2.32 (s, 3H), 2.46 (s, 3H), 3.94 (s, 3H), 7.16 (d, J=8.5 Hz, 2H), 7.24 (s, 1H), 7.58 (d, J=8.5 Hz, 2H), 7.74 (s, 1H), 7.97 (s, 1H). Mass Spectrum *m/e*: 368 (M^+).

Methylation of I——A mixture of I (30 mg), dimethyl sulfate (2.0 ml), K_2CO_3 (3.0 g) and dry acetone (30 ml) was refluxed for 5 hr. The inorganic salts were removed by filtration, and the filtrate was evaporated to give a residue which was heated with aqueous NaOH for 10 min. Resulting precipitates were collected and recrystallized from 95% EtOH to give the white crystalls (III) (15 mg), mp 178—180°. UV λ_{max}^{EtOH} nm (log ε): 263 (4.48), 320 (4.03). IR ν_{max}^{KBr} cm^{-1}: 1620, 1590, 1500, 1450, 1430.

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A New Method for the Preparation of 2-Hydroxymethyl-3-quinolinecarboxylic Acid Lactone Derivatives

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The reaction of 2-acetoxymethyl-3-acetylquinolines (V, VI) with sodium hydride gave 2-hydroxymethyl-3-quinolinecarboxylic acid lactones (IX, X), presumably formed with the migration of acetyl group. On the other hand, the fact that the reaction of 3-acetyl-2-benzoyloxymethylquinoline (VIII) with sodium hydride gave a lactone (IX) and acetophenone would strongly support for the mechanism of the lactone formation with the acyl migration.

Recently[2] we reported the convenient synthesis of 3-acetylquinaldine 1-oxide derivatives (III, IV) by the reductive cyclization of *o*-nitrobenzylideneacetylacetones (I, II).

In 1958 Fehnel[3] had reported, as studies of quinoline analogs of podophyllotoxin, for the preparation of 2-hydroxymethyl-3-quinolinecarboxylic acid lactone (IX) by a Friedländer condensation of *o*-aminobenzaldehyde with tetronic acid. In the present paper, we wish

1) Location: *2-10-65, Kawai, Matsubara, Osaka, 580, Japan.*
2) T. Kurihara, H. Sano, and H. Hirano, *Chem. Pharm. Bull.* (Tokyo), **23**, 1155 (1975).
3) E.A. Fehnel, *J. Org. Chem.*, **23**, 432 (1958); E.A. Fehnel, J.A. Deyrup, and M.B. Davidson, *ibid.*, **23**, 1996 (1958).