Hydrolysis with 5% H_2SO_4 gave quercetin (yield: 49.7%), L-rhamnose, and D-glucose, which were identified in the same manner as above.

After removal of Pb, the non-precipitable fraction was evaporated to dryness and the residue was recrystallized from H_2O to yellow needles, m.p. 181~183° (previous softening at 175°), undepressed on admixture with authentic kaempferol 3-L-rhamno-D-glucoside.*² Rf values in the three solvent systems tried and IR spectrum were also found to be the same as those of the authentic specimen. *Anal.* Calcd. for $\text{C}_{27}\text{H}_{30}\text{O}_{15} \cdot 4\frac{1}{2}\text{H}_2\text{O}$: C, 48.00; H, 5.78; H_2O , 12.0. Found: C, 47.99; H, 5.61; H_2O , 11.5.

Hydrolysis with 5% H_2SO_4 gave kaempferol (yield: 50.3%), L-rhamnose, and D-glucose. Kaempferol was identified by its m.p. 268°(decomp.), Rf values, and IR spectrum. L-Rhamnose and D-glucose were identified by paper chromatography.

The writers are deeply indebted to Mr. Kensi Kobayasi, Ex.-professor of Kumamoto College of Pharmacy, for the kind gift of a sample of kaempferol 3-L-rhamno-D-glucoside. Thanks are also due to Mrs. Yukiko Tanaka and Miss Seiko Fujishima of the Faculty of Pharmaceutical Sciences of this University for determining infrared spectra and carrying out elemental analyses.

Summary

Presence of quercitrin was proved in the leaves of *Rhus javanicus* L. (Anacardiaceae), and of rutin and kaempferol 3-L-rhamno-D-glucoside in the leaves of *Melia Azedarach* var. *subtripinnata* MIQUEL (Meliaceae).

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*² The sample isolated from the leaves of *Hyptis capitata* Jacq. (K. Kobayashi: This Journal, 72, 1 (1952)) was kindly supplied by Mr. Kensi Kobayasi.

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Masakazu Aritomi, Michiko Shimojō, and Tatsuji Mazaki: Chemical Constituents in Flowers of *Gnaphalium affine* D. DON.

(Faculty of Education, Kumamoto University*¹)

Gnaphalium affine D. DON (Japanese name "Hahako-gusa") is an annual herb of the family Compositae, which is well-known as one of the "Haru-no-nanakusa" in Japan.

In 1958, Ohta and Fukawa¹⁾ first reported the isolation from its flowering whole herb, of luteolin mono-D-glucoside (I), $\text{C}_{21}\text{H}_{20}\text{O}_{11} \cdot 2\text{H}_2\text{O}$, m.p. 224°, which was distinguished by paper chromatography from luteolin 5-D-glucoside (galuteolin) and luteolin 7-D-glucoside. Owing, however, to the material scarcity, the location of the sugar moiety in I could not be settled by them.

As described in the experimental part, a flavonoid glycoside (II), $\text{C}_{21}\text{H}_{20}\text{O}_{11} \cdot 3\text{H}_2\text{O}$, m.p. 168~178°, was isolated in pure form from the flowers of the same plant and identified as luteolin 4'-β-D-glucoside by examination of its hydrolysis products and by comparison of its infrared and ultraviolet absorption spectra with those of the authentic specimen.

Although direct comparison was unable to carry out, it may be concluded that I consists mainly of luteolin 4'-β-D-glucoside.

*² Kurokami-machi, Kumamoto (有富正和, 下城道子, 真崎辰次).

1) T. Ohta, K. Fukawa: The Annual Report of the Tokyo College of Pharmacy, 8, 50 (1958).

Experimental

Paper chromatograms of flavonoid compounds were run in BuOH-AcOH-H₂O (4:1:5 by volume, upper layer) (solv. 1) and 60% AcOH (solv. 2), and sprayed with 5% Na₂CO₃ solution. Paper chromatogram of the component sugar was run in BuOH-pyridine-H₂O (3:2:1 by volume), and sprayed with 0.1% *p*-anisidine·HCl in MeOH.

Isolation and Identification of II—The air-dried flowers of *Gnaphalium affine* D. DON were refluxed with MeOH 3 times. After removal of MeOH, the residue was digested with boiling H₂O, and the resulting aqueous solution was successively extracted with benzene, Et₂O and EtOAc (20 times). The EtOAc extract was dissolved in MeOH and treated with a saturated solution of (AcO)₂Pb in MeOH. After removal of Pb, the non-precipitating fraction was evaporated to dryness, and the yellow residue was subjected to column chromatography on Nylon powder by using MeOH as eluant. The fractions indicating the presence at R_f 0.67 (solv. 1) of only one flavonoid compound were combined, evaporated to dryness, and the residue was recrystallized from H₂O.

II was obtained as pale yellow needles, m.p. 168~178°. It gave an orange yellow color with Mg-HCl, an orange color with Zn-HCl, and a faint violet color with FeCl₃. Its R_f values, IR and UV spectra were found to be indistinguishable from those of the authentic luteolin 4'-β-D-glucoside isolated from the leaves of *Acer cissifolium* K. KOCH.²⁾ UV λ_{max} mμ (log ε): in EtOH, 269 (4.38), 340 (4.27); in EtOH-AcONa, 274 (4.38), 323 (4.11); in EtOH-AlCl₃, 282 (4.28), 290 (4.26) (shoulder), 344 (4.24), 380 (4.07); in EtOH-EtONa, 274 (4.48), 380 (4.20); in EtOH-H₃BO₃-AcONa, 270 (4.30), 340 (4.25). Anal. Calcd. for C₂₁H₂₀O₁₁·3H₂O: C, 50.38; H, 5.16; H₂O, 9.1. Found: C, 50.30; H, 5.19; H₂O, 9.7.

II was refluxed with 5% H₂SO₄ solution for 3 hr. After standing overnight, the aglucon that separated was collected, washed with H₂O, and recrystallized from MeOH-H₂O to yellow needles, m.p. 321° (decomp.), undepressed on admixture with authentic luteolin. Its R_f values, IR and UV spectra were also found to be indistinguishable from those of authentic specimen.

The acid filtrate freed of the aglucon was neutralized with BaCO₃, filtered, evaporated to a syrup *in vacuo*, and chromatographed on paper. The running distance of a sugar was found to be the same as that of D-glucose.

Infrared spectra were kindly measured by Mrs. Yukiko Tanaka of Faculty of Pharmaceutical Sciences of this University and ultraviolet spectra by Mr. Masanori Ishii of Faculty of Engineering of this University. Thanks are also due to Miss Seiko Fujishima of Faculty of Pharmaceutical Sciences of this University for carrying out elemental analyses.

Summary

A flavonoid glycoside, C₂₁H₂₀O₁₁·3H₂O, m.p. 168~178°, was isolated in a pure state from the flowers of *Gnaphalium affine* D. DON (Compositae), and identified as luteolin 4'-β-D-glucoside.

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2) M. Aritomi: Chem. Pharm. Bull. (Tokyo), 12, 841 (1964).