Notes

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Tatsuo Ohta, Toshio Miyazaki, and Susumu Mihashi : Isolation of Trifolin from the White Flowers of *Prunus Persica* BATSCH.

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The air-dried, half-opened, white flowers or flower buds of *Prunus Persica* BATSCH (*P. Persica* SIEB. ET ZUCC. VAR. *vulgaris* MAXIM.), known as "hakutôka" in Japan, are used as the diuretic and laxative in Chinese medicine. From this drug, Kariyone, *et al.*[1] obtained an amorphous flavonoid glycoside, which on hydrolysis yielded kaempferol and glucose. Kikutani[2] also obtained an amorphous glycoside, which consisted of kaempferol, rhamnose, and glucose.

A re-investigation was made on the flavonoid component of this drug. A methanolic extract of the flowers furnished a crystalline flavonoid glycoside on chromatography through Florisil or Florex XXS in ethyl acetate solution. It crystallized to pale yellow microneedles, m.p. 236° (decomp.), and corresponded to $C_{21}H_{20}O_{11}\cdot 1\frac{1}{2}H_2O$. The glycoside gave on hydrolysis kaempferol and galactose, the former was confirmed by its tetra-acetate, $C_{23}H_{18}O_{11}\cdot H_2O$ (m.p. 185~186°, showed no double m.p., differing from the report of many workers[3]), and the latter was identified as the *p*-nitrophenylhydrazone of m.p. 196°. Complete methylation of the glycoside followed by hydrolysis yielded 3-hydroxy-5,7,4'-trimethoxyflavone, showing that the glycoside is a kaempferol 3-galactoside. This glycoside agrees closely with trifolin, kaempferol 3-galactoside first isolated from *Trifolium pratense* L. by Power and Salway,[4] and whose structure was later elucidated by Hattori, *et al.*[5] Trifolin was also isolated from *Calystegia hederacea* WALL.[6] and *Menyanthes trifoliata* L.[7] Accordingly, this glycoside was found to be identical with trifolin[*2] by their direct comparison (mixed m.p., paper chromatography, and infrared spectra).

The kaempferol glycoside obtained by Kariyone, *et al.* and by Kikutani is possibly

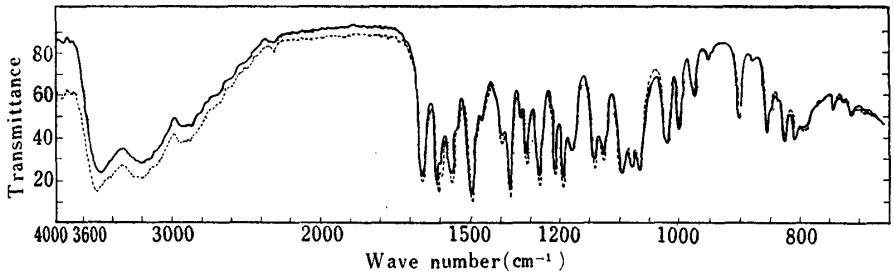

Fig. 1. Infrared Spectra of Trifolin and the Glycoside from *Prunus Persica* (in KBr pellet)

—— Glycoside from *Prunus Persica* } (Both substances were dried at
------ Trifolin from *Trifolium pratense* } 110°/1.5 mm. Hg for 6 hr.)

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*2 The sample of trifolin supplied by Prof. Hattori melted at 236° (decomp.).

1) T. Kariyone, J. Takata, Y. Yoshida : Yakugaku Zasshi, **49**, 937(1929).
2) M. Kikutani : Presented at the 5th General Meeting of the Pharmaceutical Society of Japan, May, 1952.
3) cf. K. Kobayashi : Yakugaku Zasshi, **64**, 175(1944).
4) F.B. Power, A.H. Salway : J. Chem. Soc., **97**, 231(1910).
5) S. Hattori, M. Hasegawa, M. Shimokoriyama : Acta Phytochim. (Japan), **13**, 99(1943).
6) S. Hattori, M. Shimokoriyama : Bull. soc. chim. biol., **38**, 921(1956).
7) K.G. Krebs, J. Matern : Arch. Pharm., **291/63**, 163(1958).

a very impure trifolin, since the ethyl acetate extract gave no spot except that of trifolin by paper partition chromatography.

Experimental

Isolation of the Glycoside—Approx. 1.9 kg. of the air-dried, half-opened floral parts of *Prunus Persica* (white flowers, Nara Prefecture crop in 1959) was extracted five times with cold MeOH. The combined MeOH extract was evaporated under a reduced pressure and the resulting syrup was extracted with warm water (1 L.). The filtrate was shaken with Et_2O to remove the impurity and then extracted thoroughly with AcOEt. AcOEt extract was dried and evaporated *in vacuo* to a small volume. One-half of the syrup obtained was passed through a column (5.4×22 cm.) of Florisil (Floridin Co.), flowing AcOEt (4 L.). Another half of the syrup was chromatographed using a column of Florex XXS (Floridin Co.) by the same procedure. Each effluent liquor was evaporated *in vacuo* to a small volume and allowed to stand in a refrigerator. The crystals thereby obtained were collected and recrystallized from MeOH. Both formed pale yellow microneedles, m.p. and mixed m.p. 236° (decomp.)*3 (sintering at around 232°) with trifolin. Total yield, 0.7 g. (0.037%). It gave dark greenish brown coloration with $FeCl_3$, and orange red with Mg and HCl in EtOH solution. Rf of the glycoside obtained: 0.72. Rf of trifolin: 0.72 (BuOH-AcOH-H_2O=4:1:2, Toyo Roshi No. 50 filter paper was used, developed for 16 hr. by the ascending method, the spot showed dark brown color under ultraviolet light). In 60% AcOH, Rf values were 0.81 (the glycoside obtained) and 0.81 (trifolin).*4 The foregoing AcOEt concentrate gave the chromatogram of Rf of 0.72 (BuOH-AcOH-H_2O) alone. *Anal.* Calcd. for $C_{21}H_{20}O_{11}\cdot 1\frac{1}{2}H_2O$ (trifolin): C, 53.05; H, 4.87; H_2O, 5.70. Found: C, 53.12; H, 5.32; H_2O, 5.70.

Hydrolysis of the Glycoside—The anhydrous glycoside (109.1 mg.) was hydrolyzed with 3% H_2SO_4 for 2 hr. at 100° and the deposited kaempferol (68.8 mg.), crystallized from dil. MeOH, melted at 276~278°. Its tetraacetate was crystallized from MeOH with charcoal to colorless needles, m.p. 185~186°, having one mole of water of crystallization and showing no double m.p. When dehydrated, it melted at 186.5° and absorbed one mole of water on standing in the air. *Anal.* Calcd. for $C_{23}H_{18}O_{10}\cdot H_2O$ (Kaempferol tetraacetate): C, 58.47; H, 4.27; H_2O, 3.81. Found: C, 58.87; H, 4.59; H_2O, 3.41.

The hydrolysate after separation of kaempferol was neutralized with $BaCO_3$, filtered, and evaporated. One-dimensional paper chromatograms of the residue obtained were run on Toyo Roshi No. 50 filter paper and developed with BuOH-AcOH-H_2O (4:1:2) for 16 hr. and PhOH-H_2O (3:1) for 21 hr. Rf of the former case, 0.23 and that of the latter, 0.41 (sprayed with 3% *p*-anisidine·HCl-BuOH) showed the identical values for galactose. In addition, the sugar obtained was converted into *p*-nitrophenylhydrazone of golden plates, m.p. and mixed m.p. 195~196° with authentic specimen.

Methylation of the Glycoside—A solution of the glycoside (50 mg.) in MeOH was treated repeatedly with an excess of CH_2N_2 in ether. The methylated product was hydrolyzed with 3% H_2SO_4 on a water bath. 3-Hydroxy-5,7,4′-trimethoxyflavone obtained was recrystallized from MeOH to pale yellow pillars, m.p. 143°, and mixed m.p. 143~145°, with an authentic sample of m.p. 148°.

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Summary

Trifolin, $C_{21}H_{20}O_{11}\cdot 1\frac{1}{2}H_2O$, the kaempferol 3-galactoside, m.p. 236° (decomp.), was isolated from the air-dried, half-opened, white flowers of peach tree, *Prunus Persica* Batsch, in 0.037% yield.

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*3 When heated rapidly, it melted at 249° (decomp.).
*4 Krebs and Matern (*loc. cit.*) gave Rf 0.81.