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52. Masakazu Aritomi : Homoplantaginin, a New Flavonoid Glycoside in Leaves of *Plantago asiatica* Linnaeus.

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Besides plantaginin,[1] a new flavonoid glycoside, homoplantaginin, m.p. 241~242° (decomp.) or m.p. 219~220/256~258° (decomp.), was isolated from the leaves of *Plantago asiatica* Linnaeus, and was found to be formulated as hispidulin 7-O-D-glucoside.

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Plantago asiatica Linnaeus is a perennial herb of Plantaginacaeae, which has long been used in old Chinese medicine as a diuretic.

Nakaoki, *et al.*[1] isolated a flavonoid glycoside, plantaginin, from its leaves, and identified it as scutellarein 7-O-D-glucoside. Recently, Torigoe[2] demonstrated the presence of ursolic acid, hentriacontane, palmitates of β-sitosterol and stigmasterol, and β-sitosterol in its whole herb.

Besides plantaginin, a new flavonoid glycoside, which would be named homoplantaginin hereafter, has now been isolated from the same plant source, and the present paper deals with the result of experiments carried out on the elucidation of its chemical structure.

Homoplantaginin (I) was obtained as yellow needles, $C_{22}H_{22}O_{11}\cdot 1\frac{1}{2}\ H_2O$, m.p. 241~242° (decomp.) or m.p. 219~220/256~258° (decomp.) (double m.p.).

Acid hydrolysis of I with 5% sulfuric acid gave one mole each of D-glucose and an aglycone (II), $C_{15}H_9O_5(OCH_3)$, m.p. 282~282.5° (decomp.), which gave an acetate, m.p. 169~171°.

II was found to be identical with hispidulin (6-methoxy-4′,5,7-trihydroxy flavone)[3,4] by examination of its alkali fission and methylated products, ultraviolet spectrophotometry after the procedure of Jurd[5,6] (Table I), and, finally, by direct comparison with an authentic specimen.

Methylation of I with diazomethane gave its di-O-methyl ether (III), $C_{24}H_{26}O_{11}$, m.p. 172~175°, which gave no color with ferric chloride.

Acid hydrolysis of III gave colorless needles, (IV), $C_{18}H_{16}O_6$, m.p. 194~195°, which was identified as 7-hydroxy-4′,5,6-trimethoxyflavone by direct comparison with an authentic specimen. This fact indicates that the hydroxyl group at 7 position is linked with D-glucose.

In agreement with this fact, ultraviolet spectrum of I (Table I) showed the presence of a free hydroxyl group at 4′- and 5-positions, and its absence at 7-position in its molecule.

It follows, therefore, that I has the structure of 4′,5-dihydroxy-6-methoxy-7-D-glucosyloxyflavone, *i.e.* hispidulin 7-D-glucoside, furnishing the first evidence for the occurrence of hispidulin glycoside in nature.

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Table I. Ultraviolet Absorption Maxima of I to IV
(λ_{max} mμ (log ε))

Solvent / Compound	EtOH	EtOH–EtONa	EtOH–$AlCl_3$	EtOH–AcONa
I	275(4.27)	258(4.19)	284(4.25)	274(4.24)
	337(4.43)	270(4.19)	300(4.24)	339(4.31)
		402(4.59)	348(4.41)	
II	275(4.21)	277(4.33)	295(4.26)	276(4.40)
	338(4.41)	332(4.12)[a]	301(4.27)	299(4.20)[a]
		402(4.48)	353(4.38)	364(4.20)
III	267(4.18)			
	322(4.40)			
IV	269(4.24)		267(4.16)	272(4.42)
	324(4.39)		324(4.45)	300(4.21)[b]
				360(4.18)

a) Discernible maximum in a minimum.
b) Shoulder

Experimental

Isolation of Homoplantaginin (I)——The air-dried leaves of *P. asiatica* L. were refluxed twice with MeOH. After removal of MeOH, the residue was successively extracted with benzene (5 times), ether (5 times), and EtOAc (20 times). The EtOAc extract was dissolved in MeOH and treated with $(AcO)_2Pb$ in MeOH, giving precipitable and non-precipitable fractions.

Plantaginin, m.p. 213~214° (decomp.) (reported[1] m.p. 214°(decomp.)), was isolated from the former fraction.

After removal of Pb, the latter fraction was concentrated and chromatographed on a column of Nylon powder using MeOH as eluant. After removal of MeOH and standing overnight, the fractions giving a crystalline compound were combined. The combined solid, which was shown by paper chromatography to be a mixture of two flavonoid compounds, was dissolved in a minimum amount of MeOH and treated with a large excess of $(AcO)_2Pb$ in MeOH. After removal of Pb, the non-precipitable fraction was concentrated to a small volume and allowed to stand overnight, giving paper chromatographically pure I.

I crystallized from EtOH as yellow needles, m.p. 241~242°(decomp.). After standing for several months, it showed double m.p. of 219~220/256~258°(decomp.). It gave an orange color with Mg–HCl, a red color with Zn–HCl, and a dark green color with $FeCl_3$, and had Rf values of 0.61 (BuOH–AcOH–H_2O (4:1:5)), 0.81 (60% AcOH) and 0.31 (15% AcOH). *Anal.* Calcd. for $C_{22}H_{22}O_{11}\cdot 1\frac{1}{2}H_2O$: C, 53.98; H, 5.12; H_2O, 5.5. Found: C, 53.69; H, 5.12; H_2O, 5.0.

Acid Hydrolysis of I——A solution of I (200 mg., anhydrous) in 20 ml. of 5% H_2SO_4 was refluxed for 2 hr. Next day, the aglycone that separated was collected, washed with H_2O, and dried at 100° for 1 hr. The yield of the aglycone was 61.0% to anhydrous I.

The aqueous solution freed from the aglycone was neutralized with $BaCO_3$, evaporated *in vacuo*, and chromatographed on paper. Only one spot of a sugar was revealed on the paper chromatogram, the running distance of which was found to be the same as that of D-glucose.

Identification of II——The above aglycone was recrystallized from MeOH–H_2O to yellow needles, m.p. 282~282.5°(decomp.) (reported[3] m.p. 291~292°). Its IR spectrum was found to be superimposable with that of authentic hispidulin. *Anal.* Calcd. for $C_{15}H_9O_5(OCH_3)$: C, 64.00; H, 4.00; OCH_3, 10.33. Found: C, 64.20; H, 3.96; OCH_3, 10.24.

Alkali fission of II after the procedure of Lindstedt[7] furnished *p*-hydroxybenzoic acid as the only detectable fission product.

Acetylation of II with Ac_2O and AcONa in the usual manner gave its acetate as colorless needles, m.p. 169~171°(MeOH) (reported[3] m.p. 168~170°).

Methylation of II with CH_2N_2 in the usual manner gave pale yellow needles (acetone–ligroin or benzene–ligroin), m.p. 157~159°, undepressed on admixture with authentic scutellarein tetra-O-methyl ether. Its IR spectrum was found to be hardly distinguishable from that of the authentic specimen. UV λ_{max}^{EtOH} mμ (log ε): 267 (4.20), 322 (4.47). *Anal.* Calcd. for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.73; H, 5.35.

7) G. Lindstedt, A. Misiorny: Acta Chim. Scand., **5**, 1 (1951) [C. A., **45**, 8010 (1951)].

Methylation of I (III)——To a solution of I (0.5 g.) in MeOH (100 ml.) an ether solution of CH_2N_2, prepared from 10 g. of nitrosomethylurea, was added. After standing at room temp. for 48 hr., the solvent was removed and the residue was allowed to stand for several days in a refrigerator. Almost colorles needles that separated were collected, washed with H_2O, and recrystallized from MeOH to colorless needles (III), m.p. 172~175°, which gave no color with $FeCl_3$. *Anal.* Calcd. for $C_{24}H_{26}O_{11}\cdot 2\frac{1}{2}H_2O$: C, 53.83; H, 5.79. Found : C, 54.12; H, 5.78.

Acid Hydrolysis of III (IV)——To a solution of III (0.1 g.) in a minimum amount of MeOH was added 10% H_2SO_4 (5 ml.) and the mixture was refluxed for 3 hr. After removal of MeOH, the reaction mixture was allowed to stand overnight. The precipitate that separated was collected, washed with H_2O, and purified by column chromatography on Al_2O_3 using MeOH as eluant and subsequent recrystallization from acetone–ligroin. The product was obtained as almost colorless needles, m.p. 194~195°, undepressed on admixture with authentic 7-hydroxy-4′,5,6-trimethoxyflavone. Its IR spectrum was found to be superimposable with that of the authentic specimen. *Anal.* Calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91. Found : C, 65.75; H, 5.16.

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