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Masakazu Aritomi: Chemical Constituents in Aceraceous Plants. I.

Flavonoid Constituents in the Leaves of

***Acer palmatum* THUNBERG.**

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Previously it was reported¹⁾ that vitexin was isolated from the leaves of *Acer palmatum* THUNBERG.*² Besides vitexin, the presence of three other flavonoid compounds was indicated by paper chromatography, but they were not isolated in pure state.

This paper is concerned with the result of further detailed experiments carried out on the isolation and identification of these co-existent flavonoids.

As described in detail in the experimental part, complete separation of four flavonoids was achieved, i.e. compound (I), m.p. 260~261°(decomp.); compound (II), m.p. 225°(decomp.); compound (III), m.p. 283°(decomp.); and compound (IV), m.p. 236~237°(decomp.).

As previously reported,¹⁾ compound (I) was proved to be identical with vitexin.

Compound (II) was found to be non-glycosidic because of its negative Molisch reaction. As can be seen in Table I, the ultraviolet spectrum of II closely resembles those of vitexin and apigenin even after addition of sodium acetate.³⁾ This fact suggests that the side chains in the molecules of vitexin and saponaretin exhibit no marked effect on the ultraviolet spectrum of apigenin.^{3,4)} As was expected, compound (II) was proved as being saponaretin^{5,6)} by direct comparison with an authentic specimen.

Compounds (III) and (IV) agreed with the formulae $C_{21}H_{20}O_{11}$ and $C_{21}H_{22}O_{12}$, respectively. They were also found to be non-glycosidic because of their negative Molisch reaction and non-formation of sugar even after drastic treatment with conc. hydrochloric acid.

At the same time, it was found that they are interconvertible into each other, reminiscent of the interrelationship between vitexin and saponaretin.^{3,6)}

Alkali fission of III afforded protocatechuic acid and phloroglucinol, showing its similarity to luteolin. As listed in Table I, the ultraviolet spectra of III and IV were found to closely resemble that of luteolin even after addition of sodium acetate, as well as boric acid and sodium acetate.⁷⁾

From the foregoing findings, III and IV may be respectively identical with orientin⁸⁾

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and homo-orientin.^{9,10)}

Finally, mixed melting point determination,*³ co-chromatography on paper, and comparison of infrared spectra with authentic specimens established the identity of III and IV with orientin and homo-orientin, respectively.

Table I. Ultraviolet Absorption Spectra of I~IV

Solvent	($\lambda_{\max}$ m μ (log ϵ))					
	I	II	Apigenin	III	IV	Luteolin
EtOH	271 (4.26)	273	270 (4.25)	260 (4.26) ^{a)}	262 (4.23) ^{a)}	257 (4.23)
	337 (4.26)	340	342 (4.27)	270 (4.27)	271 (4.27)	268 (4.21) ^{a)}
				354 (4.32)	354 (4.29)	354 (4.25)
EtOH-AcONa	280 (4.39)	280	276 (4.34)	273 (4.24) ^{a)}	272 (4.29) ^{a)}	270 (4.27)
	299 (4.17) ^{a)}	299 ^{a)}	301 (4.16) ^{a)}	279 (4.27)	279 (4.30)	
	376 (4.14)	384	376 (4.17)	325 (3.99) ^{b)}	327 (4.03) ^{b)}	330 (4.00) ^{b)}
				388 (4.20)		389 (4.19)
EtOH-H ₃ BO ₃ -AcONa				264 (4.31)	264 (4.25)	262 (4.32)
						294 (3.80) ^{b)}
				379 (4.31)	382 (4.23)	376 (4.33)

a) shoulder.

b) barely discernible maximum in a minimum.

Table II. Rf Values on Paper Chromatogram

Solvent	I	II	III	IV	Products after treatment with conc. HCl		Lespecapitoside
					of III	of IV	
Solv. 1	0.44	0.59	0.32	0.45	0.32	0.32	0.45
					0.45	0.45	0.60 (trace)
Solv. 2	0.69	0.83	0.57	0.72	0.57	0.57	0.72
					0.72	0.72	0.75 (trace)
Solv. 3	0.32	0.52	0.23	0.39	0.23	0.23	0.39 (tailing)
					0.39	0.39	0.52 (trace)

Fig. 1.
Infrared Absorption
Spectra (KBr)

a : orientin
b : homo-orientin
c : lespecapitoside

*³ Reported m.p. 225~257°⁸⁾ and m.p. 265~267°(decomp.) (Kofler).¹⁰⁾ It was observed that the original sample of orientin furnished by Prof. Dr. B.H. Koeppen also decomposes at 284° with the foregoing change of color in a sulfuric acid-bath.

9) L. Hörhammer, H. Wagner, H. Nieschlag, G. Wildt: *Ibid.*, 292, 380 (1959).

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Thus, it has now become evident that flavonoid compounds in the leaves of *A. palmatum* THUNBERG consist of four flavone C-glycosides, i.e. vitexin, saponaretin, orientin, and homo-orientin, furnishing an example of their co-existence in nature.

Lespecapitoside isolated from the leaves of *Lespedeza capitata* MICHAUX was reported to be one of the flavone C-glycosides of the luteolin type.¹¹⁾ There can be little doubt that lespecapitoside is a mixture of flavone C-glycosides which consists mainly of homo-orientin.

Experimental

UV spectra were measured after Jurd^{2,7)} using a self-recording spectrophotometer (Hitachi EPS 2 type).

Paper chromatography was carried out by the ascending method, using Toyo Filter Paper No. 50 and solvent systems of (1) BuOH-AcOH-H₂O (4 : 1 : 5 by volume) (solv. 1), unless otherwise indicated, (2) 60% AcOH (solv. 2), and (3) 15% AcOH (solv. 3). Spray reagent was 5% Na₂CO₃ solution.

Extraction and Isolation of Pigments—The air-dried leaves of *A. palmatum* THUNBERG (4 kg.) were refluxed twice with MeOH. After removal of MeOH, the residue was extracted several times with boiling H₂O. Next day, the deposited resinous matter was removed by decantation and the supernatant was successively extracted with benzene, Et₂O, and then with AcOEt (20 times). The AcOEt extract was passed through a column of Magnesol (50 g.) to remove resinous matter. The effluent was evaporated to dryness, giving a brown syrupy residue, which was dissolved in a minimum of MeOH (ca. 50 cc.) and allowed to stand several weeks in an ice box. A mixture of flavonoids gradually deposited as powdery crystals was dissolved in a minimum of dioxane, diluted with MeOH to ca. 500 cc., and chromatographed on a column of Nylon powder (1 kg.), using MeOH as an eluant. The faster-moving fraction (fraction A) consisted of a mixture of I and II, and the slower-moving fraction (fraction B) consisted of a mixture of III and IV, respectively.

Fraction A was concentrated to a small volume, allowed to stand several days in an ice box, and gave crude I as yellow plate crystals.

The mother liquor was evaporated to dryness and re-extracted with boiling MeOH, whereby I remained undissolved. The MeOH-soluble residue was digested with hot H₂O and the resulting aqueous solution was subjected to chromatography on a column of cellulose powder, using AcOEt as an eluant. The eluates indicating only one spot at R_f 0.59 on a paper chromatogram of the flavonoid were combined and evaporated to dryness, giving crude II as yellow crystals.

Fraction B was digested several times with hot H₂O. The sparingly soluble fraction (fraction C) consisted chiefly of III and the more-soluble fraction (fraction D) consisted of a mixture of IV (predominant) and III (minor). Fraction C was dissolved in a minimum of dioxane and diluted with MeOH. The resulting solution was subjected to chromatography on a column of Nylon powder, using MeOH as an eluant. The eluates indicating only one spot at R_f 0.32 on a paper chromatogram of the flavonoid were combined and evaporated to dryness, giving crude III.

Fraction D was subjected to chromatography on a column of cellulose powder, using AcOEt as an eluant. The eluates indicating only one spot at R_f 0.45 on a paper chromatogram of the flavonoid were combined, evaporated to dryness, and gave crude IV.

Vitexin (I)—Crude I was recrystallized from dioxane-H₂O (1 : 2) as yellow, lustrous plates, m.p. 260~261° (decomp.), either alone or on admixture with authentic vitexin.¹⁾ Yield, 500 mg.

Saponaretin (II)—Crude II was recrystallized from MeOH-AcOEt as yellow needles, m.p. 225° (decomp.). Yield, 10 mg. It gave a brown color with FeCl₃, an orange color with Mg-HCl, and a red color with Zn-HCl. R_f values (Table II) agreed with those of authentic saponaretin.*⁴ Admixture with authentic saponaretin did not depress the melting point.

Orientin (III)—Recrystallization of crude III from dioxane-H₂O (1 : 2) yielded pure III as yellow needles, which began to blacken at ca. 250° and decomposed at 283° to a black liquid with effervescence. Yield, 50 mg. It gave a greenish brown color with FeCl₃, an orange color with Mg-HCl, and an orange color with Zn-HCl. R_f values (Table II) agreed with those of authentic orientin.*⁵ Admixture with authentic orientin did not depress the melting point and the IR spectrum was also found to be superimposable with that of orientin. Anal. Calcd. for C₂₁H₂₀O₁₁: C, 56.25; H, 4.50. Found: C, 55.77; H, 4.36.

Homo-orientin (IV)—Crude IV was recrystallized from MeOH-H₂O as yellow needles, m.p. 236~237° (decomp.). Yield, 10 mg. It gave a greenish brown color with FeCl₃, an orange color with Mg-

*⁴ The sample was furnished through the courtesy of Prof. Dr. Naokata Morita.

*⁵ The sample was furnished through the courtesy of Prof. Dr. B.H. Koeppen.

11) R. Paris, A. Charles: Compt. rend., 254, 352 (1962).

HCl, and an orange color with Zn-HCl. Rf values (Table II) agreed with those of authentic homo-orientin.*⁶ Admixture with authentic homo-orientin did not depress the melting point and the IR spectrum was also found to be superimposable with that of homo-orientin. *Anal.* Calcd. for $C_{21}H_{22}O_{12}$: C, 54.08; H, 4.72. Found: C, 53.70; H, 4.77.

Interconversion of III and IV—Ten mg. each of III and IV was respectively heated on a water bath with conc. HCl for 10 min. After being cooled, the reaction mixture was diluted with H_2O and extracted 3 times with AcOEt. No sugar was detected in the mother liquor.

The AcOEt extract was evaporated to dryness and the residue was subjected to paper chromatography. In each case, two spots were revealed on the paper chromatograms of the two flavonoids (Table II), indicating the occurrence of III and IV in each reaction mixture.

Alkali Fission of III—A mixture of 2 mg. of III and 0.5 cc. of KOH solution (50%) was refluxed for 15 min. After cooling and dilution with H_2O , the reaction mixture was acidified with dil. HCl and extracted 3 times with Et_2O . The Et_2O extract was chromatographed on paper using diazotized sulfanilic acid as a spray reagent. Two spots were revealed on the paper chromatograms of the two phenolic substances, the Rf values of which agreed with those of protocatechuic acid and phloroglucinol, respectively.

Lespecapitoside—Lespecapitoside*⁶ revealed two spots on the paper chromatograms of two flavonoids (Table II), one of which (predominant) agreed in every respect (Rf values, color reactions) with authentic homo-orientin. The IR spectrum was found to be hardly distinguishable from that of homo-orientin (Fig. 1).

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Summary

Besides vitexin, saponaretin, orientin, and homo-orientin have now been isolated in pure state from the leaves of *Acer palmatum* THUNBERG and identified as such.

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*⁶ The sample was furnished through the courtesy of Prof. Dr. R. Paris. Recorded m.p. 286° (bloc Maquenne) and m.p. 246~248° (Kofler).¹¹ It had m.p. 234° (decomp.) (H_2SO_4 -bath).