Isolation and Structure of New Flavonoids, Flavoyadorinin-A, Flavoyadorinin-B and Homo-flavoyadorinin-B, in the Leaves of *Viscum album* Linnaeus var. *coloratum* Ohwi Epiphyting to *Pyrus communis* Linnaeus[†]

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The flavonoid constituents of the leaves of *Viscum album* Linnaeus var. *coloratum* Ohwi (*Viscaceae*) epiphyting to *Pyrus communis* Linnaeus were examined. Three kinds of new flavonoids named Flavoyadorinin-A (I), $C_{23}H_{24}O_{12}$, mp 245~246°C, Flavoyadorinin-B (II), $C_{23}H_{24}O_{11} \cdot 3H_2O$, mp 203~206°C, and Homo-flavoyadorinin-B (III), $C_{28}H_{32}O_{15} \cdot 3H_2O$, mp 208~210°C (sint. at 130°C) were isolated from the leaves of this plant.

The structures of the Compound (I), (II), and (III) were showed to be 7,3'-di-*O*-methyl-quercetin-3-*O*-mono-D-glucoside (rhamnazin-3-*O*-mono-D-glucoside), 7,3'-di-*O*-methyl-luteolin-4'-*O*-mono-D-glucoside, and 7,3'-di-*O*-methyl-luteolin-4'-*O*-D-glucosapioside, respectively.

Japanese mistletoe, *Viscum album* Linnaeus var. *coloratum* Ohwi is a perennial shrub of *Viscaceae*, which grows wild in Saghalien, Kurles, Honshu, Shikoku, Kyushu, Formosa, Korea and China.

In 1957, the isolation of four kinds of flavonoids, Compound A, mp 208~210°C (positive Molish reaction), Compound B, mp 107~109°C, Compound C, mp 142~143°C, and Compound D, mp 219~220°C (all were positive to Mg-HCl reaction) were reported by Ts'eng Kuang-Fang *et al.*¹⁾ No further investigations of their structures, however, have been made.

The present paper deals with the isolation and the structural elucidation of flavonoid compounds in the fresh leaves of Japanese

mistletoe (*Viscum album* Linnaeus var. *coloratum* Ohwi) epiphyting to *Pyrus communis* Linnaeus.

As described in the experimental part, three flavonoids were isolated in pure state on a cellulose-column chromatography, Compound (I), mp 245~246°C, Compound (II), mp 203~206°C, and Compound (III), mp 208~210°C. All of these compounds, (I), (II) and (III), were new flavonoids, which would be named Flavoyadorinin-A Flavoyadorinin-B, and Homo-flavoyadorinin-B, respectively.

The compound (I), named flavoyadorinin-A, was obtained as pale yellowish needles, mp 245~246°C, and from its analytical values was suggested the formula $C_{23}H_{24}O_{12}$ containing two methoxyl groups. It exhibited a dark green color with ferric chloride, and the reduction tests for flavonoid (Mg-HCl, Zn-HCl) were positive. It also was positive in the Gibbs indophenol test. The UV-spectrum of this compound (I), maxima 253~4, 268, and 350 m μ , and its pattern,

[†] A part of this work was presented at the General Meeting of the West Japan Branch of the Agricultural Chemical Society of Japan, held on October 20, 1968, at the Shimane University, Japan.

1) Ts'eng Kuang-Fang and Li Shil-Chuen, *Yao Hsueh Hsueh Pao* (Acad. Sinica), 5, 169 (1957).

were very similar to those reported for a number of flavonoids of the quercetin type. This was also supported by the fact that demethylation of the compound (I) with hydriodic acid in phenol gave quercetin, mp 314~317°C, which was further characterized as its penta-acetate (mp 201°C).

On the hydrolysis with 3% sulfuric acid, the compound (I) gave an aglycon (IV) as pale yellowish needles (mp 210~216°C, 66.79%), which was identical with the authentic specimen of rhamnazin (7,3'-di-*O*-methyl-quercetin)* obtained from the leaves of *Polygonum hydropiper* Linnaeus. From the hydrolyzate freed of the aglycon (IV), D-glucose was proved to be present by paper chromatography. Alkali fusion of the compound (I) under the usual manner gave mono-*O*-methyl-phloroglucinol and vanillic acid. The compound (I) and its aglycon (IV) were confirmed by comparing their UV-spectra with those in presence of the following reagents:²¹ 1) sodium acetate (both the compound (I) and its aglycon (IV):—no change); 2) aluminum chloride (the compound (I):—no change, its aglycon (IV):—the bathochromic shift of 59 m μ); and 3) sodium ethylate (the compound (I):—the long wavelength bands disappearing, its aglycon (IV):—the bathochromic shift of 57 m μ).

On the basis of these data the structure of the compound (I) might be rhamnazin-3-*O*-mono-D-glucoside.

The compound (II), named flavoyadorinin-B, was obtained as yellowish needles, mp 203~206°C, and from its analytical values was suggested the formula $C_{21}H_{18}O_9(OCH_3)_2 \cdot 3H_2O$. It gave brown color with ferric chloride, and the reduction tests for flavonoid

(Mg-HCl, Zn-HCl) were positive. It was also positive in the Gibbs test. The UV-spectrum of this compound (II), maxima 243~6, 271~2, and 334~6 m μ , and its pattern, were very similar to those reported for a number of flavonoids of the luteolin type. This also was supported by the fact that demethylation of the compound (II) with hydriodic acid in phenol gave luteolin, mp 326~329°C. Its acetate, mp 226~227°C.

On the hydrolysis with 3% sulfuric acid the compound (II) gave a new aglycon (V) as yellowish granular crystals (58.51%), $C_{15}H_8O_4 \cdot (OCH_3)_2$, mp 218~220°C, which would be named flavoyadorigenin-B, hereafter. Acetate of this aglycon (V), mp 182~186°C. In the hydrolyzate freed of the aglycon (V), only D-glucose was detected as the sugar component by paper chromatography. Alkali fusion of the compound (II) under the usual manner gave mono-*O*-methyl phloroglucinol, and vanillic acid. In the UV-absorption determination, the following results were confirmed: 1) both the compound (II) and its aglycon (V) showed no shift of the absorption maximum by adding sodium acetate, suggesting the presence of 7-methoxyl group, and also showed a significant bathochromic shift (about 10 m μ) by adding aluminum chloride, indicating the presence of 5-hydroxyl group in their nucleus; 2) the aglycon (V) showed a characteristic bathochromic shift (70 m μ) of absorption maximum by adding sodium ethylate, suggesting the presence of 4'-hydroxyl group in its structure.

From these results the structure of the compound (II) and its aglycon (V) might be 7,3'-di-*O*-methyl-luteolin-4'-*O*-mono-D-glucoside and 7,3'-di-*O*-methyl-luteolin, respectively.

The compound (III), named homo-flavoyadorinin-B, was obtained as yellowish needles, mp 208~210°C (sint. at 130°C), and analyzed for $C_{26}H_{26}O_{13}(OCH_3)_2 \cdot 3H_2O$. It gave brown color with ferric chloride, and the reduction tests for flavonoids were positive. Gibbs: positive. The UV-spectrum of the compound

* The authentic sample of rhamnazin was furnished through the courtesys of Dr. M. Aritomi (Kumamoto University) and Dr. A. Kawase (Hiroshima Women's University).

2) L. Jurd, "The Chemistry of Flavonoid Compounds," ed. by T. A. Geissman, Pergamon Press, 1962, p. 107.

(A)

I: R=glucosyl
IV: R=H; (rhamnazin)

(B)

II: R=glucosyl
III: R=glucoapiosyl
V: R=H

(A): position

3	5	7	3'	4'
OH	OMe	OH	OH	OH
OH	OH	OMe	OH	OH
OH	OH	OH	OMe	OH
OH	OH	OH	OH	OMe
OH	OH	OMe	OMe	OH
OH	OH	OMe	OH	OMe
OMe	OH	OMe	OH	OMe

(B): position

5	7	3'	4'
OH	OH	OH	OMe
OH	OH	OMe	OH

(azaleatin) E. Wada, *J. Am. Chem. Soc.*, **78**, 4725 (1956).

(rhamnetin) A. G. Perkin and J. R. Allison, *J. Chem. Soc.*, **81**, 468 (1902).

(isorhamnetin) R. Chatterjee and D. K. Datt, *Indian J. Physiol.*, **4**, 61 (1950).

(tamarixetin) A. G. Perkin and P. J. Wood, *J. Chem. Soc.*, **73**, 374 (1898).

(rhamnazin) A. G. Perkin and J. Geldard, *ibid.*, **67**, 496 (1895).

(ombuin) G. B. Marini-Bettolo, V. Deulofeu and E. Hug, *Gazz. chim. ital.*, **80**, 63 (1950).

(ayanin) F. E. King, T. J. King and K. Sellars, *J. Chem. Soc.*, **1952**, 92.

(diosmetin) E. Vongerichten, *Ber.*, **33**, 2334 (1900).

(chrysoeriol) F. B. Power and F. Tutin, *Proc. Am. Pharm. Assoc.*, **54**, 352 (1906).

(III), maxima 243~6, 271~2, and 340 m μ , and its pattern, were almost the same as that of the compound (II), suggesting a close similarity in the structure between the compound (II) and the compound (III). This was also proved by the fact that the compound (III) with hydriodic acid in phenol in the same manner as before gave luteolin, mp 326~329°C.

On the hydrolysis with sulfuric acid in the usual manner, the compound (III) produced an aglycon as yellowish granular crystals (50.1%), C₁₅H₈O₄(OCH₃)₂, mp 218~220°C, which was proved to be identical with the compound (V), by mixed mp and IR-absorption data. In the hydrolyzate freed of the aglycon (V), two kinds of sugar components were proved to be present by paper chromatography. One of these was identical in

R_f value with that of D-glucose and the other was also proved to be apiose, by cochromatography on paper using the sugar solution obtained from the hydrolyzate of authentic apiin.* Moreover, partial hydrolysis of the compound (III) with 5% acetic acid gave the compound (II) and apiose.

The compound (III) was also confirmed by comparing its UV-spectrum with those in presence of the following reagents: 1) sodium acetate (no change), 2) aluminum chloride (the bathochromic shift of about 10 m μ), 3) sodium ethylate (the long wavelength bands disappearing).

Consequently, the structure of the com-

* The authentic specimen of apiin was furnished through the courtesy of Dr. A. Kawase (Hiroshima Women's University).

compound (III) might be 7,3'-di-*O*-methyl-luteolin-4'-*O*-D-glucoside.

In this way, it has now become evident

FIG. 1. Infrared Absorption Spectra of a: Flavoyadorinin-A (I), b: Its Aglycon (IV), c: Flavoyadorinin-B (II), d: Homo-flavoyadorinin-B (III), e: Flavoyadorigenin-B (V), and f: Its Acetate (in KBr).

that flavonoid compounds in the leaves of *Viscum album* Linnaeus var. *coloratum* Ohwi epiphyting to *Pyrus communis* Linnaeus consist of three kinds of 7,3'-di-*O*-methyl flavonoids, i.e., flavoyadorinin-A (I), flavoyadorinin-B (II), and homo-flavoyadorinin-B (III). The ratio of the above mentioned flavonoid contents, i.e., the compound (I) to (II) and to (III) was about 4:1:60.

In addition, it is chemotaxonomically interesting to note that from the same plant source epiphyting to the different host plant, i.e. *Zelkova serrata* Makino, only two kinds of flavonoids, i.e., flavoyadorinin-B (II) and homo-flavoyadorinin-B (III) have been proved to be present by paper chromatographical examination.

EXPERIMENTAL

All melting points were uncorrected. Ultraviolet spectra were measured after Jurd,²⁾ by using a Hitachi EPS-2U recording spectrophotometer, and infrared spectra in KBr discs by using a Nihon Koken DS-301 spectrophotometer.

Paper chromatography. The flavonoid compounds were run in the following solvent systems by the ascending method: (I) BuOH-AcOH-H₂O (4:1:5, upper phase) (solv. 1), (II) 60% AcOH (solv. 2), (III) 30% AcOH (solv. 3), and (IV) 15% AcOH (solv. 4). A spray reagent used 3% sodium carbonate solution

TABLE I. ULTRAVIOLET ABSORPTION SPECTRA ($\lambda_{\max}$ m μ (log ϵ))

	Compound (I)	Compound (IV)	Compound (II)	Compound (III)	Compound (V)
EtOH	253~4 (3.44) 258 (3.40) 350 (3.36)	255~6 (4.46) 372~3 (4.42)	243~6 (4.21) 271~2 (4.28) 334~6 (4.31)	243~4 (4.33) 270~1 (4.32) 340 (4.36)	251~3 (4.20) 268 (4.16) 350 (4.31)
EtOH+NaOAc	253~4 (3.43) 268 (3.39) 354 (3.34)	256 (4.42) 372 (4.39)	241~5 (4.33) 269~71 (4.36) 338~42 (4.37)	244~7 (4.26) 270~1 (4.27) 336~8 (4.33)	257 (4.18) 268 (4.16) 350 (4.19)
EtOH+AlCl ₃	269~70 (3.40) 350~2 (3.30)	266~7 (4.45) 432 (4.50)	282~3 (4.26) 343~46 (4.37)	283 (4.26) 246~8 (4.28)	279 (4.21) 358~60 (4.25)
EtOH+EtONa	286 (3.51)	267 (4.42) 430 (4.46)	286~88 (4.37)	287~9 (4.36)	262 (4.30) 420 (4.56)
EtOH+H ₃ BO ₃ +NaOAc	253 (3.45) 268~9 (3.40) 348~50 (3.35)	256 (4.38) 372 (4.41)	243 (4.24) ^{a)} 271~3 (4.26) 334~38 (4.29)	245~7 (4.24) 270~71 (4.26) 338~40 (4.33)	251~3 (4.20) 268 (4.17) 350 (4.24)

^{a)} shoulder

or 1% ferric chloride ethanolic solution. The component sugar was run in the following solvent systems: solv. 1, (V) BuOH-pyridine-H₂O (3:2:1.5) (solv. 5), (VI) PhOH-H₂O (5:1) (solv. 6), (VII) BuOH-EtOH-H₂O (4:1:5, upper phase) (solv. 7), by ascending method, and detected by spraying with the following reagents: benzidine-trichloroacetic acid, diphenylamine-aniline, aniline-phthalic acid, naphthoresorcinol-phosphoric acid or silver nitrate reagents. The phenolic and acidic compounds were run in the solvent of BuOH saturated with 2% ammonium hydroxide (solv. 8) and detected by spraying with diazotized sulfanilic acid.

Extraction and isolation of pigments. The fresh leaves (4.2 kg)* were extracted with boiling ethanol and the extract was condensed to a syrup *in vacuo*. The syrupy substance was digested with boiling water and the resulting aqueous solution was repeatedly shaken with chloroform, giving a reddish brown aqueous solution, whereby a pale yellowish gray color substance (4.1 g, ferric chloride and sodium carbonate: both negative) was observed between chloroform and aqueous layers. The yield of the chloroform soluble fraction** was 121.0 g (2.85% of the fresh leaves). The aqueous solution freed of the chloroform soluble fraction was further extracted with ethyl acetate exhaustively, giving a reddish brown extract (4 spots in solv. 1; *R_f* values: 0.88, 0.73, 0.48, and 0.28). After removing the solvent, the ethyl acetate extract was dissolved in a suitable amount of ethanol and was left to stand in a refrigerator for a week or more. The pale yellowish granular crystals (Substance A), mp 232~240°C, gradually separated were collected by filtration. Yield, 400 mg (0.0095%).

The remaining filtrate freed of the Substance A was dried up to remove the solvent, and was dissolved in a suitable amount of water. One half of this resulting aqueous solution was chromatographed on a column of cellulose (3.0×30 cm). Elution with water-saturated ethyl acetate of the column gave a fraction (yellow color) containing yellowish needles (Substance B) (67 mg, 0.0015%), mp 194~200°C. The column freed of the Substance B was further eluted with ethanol, giving a fraction (yellowish brown color)

containing yellowish needles (Substance C) (1.2 g, 0.028%), mp 197~202°C (sint. at 130°C).

Another half volume of the resulting aqueous solution was treated by the same procedure described above. Yield of the total Substance B and C, were 130 mg and 2.3 g, respectively.

Isolation of the compound (I) (flavoyadorinin-A). The Substance A (400 mg), mp 232~240°C, was recrystallized several times from ethanol, giving pale yellowish needles (370 mg) mp 245~246°C. It gave the following color reactions: ferric chloride: dark brown; sodium carbonate: yellow; basic lead acetate: yellow; Mg-HCl: yellow; Zn-HCl: orange red. Under UV-light: brown. Gibbs: positive. This compound is soluble in 30~80% ethanol or methanol, ethyl acetate, and pyridine, hardly soluble in ethanol and methanol, and insoluble in chloroform, benzene, ethyl ether, petroleum ether, acetone, hexane and water. The *R_f* values: 0.28 (solv. 1); 0.81 (solv. 2); 0.80 (solv. 3); 0.72 (solv. 4). $\lambda_{\text{max}}^{\text{EtOH}}$ $m\mu$ (log ϵ): 253~4 (3.44), 268 (3.40), 350 (3.36). *Anal.* Found: C, 50.23; H, 5.44; OCH₃, 11.22. Calcd. for C₂₁H₁₈O₁₀(OCH₃)₂·3H₂O: C, 50.54; H, 5.49; OCH₃, 11.35%.

Hydrolysis of the compound (I). The compound (I) (40.5 mg) was refluxed with 5% sulfuric acid in 50% ethanol solution for 3 hr. After removing the ethanol *in vacuo*, the resulting solution was diluted with a small amount of water, and was extracted with ethyl acetate several times. The ethyl acetate soluble fraction were combined together, washed with a small amount of water, and then evaporated off. The residue was recrystallized several times from ethanol giving pale yellowish fine needles (IV) (27.1 mg, 66.79%), mp 210~216°C either alone or on admixture with an authentic specimen of rhamnazin isolated from the leaves of *Polygonum hydropiper* Linnaeus.* *R_f* values: 0.85 (solv. 1), 0.53 (solv. 2), 0.10 (solv. 3), and 0.05 (tailing) (solv. 4), were found to be the same as those of authentic rhamnazin. $\lambda_{\text{max}}^{\text{EtOH}}$ $m\mu$ (log ϵ): 255~6 (4.44), 372~3 (4.42). *Anal.* Found: C, 61.52; H, 4.43; OCH₃, 18.35. Calcd. for C₁₅H₈O₆(OCH₃)₂: C, 61.81; H, 4.23; OCH₃, 18.78%.

Hydrolyzate freed of the compound (IV) was neutralized with barium carbonate, and filtered to yield a clear solution, which was used for detection of the sugar component. D-Glucose was proved to be the sugar component of the compound (I) by paper chromatography (solv. 5) using authentic D-glucose as check.

* The fresh leaves were collected at Nango-dani, Mt. Aso in the spring of 1967.

** In this chloroform soluble fraction, oleanolic acid, β -amyrin, lupeol and myristic acid have been proved to be present.

Demethylation of the compound (I); Formation of quercetin.

A mixture of the compound (I) (110 mg), hydriodic acid (4 ml), and phenol (6 ml) was heated at 100°C in nitrogen stream for 1.5 hr. After cooling, the reaction mixture was diluted with a suitable amount of water, and treated with sodium bisulfate. A crystalline substance was obtained, which was crystallized from ethanol as yellowish needles (45 mg), mp 314~317°C either alone or on admixture with an authentic specimen of quercetin. Acetylation of this demethylated compound with acetic anhydride-pyridine in the usual manner gave almost colorless needles, mp 200~201°C, which were identical with authentic quercetin pentaacetate (mp 201°C).

Alkali fusion of the compound (I). A mixture of the compound (I) (24 mg) and 20% potassium hydroxide solution (10 ml) was heated at 120°C in nitrogen for 30 min. After cooling and diluting with water, the resulting reaction mixture was acidified with dil. hydrochloric acid and extracted with ether. The ethereal extract was subjected to paper chromatography (solv. 8). Two spots were revealed on the chromatogram, the *R_f* values of which agreed with those of authentic mono-*O*-methyl phloroglucinol (*R_f*: 0.88, blood color) and vanillic acid (*R_f*: 0.09, reddish brown color), respectively.

Isolation of the compound (II) (flavoyadorinin-B). The Substance B (130 mg), mp 194~200°C, was recrystallized several times from 50% ethanol, giving pale yellowish needles (112 mg), mp 203~206°C. It gave the following colorations: ferric chloride: purplish brown; sodium carbonate: yellow; basic lead acetate: yellow; Mg-HCl; orange; Zn-HCl: orange red. Under UV-light: brown. Gibbs: positive. This compound is soluble in 30~80% ethanol or methanol, water-saturated ethyl acetate, and pyridine, hardly soluble in ethanol, methanol, and ethyl acetate, and insoluble in chloroform, benzene, ethyl ether, petroleum ether, acetone, hexane and water. *R_f* values: 0.76 (solv. 1); 0.83 (solv. 2); 0.62 (solv. 3); 0.33 (solv. 4). $\lambda_{\max}^{\text{EtOH}}$ m μ (log ϵ): 243~6 (4.21), 271~2 (4.28), 334~6 (4.31). *Anal.* Found: C, 55.07; H, 5.66; OCH₃, 11.62. Calcd. for C₂₂H₁₈O₉(OCH₃)₂·3H₂O: C, 55.14; H, 5.51; OCH₃, 11.69%.

Hydrolysis of the compound (II); Formation of flavoyadorinin-B (V). The compound (II) (40.3 mg) was refluxed with 5% sulfuric acid in 50% ethanol for 3 hr. After removing ethanol *in vacuo*, the resulting

solution was left to stand in a refrigerator over night. The aglycon separated was collected, washed with ice-cooled water, and recrystallized from ethanol giving yellowish fine needles (V) (23.6 mg, 58.51%), mp 220°C. Color reactions of this compound (V): ferric chloride: brown; sodium carbonate: yellow; basic lead acetate: yellow; Mg-HCl: orange red; Zn-HCl: orange yellow. Its solubilities: soluble in 30~100% ethanol or methanol, acetone, and pyridine; insoluble in chloroform, benzene, ethyl ether, petroleum ether, hexane and water. *R_f* values: 0.92 (solv. 1), 0.76 (solv. 2), 0.3 (tailing) (solv. 3), 0.09 (tailing) (solv. 4). $\lambda_{\max}^{\text{EtOH}}$ m μ (log ϵ): 251~3 (4.20), 268 (4.16), 350 (4.31). *Anal.* Found: C, 70.03; H, 4.75; OCH₃, 21.05. Calcd. for C₁₅H₈O₄(OCH₃)₂: C, 70.34; H, 4.82; OCH₃, 21.37%.

Hydrolyzate freed of the aglycon (V) was neutralized with barium carbonate, and filtered to yield a solution, which was used for detection of the sugar component. D-Glucose was proved to be the sugar component of the compound (II) by cochromatography on paper.

Acetylation of the compound (V). To a solution of the compound (V) (25 mg), mp 220°C, in pyridine (1 ml) was added acetic anhydride (1.5 ml), and the mixture was allowed to stand at room temperature for 2~3 days. The reaction mixture was poured into ice-cooled water, and the crude acetate separated was filtered, washed with water, and then dried. After recrystallizations twice from ethanol, the pure acetate obtained as almost colorless needles (20 mg), mp 182~186°C. Color reactions: ferric chloride: negative; sodium carbonate: yellow; basic lead acetate: pale yellow. Under UV-light: fluorescence. Its solubilities: easily soluble in chloroform, hexane, dioxane and pyridine; soluble in ethanol, methanol, acetone, benzene, ethyl acetate, ethyl ether and petroleum ether; and insoluble in water. *Anal.* Found: C, 63.07; H, 4.67; OCH₃, 15.15. Calcd. for C₁₅H₈O₄(OCH₃)₂(CH₃CO)₂: C, 63.31; H, 4.52; OCH₃, 15.57%.

Demethylation of the compound (II); Formation of luteolin. A mixture of the compound (II) (51 mg), hydriodic acid (4 ml), and phenol (6 ml) was heated at 100°C in nitrogen for 1.5 hr. After cooling the reaction mixture was worked up as described before. The demethylated compound obtained was crystallized from ethanol as yellowish needles (22 mg), mp 325~327°C either alone or on admixture with an authentic specimen of luteolin. Acetylation of this demethylated

TABLE II-1. *R_f* VALUES OF SUGARS AFTER HYDROLYSIS

Compound	<i>R_f</i> values in							
	solv. 5		solv. 1		solv. 6		solv. 7	
Sugars in hydrolyzate	0.39	0.59	0.16	0.34	0.36	0.55	0.24	0.39
Glucose	0.39		0.16		0.36		0.24	
Apiose		0.59		0.34		0.55		0.39
Rhamnose		0.55		0.36		0.55		0.39

TABLE II-2. Color REACTIONS OF SUGARS PRESENT IN HYDROLYZATE

Spray reagent	Glucose	Apiose	Authentic rhamnose
Benzidine-trichloroacetic acid	yellowish brown	yellowish brown	yellowish brown
Diphenylamine-aniline ^{a)}	gray	pinkish yellow	purplish brown
Aniline-phthalic acid ^{b)}	yellowish brown	yellowish brown	brown
Naphtho-resorcinol-phosphoric acid ^{c)}	blue	dark green	blue
AgNO ₃ ^{d)}	dark brown	dark brown	dark brown

^{a)} R. W. Bailey and E. J. Boure, *J. Chromato.*, **4**, 206 (1960).

^{b)} S. M. Partidge, *Nature*, **164**, 443 (1949).

^{c)} V. Prey, H. Berbalk and M. Kausz, *Mikrochim. Acta*, **1961**, 968.

^{d)} W. E. Trevelyan, D. P. Procter and J. B. Harrison, *Nature*, **166**, 444 (1950).

compound with acetic anhydride-pyridine in the usual manner gave almost colorless needles, mp 224~227°C, which were identical with an authentic specimen of luteolin-tetraacetate, mp 226~227°C.

Alkali fusion of the compound (II). A mixture of the compound (II) (19 mg) and 20% potassium hydroxide solution was heated at 120°C in nitrogen for 30 min. After cooling and diluting with water, the resulting mixture was acidified with dil. hydrochloric acid and extracted with ether. The ethereal extract was subjected to paper chromatography (solv. 8). Two spots were revealed on the chromatogram, the *R_f* values of which agreed with those of authentic mono-*O*-methyl phloroglucinol and vanillic acid, respectively.

Isolation of the compound (III) (homo-flavoyadorinin-B). The Substance C (2.3 g), mp 197~202°C (sint. at 130°C), was recrystallized several times from 50%

ethanol, giving pale yellowish needles (2.15 g), mp 208~210°C (sint. at 130°C). It gave the following colorations: ferric chloride: purplish brown; sodium carbonate: yellow; basic lead acetate: yellow; Mg-HCl: orange; Zn-HCl: orange red. UV-light: brown. Gibbs: positive. Its solubilities: soluble in 30~80% ethanol or methanol, ethyl acetate, and pyridine; hardly soluble in ethanol and methanol; and insoluble in acetone, chloroform, benzene, ethyl ether, petroleum ether, hexane and water. *R_f* values: 0.46 (solv. 1); 0.90 (solv. 2); 0.75 (solv. 3); 0.49 (solv. 4). $\lambda_{\text{max}}^{\text{EtOH}}$ m μ (log ϵ): 243~4 (4.39), 270~1 (4.32), 340 (4.36). *Anal.* Found: C, 51.47; H, 5.72; OCH₃, 9.07. Calcd. for C₂₆H₂₆O₁₃(OCH₃)₂·3H₂O: C, 51.77; H, 5.29; OCH₃, 8.93%.

Hydrolysis of the compound (III); Formation of the compound (V) and detection of apiose. The compound (III) (50 mg) was refluxed with 5% sulfuric acid in 50% ethanol for 3 hr. After removing ethanol *in vacuo*, the resulting solution was treated as described before. The aglycon separated was recrystallized several times from 80% ethanol, giving yellowish fine needles (25.0 mg, 50.1%), mp 218~220°C, which was proved to be identical with the compound (V) by mixed mp and IR spectrum.

Hydrolyzate freed of the aglycon was neutralized with barium carbonate, and filtered to yield a solution, which was subjected to paper chromatography for detection of the sugar component. Two spots were detected on the chromatogram, the *R_f* values of which respectively agreed with those of D-glucose and apiose obtained from the hydrolyzate of authentic apiin* (Table II).

Partial hydrolysis of the compound (III); Formation of the compound (II). A suspension of the compound (III) (a few mg) in 5% acetic acid (10 ml) was heated on a water-bath for 3 hr. After cooling and diluting with a small amount of water (ca. 5 ml), the resulting reaction solution was subjected to paper chro-

matography using solv. 1~4 (for flavonoid component) and solv. 1 and 5~7 (for sugar component). Two components were detected on the chromatograms, the *R_f* values of which agreed with those of the compound (II) and apiose, respectively (Table II).

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