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On a Flavonol Glycoside Isolated from Flowers of a White Azalea (*Rhododendron mucronatum* G. Don)

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RECEIVED MARCH 26, 1956

The flavonol glycoside isolated from white flowers of an azalea (*Rhododendron mucronatum* G. Don) yielded, on hydrolysis, rhamnose and an apparently new flavonol. The structure 3,3′,4′,7-tetrahydroxy-5-methoxyflavone is proposed for the aglycon. The glycoside is the 3-rhamnoside of this aglycon. A small amount of the aglycon also has been isolated from the flowers in a free form. The names "azaleatin" and "azalein" are proposed for the aglycon and its rhamnoside, respectively.

Rhododendron mucronatum G. Don is a white azalea very commonly cultivated in Japanese gardens. Nakaoki[1] reported the isolation, from an extract of the white flowers, of a glycoside in an amorphous form which, on hydrolysis, yielded quercetin, glucose and rhamnose; however, further details were obscure.

The present study has been made in order to clarify the structure of the glycoside. Contrary to expectation, an apparently new flavonol and its glycoside were isolated from the extract of white flowers of the azalea.

The glycoside melted at 181–185° and a methanol solution gave a green color with ferric chloride. The methanol solution was effectively oxidized by pentammine cobaltichloride (purpureo salt), indicating the presence of two or more adjacent phenolic hydroxyl groups. On hydrolysis of the glycoside, rhamnose and an aglycon which melted at about 320° were found. The melting point was depressed when the aglycon was mixed with quercetin or isorhamnetin.[2] The acetate of the aglycon melted at 196–198°. The R_f values of the aglycon in three solvent systems did not agree with those of quercetin, isorhamnetin and rhamnetin. Methylation of the aglycon with dimethyl sulfate yielded a compound that melted at 148° and showed no depression in melting point on mixing with an authentic sample of quercetin pentamethyl ether. The product obtained by hydrolysis of the fully-methylated glycoside had a melting point of 193°, and no melting point depression was observed when it was mixed with 3′,4′,5,7-tetramethylquercetin. This indicates that the sugar residue is attached at the 3-position of the aglycon. This is also confirmed by the absorption spectra of the glycoside and the aglycon. The maximum absorption of the latter is at 367 mμ, more than 20 mμ greater than the former, indicating that the hydroxyl in the 3-position of the glycoside is substituted by the sugar.[3] Micro Zeisel determinations showed the presence of one methoxyl group in each. The absorption spectrum of the aglycon is quite similar to that of fisetin (3,3′,4′,7-tetrahydroxyflavone).[4]

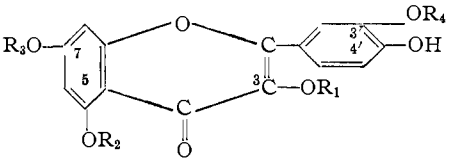
The only monomethyl ethers of quercetin (3,3′,4′,5,7-pentahydroxyflavone) hitherto known in nature are rhamnetin and isorhamnetin; these are 7- and 3′-monomethylquercetin, respectively. The author's aglycon is identical with neither rhamnetin nor isorhamnetin. However, since the methyl ethers of the aglycon and the glycoside are identical with those of quercetin and since the evidence mentioned above indicates that there should be at least two adjacent hydroxyl groups and that the hydroxyl at the 3-position is free, the only possible location for the methoxyl in the aglycon is the 5-position.

TABLE I

R_f VALUES[a] OF SOME FLAVONOLS IN THREE SOLVENT SYSTEMS

Samples	Solvents: *n*-Butanol–acetic acid: water (40:10:50)	*m*-Cresol satd. with water	Phenol satd. with water
Quercetin	0.73	0.11	0.33
Isorhamnetin	.83	.53	.71
Rhamnetin[b]	.80	...	.71
Author's aglycon	.56	0.29	.66

[a] The R_f values listed are averages of three determinations on Whatman No. 1 filter paper, at 20°. [b] Since rhamnetin was not available, the R_f values of rhamnetin are taken from the report of Wender, *et al.*[5]



R_1, R_2, R_3, R_4 = H	Quercetin
R_1, R_2, R_4 = H; R_3 = CH_3	Rhamnetin
R_1, R_2, R_3 = H; R_4 = CH_3	Isorhamnetin
R_1, R_3, R_4 = H; R_2 = CH_3	Azaleatin
R_3, R_4 = H; R_2 = CH_3	Azalein

R_1 = rhamnose residue

Kuhn and Löw[6] synthesized a number of quercetin methyl ethers and mentioned that none of the methyl ethers having a free hydroxyl group at the 5-position showed fluorescence in acetic anhydride; however, the methyl ethers in which the hydroxyl group at the 5-position was methylated showed a strong fluorescence in that solvent. The author's aglycon fluoresced in acetic anhydride. Although Kuhn and Löw's quercetin-5-methyl ether melted at 301–302°, the acetate melted at 197–198.5°, which is in good agreement with that of the acetate of the author's aglycon (196–198°). From this evidence, their results support the proposed structure of azaleatin.

There is no doubt, therefore, that the glycoside is the 3-rhamnoside of 5-methoxyquercetin; and since both the aglycon and its glycoside are new in nature, the names "azaleatin" and "azalein" are given to the aglycon and the glycoside, respectively.

(1) T. Nakaoki, *J. Pharm. Soc. Japan*, **52**, 1089 (1932).

(2) The specimen was generously supplied by Dr. H. Tatsuta of the Tôhoku University, Sendai, Japan.

(3) S. Hattori, *Acta Phytochim. (Japan)*, **6**, 131 (1932).

(4) M. Hasegawa, personal communication.

(5) T. B. Gage, C. D. Douglass and S. H. Wender, *Anal. Chem.*, **23**, 1582 (1951).

(6) R. Kuhn and I. Löw, *Ber.*, **77**, 211 (1944).

Experimental[7]

Isolation of the Glycoside.—Fresh white flowers (4.3 kg.) of *Rhododendron mucronatum*, collected on the campus of the College of General Education, University of Tokyo, were extracted twice with methanol for 1 hr. on a water-bath. The extracts were combined and filtered after cooling. The methanol was removed *in vacuo* with the occasional addition of water. The residue of approximately 1.5 l. was filtered while hot. The aqueous filtrate was extracted with *n*-butanol several times and the *n*-butanol extracts were combined and evaporated to dryness at reduced pressure. The dark, tarry residue was then dissolved in 300 ml. of hot water and the insoluble material filtered off. After standing overnight, the filtrate was extracted twice with ether. The ether extract yielded a small quantity of yellow precipitate, but no further investigation of this substance was made.

The aqueous layer was extracted with ethyl acetate for 24 hr., using a liquid–liquid extractor. After removal of the solvent, a yellow precipitate was obtained, which proved to be identical with the aglycon obtained by hydrolysis from the glycoside. The residue from the ethyl acetate extract was dissolved in water and treated with a saturated solution of normal lead acetate until no further precipitation occurred. The precipitate was collected and deleaded by hydrogen sulfide. The filtrate of lead sulfide gave no test for flavonoids.

The filtrate from which the lead precipitate had been removed was neutralized with an aqueous ammonia solution, and during this treatment an orange-yellow precipitate was formed. At *p*H 7, a basic lead acetate solution was added. The precipitates were combined, separated by centrifugation and washed with water several times. They were then suspended in methanol and after removal of lead with hydrogen sulfide, the methanol solution was concentrated *in vacuo* to approximately 50 ml. The resulting concentrate, now quite aqueous, was allowed to stand at room temperature for several days.

After keeping the concentrate in the refrigerator for an additional several days and occasionally stirring it with a glass rod, it turned into a light yellow creamy mass. The precipitated material was collected and recrystallized from 40% methanol and then several times from water; the yield was 3.6 g.

Purification of the Glycoside.—The substance obtained above was not yet pure, and paper chromatographic study revealed that it contained another flavonoid substance. The substance was finally purified by paper chromatography on Whatman No. 1 filter paper according to the method of Ice and Wender.[8] The strips containing the glycoside were cut off and extracted twice with methanol under reflux. The combined methanol extracts were concentrated and allowed to stand in the refrigerator until crystals were formed. The crystals were filtered off and then recrystallized from 40% methanol and from water; the m.p. was 181–185°.

The crystals of the glycoside are minute, light yellow needles, readily soluble in hot water, methanol and acetone but not in ethyl acetate. The methanol solution of the glycoside showed a green coloration with ferric chloride solution. The methanol solution gave a deep red color with concentrated hydrochloric acid and magnesium. The glycoside dried in air had 5 molecules of water of crystallization and lost the water when heated *in vacuo* over phosphorus pentoxide at 100°. Absorption: λ_{max} 250 mμ, log ϵ 4.34; λ_{max} 340 mμ, log ϵ 4.25; λ_{min} 282 mμ, log ϵ 3.90.

Calcd. for $C_{22}H_{22}O_{11}\cdot 5H_2O$: water of crystn., 15.53. Found: water of crystn., 15.54.

Anal. Calcd. for $C_{22}H_{22}O_{11}\cdot H_2O$: C, 55.00; H, 5.00; OCH_3, 6.44; water of crystn., 3.75. Found: C, 55.12; H, 4.98; CH_3O, 6.76; water of crystn., 4.0.

Hydrolysis of the Glycoside.—The anhydrous glycoside (42.5 mg.) was hydrolyzed by refluxing with 2% sulfuric acid. The hydrolysis was complete within a few minutes. After cooling overnight, the aglycon was filtered off, dried and weighed (28.4 mg.). Thus, the ratio of the aglycon to the glycoside is 68.5%, and this ratio indicates the presence of one mole of sugar per mole of aglycon.

The aglycon was recrystallized from 80% methanol, and bright yellow needles, which melted at about 320°, were obtained. Absorption: λ_{max} 252 mμ, log ϵ 4.38; λ_{max} 368 mμ, log ϵ 4.42; λ_{min} 286 mμ, log ϵ 3.89.

Anal. Calcd. for $C_{15}H_{12}O_7\cdot H_2O$: C, 57.48; H, 4.16; OCH_3, 9.28; water of crystn., 5.5. Found: C, 57.07; H, 4.07; OCH_3, 7.91; water of crystn., 5.5.

The filtrate from which the aglycon was removed was neutralized with barium hydroxide, filtered and concentrated *in vacuo*. The sugar was identified chromatographically in a solvent system of *n*-butanol–acetic acid–water (4:1:5) using authentic sugars as checks. The R_f value of the sugar was identical with that of rhamnose (R_f 0.40). The osazone of the sugar was also prepared from the concentrate by the usual method; it melted at 181–184°, showing no depression of the melting point when mixed with the authentic specimen.

Acetate of the Aglycon.—The aglycon (0.1 g.) was acetylated with acetic anhydride and one drop of pyridine by gently heating on a water-bath. The acetate formed white needles which melted at 196–198° after several recrystallizations; the yield was 0.12 g. The acetate caused a notable depression of the melting point (167–171°) when mixed with an authentic specimen of quercetin pentaacetate (192°).

Anal. Calcd. for $C_{15}H_8O_7(CH_3CO)_4$: C, 59.50; H, 4.13. Found: C, 59.39; H, 4.43.

Position of the Sugar of the Glycoside.—The methyl ether of the glycoside was prepared by boiling a mixture of the glycoside (0.3 g.), acetone (50 ml.), dimethyl sulfate (1.5 ml.) and potassium carbonate (4 g.) on a water-bath until a few drops of the mixture showed no more coloration with ferric chloride. After filtration, the acetone was removed by distillation, and the filtrate was immediately hydrolyzed. Several recrystallizations yielded yellow needles melting at 193°, and a mixture of this ether and an authentic specimen of 3′,4′,5,7-tetramethylquercetin showed no depression of melting point.

Methylation of the Aglycon.—One-tenth gram of the methyl ether described above was further methylated by the same method. It yielded 80 mg. of colorless needles after recrystallization from methanol. The ether melted at 148°, and no depression of melting point was observed when admixed with an authentic pentamethylquercetin.

Acknowledgments.—The author thanks Prof. S. Hattori, the University of Tokyo, and Dr. M. Hasegawa, Government Forest Experiment Station of Japan, for their many helpful suggestions. The author is grateful to Prof. T. A. Geissman, University of California at Los Angeles, for his kindness in revising this manuscript.

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(7) All the melting points are not corrected.

(8) C. H. Ice and S. H. Wender, THIS JOURNAL, **75**, 50 (1953).