Eriodictyol-7-glucoside and Other Phenolics in the Blue Fruits of *Lasiauthus japonica*

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Eriodictyol-7-glucoside, a flavanone glycoside, was isolated as cream-white needles from the deep-blue fruit skins of *Lasianthus japonica*, and in the same tissue eriodictyol and scopoletin were also found to occur in a small amount by means of paper chromatography.

Lasianthus japonica Miq. belonging to Rubiaceae bears deep blue fruits in fall. In the fruits, the occurrence of anthocyanins, keracyanin and chrysanthemin, have been reported [1]. We now isolated a flavanone glycoside, eriodictyol-7-glucoside, from the blue epicarp. In the same tissue eriodictyol and a coumarin derivative, scopoletin, were also found to occur in a small amount, and they were identified by means of PC, TLC and UV spectrum.

The flavanone glycoside isolated from the ethyl acetate extract gave aglycone and sugar on acid hydrolysis. The former was identified as eriodictyol, 5,7,3′,4′-trihydroxyflavanone, based on the agreement with PC, TLC, UV spectrum and NMR with those of the authentic sample, and the R_F values and color reactions of the latter were completely coincided with those of glucose. The quantitative determination of aglycone showed that the glycoside gave the value corresponding to a monoglucoside. An alcoholic solution of the glycoside gave a red-purple color with Mg – HCl indicating the presence of flavanone structure. The absorption spectrum of the glycoside showed a bathochromic shift on the addition of $AlCl_3$ and there was no spectral reversion on the addition of dilute HCl indicating the presence of free C5 hydroxyl group [2]. The NMR spectrum of the glycoside has also strengthened this possibility. The methylated product of the glycoside with diazomethane was degraded with alkali into phlorogluci-

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nol monomethyl ehter and veratric acid, which were derived from the A and B rings, respectively. These results indicate that the flavanone glycoside is eriodictyol-7-glucoside, which has been isolated for the first time, except that it has been isolated only as the heptaacetate from the rhizome of *Lophophytum leandri* [3].

Experimental

Plant material and extraction. Fresh deep-blue epicarps (ca. 50 g from 600 g fresh fruits) of *Lasianthus japonica* were collected from the trees growing in a copse of Sakamoto, Kumamoto prefecture, and ground for 5 min at full speed in a Polytron homogenizer with 300 ml of distilled water. The homogenate was centrifuged at $10{,}000 \times g$ for 15 min. The supernatant was acidified with HCl to pH 2 and extracted thoroughly with ether and then with ethyl acetate.

Eriodictyol-7-glucoside. The ethyl acetate extract obtained above was evaporated to dryness, dissolved with 10 ml of MeOH and allowed to stand in a refrigerator to give a brownish white needles. Yield 300.2 mg. This crop was recrystallized from MeOH – petroleum ether (b. p. 30 –70 °C) as cream-white needles, m. p. 208 °C. Found: C, 55.35; H, 4.99; H_2O, 1.84. Calcd. for $C_{21}H_{22}O_{11} \cdot \frac{1}{2}H_2O$: C, 56.00; H, 4.92; H_2O, 1.96%. Black in UV; yellow in UV + NH_3. Colors with diazotized *p*-nitroaniline, brown; with Gibbs reagent, dark blue; with Mg–HCl, red-purple; with MgOAc, blue fluorescence in UV. PC: R_F 0.68, *n*-BuOH : HOAc : H_2O (4 : 1 : 5) (BAW); 0.70, 30% HOAc; 0.58, HOAc : HCl : H_2O (15 : 3 : 82) (AAH-II). TLC: R_F 0.50, H_6O_6 : MeOH : HOAc (10 : 3 : 3) (BzMA); 0.48, $CHCl_3$: HOAc (4 : 1). UV λ_{max}^{MeOH} nm 288 (100%), 329 sh (41%); $AlCl_3$ 311 (100%), 378 (33%); $AlCl_3$/HCl 311 (100%), 378 (33%); NaOAc 288 (87%), 330 (100%); NaOAc/H_3BO_3 288 (87%), 330 (100%). NMR (DMSO-d_6) δ = 2.7–3.8 (8H, m, aliphatic CH), 4.1–5.6 (6H, m, aliphatic CH and OH), 5.84 (2H, s, $H_{(6)}$ and $H_{(8)}$), 6.4–7.3 (3H, m, $H_{(2')}$, $H_{(3')}$ and $H_{(6')}$), 8.65 (1H, broad s, OH), 10.7 (1H, broad s, OH), and 12.10 (1H, s, $C_{(5)}$-OH). On complete acid hydrolysis [4], the glycoside gave aglycone (eriodictyol) and glucose. PC of glucose as lit. [4]. 21.94 mg the glycoside (anhydrous) gave 14.10 mg eriodictyol (anhydrous) and 3.70 mg glucose, which was determined by the method of Somogyi [5]. Found: aglycone

64.27%, glucose 16.86%. Calcd. for $C_{21}H_{22}O_{11} \rightarrow C_{15}H_{12}O_6 + C_6H_{12}O_6$: aglycone 64.00%, glucose 40,00%. Methylation of the glycoside with diazomethane followed by hydrolysis with 2 *N* HCl gave eriodictyol 5,3′,4′-trimethyl ether. The product was degraded with 12% NaOH in N_2 under reflux into phloroglucinol monomethyl ether, which was identified by TLC as lit. [6], and veratric acid (TLC R_F 0.27, C_6H_6: MeOH : HOAc, 45 : 8 : 4; color with $FeCl_3$, dark yellow).

Eriodictyol (aglycone). Colors with diazotized *p*-nitroaniline, yellow; with Gibbs reagent, dark blue; with $FeCl_3$, purple; with MgOAc, blue fluorescence in UV. PC behaviors and UV spectra of the aglycone were completely coincided with those of the authentic eriodictyol. PC: R_F 0.93, BAW; 0.32, AAH-II; 0.55, 30% HOAc. TLC: R_F 0.88, BzMA; 0.46, C_6H_6: dioxane : H_2O (90 : 25 : 4). UV λ_{max}^{MeOH} nm 299 (100%), 320 sh (39%); $AlCl_3$ 312 (100%), 378 (35%); $AlCl_3$/HCl 312 (100%), 378 (35%); NaOAc 295 sh (59%), 326 (100%); NaOAc/H_3BO_3 292 (100%), 322 (63%). NMR (DMSO-d_6) δ = 2.7–3.5 (3H, m, $2H_{(3)}$ and OH), 5.42 (1H, dd, J = 11 and 3Hz, $H_{(2)}$), 5.91 (2H, s, $H_{(6)}$ and $H_{(8)}$), 6.7–7.0 (3H, m, $H_{(2')}$, $H_{(3')}$ and $H_{(6')}$), 9.0 (2H, broad, OH), and 12.0 (1H, s, OH). MS *m/e* 288 (M^+), 179, 166, 153 and 136.

Eriodictyol and scopoletin. Eriodictyol and scopoletin in the ether extract obtained above were identified paperchromatographically. PC behaviors and UV spectra of eriodictyol were completely coincided with those of the aglycone as mentioned above. PC behaviors and UV spectra of scopoletin was completely identical with those of the authentic sample: PC (R_F 0.60, AAH-II; 0.87, BAW; 0.30, H_2O; 0.52, 10% HOAc). Purple in UV; sky blue in UV + NH_3. Color with Pauly reagent, brown. UV λ_{max}^{EtOH} nm 299 (68%), 348 (100%); NaOH 400.

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