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AN ACYLATED PELARGONIDIN DIGLYCOSIDE FROM *PULSATILLA CERNUA*

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Key Word Index—*Pulsatilla cernua*; Ranunculeaceae; acylated anthocyanin; pelargonidin 3-[2″-(2‴-*trans*-caffeoyl-β-D-glucopyranosyl)-β-D-galactopyranoside].

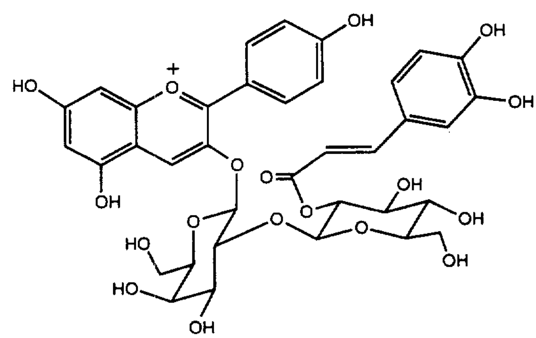
Abstract—A new acylated pelargonidin glycoside was isolated from the sepals of *Pulsatilla cernua*. The structure was established as pelargonidin 3-[2″-(2‴-*trans*-caffeoyl-β-D-glucopyranosyl)-β-D-galactopyranoside] by spectroscopic methods. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Pulsatilla cernua Spreng. (Ranunculaceae) is a perennial herb, whose flowers open with dark brownish red sepals in the spring. A wide variety of anthocyanins have been reported from the members of Ranunculaceae e.g. *Delphinium* species [1–3]. *Anemone* species [4–6], *Clematis terniflora* [7] and 17 *Aquilegia* species [8]. Recently, a polyacylated anthocyanin with 4 moles of *p*-hydroxybenzoic acid and malonated anthocyanins have been identified in the blue flowers of *Delphinium hybridum* [9] and in the flowers of *Ranunculus asiaticum* [10], respectively. We now report the isolation and characterization of a new acylated anthocyanin (1) from the sepals of *P. cernua*.

RESULTS AND DISCUSSION

The new anthocyanin (1) was isolated by repeated paper and column chromatography from an ethanol–acetic acid–water (9:1:10) extract. The R_f-values (×100) of 1 on TLC were 49, 75, 38 and 10 with 1% HCl, AAH-II, BAW and BuN, respectively (see Experimental). Compound 1 showed λ_{max} (0.1% HCl–MeOH) at 512, 332 and 287 nm with a shoulder at 433 nm, and no bathochromic shift was observed after addition of $AlCl_3$. The values of $E_{440}/E_{vis\,max}$ (42%) and $E_{acid\,max}/E_{vis\,max}$ (82%) indicated that 1 was a 3-*O*-glycoside acylated with a cinnamic acid group [11]. The absence of fluorescence in UV light also suggested 1 is a 3-*O*-glycoside. Acid hydrolysis of 1 gave pel-



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argonidin, glucose, galactose and caffeic acid (PC and TLC). Alkaline hydrolysis of 1 yielded the deacylated compound (2) and caffeic acid, while H_2O_2 degradation of 2 released a diglycoside, which gave a similar colour reaction with diphenylamine–aniline reagent to sophorose but the R_fs were very slightly different. Compound 2 was degradated *via* two steps to pelargonidin by controlled acid hydrolysis (TLC), indicating 1 was a diglycoside. The first product was not pelargonidin 3-*O*-glucoside (TLC), suggesting that 1 was a pelargonidin 3-*O*-glucosylgalactoside. The positive FAB mass spectrum of 1 gave $[M+H]^+$ at *m/z* 757, indicating that 1 was composed of one molecule each of pelargonidin, glucose, galactose and caffeic acid. This was confirmed by ^1H and ^{13}C NMR spectra. Thus, seven aglycone protons and 15 aglycone carbons were assigned to be protons and carbons of pelargonidin, respectively (Table 1). Two anomeric protons of galactose and glucose were assigned at δ 5.50 (*d*, $J = 7.3$ Hz) and 5.17 (*d*, $J = 8.2$ Hz), respectively, and the large coupling constants indicated that 1 has

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✠ Deceased.

Table 1. ^{13}C and ^{1}H NMR assignments and ^{1}H-^{13}C long-range correlations of **1** by ^{1}H-^{1}H COSY, HMBC and HMQC in DMSO-d_6 containing 10% TFA

C	δ_C	δ_H	Cross-peaks (δ_0) in HMBC spectrum
Aglycones			
2	162.1		
3	143.6		
4	135.6	9.02 (*s*)	112.1(10), 119.3(1′), 143.6(3), 156.0(9), 162.1(2)
5	168.6		
6	102.4	6.80 (*br d*, 1.9)	94.1(8), 168.6(5,7)
7	168.6		
8	94.1	6.90 (*br s*)	102.4(6), 156.0(9), 168.6(5,7)
9	156.0		
10	112.2		
1′	119.3		
2′	134.8	8.56 (*d*, 9.2)	162.1(2), 164.8(4′)
3′	116.9	7.15 (*d*, 9.2)	164.8(4′)
4′	164.8		
5′	116.9	7.15 (*d*, 9.2)	164.8(4′)
6′	134.8	8.56 (*d*, 9.2)	162.1(2), 164.8(4′)
Galactose			
1	100.0	5.50 (*d*, 7.3)	143.6(3)
2	75.4	4.21 (*t*, 9.2)	73.4(gal-3)
3	73.4	3.70 (*dd*, 2.7, 9.2)	
4	68.4	3.78 (*br s*)	73.4(gal-3), 75.4(gal-2)
5	76.1	3.78 (*br s*)	60.2(gal-6), 68.4(gal-4)
6	60.2	3.54 (*dd*, 6.5, 11.0)	68.4(gal-4), 76.1(gal-5)
		3.66 (*dd*, 4.1, 11.0)	68.4(gal-4)
Glucose			
1	99.6	5.17 (*d*, 8.2)	75.4(gal-2)
2	74.0	4.66 (*t*, 9.2)	74.7(glc-3), 165.9(caff-9)
3	74.7	3.40 (*t*, 9.2)	70.8(glc-4)
4	70.8	3.12 (*br s*)	77.4(glc-5)
5	77.4	3.12 (*br s*)	70.8(glc-4)
6	61.4	3.33 (*m*)	77.4(glc-5)
		3.66 (*d*, 11.9)	
Caffeic acid			
1	125.9		
2	114.7*	7.00 (*br s*)	145.0(caff-7), 145.7(caff-3), 148.4(caff-4)
3	145.7		
4	148.4		
5	116.0	6.77 (*d*, 8.3)	145.7(caff-3), 148.4(caff-4)
6	121.4	6.89 (*br d* 8.3)	145.0(caff-7), 148.4(caff-4)
7	145.0	7.41 (*d*, 15.6)	165.9(caff-9)
8	114.8*	6.81 (*d*, 15.6)	165.9(caff-9)
9	165.9		

* Signals may be interchanged.

β-configurations. The ^{13}C NMR spectrum of **1** (Table 1) clearly showed that both C-6 of the glucose and the galactose were free because of the absence of shifts. The glycosylation shift to lower field of the C-2 carbon of galactose (δ 75.4) indicated that the glucose was attached to 2-OH of galactose, which was supported by FGHMBC of **1** showing a correlation between H-1 of glucose and C-2 of galactose. The acylation shift to lower field of a H-2 proton of glucose (δ 4.66, *t*, $J = 9.2$ Hz) indicated that the caffeic acid was attached to 2-OH of glucose, which was supported by HMBC (8 Hz) and HMQC analyses of **1**. Thus, **1** is pelargonidin 3-[2″-(2‴-*trans*-caffeoyl-β-D-glucopyranosyl)-β-D-galactopyranoside].

EXPERIMENTAL

General. PC and TLC were carried out on Toyo No. 51 and micro-crystalline cellulose plate (Avicel SF). The following solvent systems were employed;

BAW: *n*-BuOH–HOAc–H_2O (4:1:5), BuN: *n*-BuOH–2N HCl (1:1), AAH-II: HOAc–HCl–H_2O (15:3:82), 1% HCl, Forestal: HOAc–HCl–H_2O (30:3:10), FHW: HCOOH–HCl–H_2O (5:2:3), BzA: C_6H_6–HOAc–H_2O (125:72:3) for TLC and BAW, *n*-PEtAW: *n*-PrOH–EtOAc–H_2O (3:1:1) and BPW: *n*-BuOH–pyridine–H_2O (6:1:3) for PC. Purity of **1** was checked by HPLC on a Lichrospher 100 RP-18 column ($4.6\phi \times 250$ mm) at a flow rate of 0.5 ml min^{-1}, at 280 and 530 nm, using an isocratic solvent system H_3PO_4–HOAc–MeCN–H_2O (3:8:10:79). FAB-MS was measured in the positive ion mode with glycerol-HCl as the matrix. ^1H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, in TFA-DMSO-d_6 (1:9).

Plant material. Pulsatilla cernua flowers were collected from the foot of Mt. Nekodake in Kyushu, Japan, in April 1995. The plant was authenticated by Dr M. Takamiya, Department of Environmental Science, Kumamoto University. A voucher specimen was deposited in the Herbarium of Kumamoto University (KUMA), Kumamoto, Japan.

Extraction and Isolation. Air-dried sepals were extracted with EAW (EtOH–HOAc–H_2O, 9:1:10) or 0.1% HCl–MeOH at 5°, concd *in vacuo*, and washed thoroughly with Et_2O and EtOAc. The extract was purified by Polyamide C-200 and Sephadex LH-20 CCs. The eluate containing **1** was concd and further purified by PPC with AAH-II. The eluate from PC was again applied to Sephadex LH-20 CC, concd and crystallized in cold H_2O.

Acid and alkaline hydrolyses. Compound **1** was refluxed in 2N HCl at 95° for 1 hr. The organic acid and aglycone were extracted with Et_2O and *iso*-amylalcohol, respectively, and identified by co-TLC with authentic markers in Forestal, BzA and BAW. The sugars were identified by co-PC and co-TLC with standard markers (BAW, *n*-PEtAW, BPW). Controlled acid hydrolysis was carried out with 10% HCl at 75° for 50 min (AAH-II, BuN) and alkaline hydrolysis with 8% NaOH at 25° for 1 hr in N_2. H_2O_2 degradation was performed by the method of Takeda and Hayashi [12].

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