

PELARGONIDIN 3-DI (*p*-HYDROXYBENZOYL) RUTINOSIDE-7-GLUCOSIDE FROM FLOWERS OF *CAMPANULA*

SAM ASEN, ROBERT N. STEWART and KARL H. NORRIS
USDA, Science and Education Administration, AR, Beltsville, MD 20705, U.S.A.

(Received 8 December 1978)

Key Word Index—*Campanula* sp.; Campanulaceae; bellflower; acylated anthocyanin; pelargonidin 3-di (*p*-hydroxybenzoyl) rutinoid-7-glucoside; *p*-hydroxybenzoic acid.

Among the several hundred *in vivo* absorption spectra of flowers that we have recorded, the biennial bellflower (*Campanula* sp. cv Rose) exhibited characteristics of special interest. The pH of epidermal cells was *ca* 6.4 and the flowers had 3 distinctively sharp visible absorption bands with λ_{\max} of 496, 532 and 571 nm. Anthocyanins with sugars in the 7-position are rare [1, 2] and we now report one of the anthocyanins in Rose bellflower as a new acylated 3,7-disubstituted pelargonidin.

The absorption spectrum of the isolated anthocyanin in 1% HCl–MeOH showed λ_{\max} at 513 nm (log ϵ 4.34) and 250 nm (log ϵ 4.48). Typical R_f 's ($\times 100$) were 42 (1% HCl); 77 (HOAc–HCl–H₂O, 15:3:82); 15 (*n*-BuOH–HOAc–H₂O, 6:1:2); and 31 (*n*-BuOH–2 N HCl, 1:1). After alkaline hydrolysis, R_f 's in the same solvents were 64, 86, 18, 15, respectively and λ_{\max} 's were 502 (log ϵ 4.45), 279 (log ϵ 4.26), and 260 (sh) (log ϵ 4.23) nm. The $E_{440}/E_{\text{vis max}}$ (0.42), $E_{\text{UV max}}/E_{\text{vis max}}$ (0.65) and the absorption spectra were characteristic of a 3,7-disubstituted pelargonidin [3].

The mass spectral fragmentation pattern, chromatographic characteristics and absorption spectra of the acyl moiety obtained from alkaline hydrolysis were indistinguishable from those of an authentic sample of *p*-hydroxybenzoic acid [4]. Solutions in 0.5% HCl–MeOH that contained molar ratios of *p*-hydroxybenzoic acid to pelargonidin 3-rutinoside-7-glucoside of 0:1, 1:1, 2:1 and 3:1 exhibited E_{255}/E_{502} (UV and visible λ_{\max}) of 0.40, 0.96, 1.49 and 2.00, respectively. In the same solvent, E_{250}/E_{513} (UV and visible λ_{\max}) for the isolated

anthocyanin was 1.47, which indicated that the molar ratio of *p*-hydroxybenzoic acid to anthocyanin was 2:1.

Complete acid hydrolysis of the deacylated anthocyanin yielded pelargonidin, glucose and rhamnose, whereas partial hydrolysis with acid yielded four intermediate pelargonidin glycosides designated as Pg 1, Pg 2, Pg 3 and Pg 4 (Table 1). They were resolved by TLC with HOAc–HCl and BuHCl, respectively. The products of partial and complete acid hydrolysis, color in UV, R_f 's and absorption spectra of Pg 1, Pg 3 and Pg 4 were indistinguishable from 3 of the 4 intermediate pelargonidin glucosides obtained from the controlled acid hydrolysis of pelargonidin 3-sophoroside-7-glucoside isolated from *Papaver orientale* [5]. Pg 1 was identified as pelargonidin 3,7-diglucoside, Pg 3 as pelargonidin 3-glucoside, and Pg 4 as pelargonidin 7-glucoside. Pelargonidin 3-sophoroside and Pg 2 were chromatographically different. The disaccharide obtained from the H₂O₂ oxidation of Pg 2 was chromatographically indistinguishable from rutinose (prepared from rutin) and could not be distinguished from rutinose by electrophoresis on paper in borate buffer, pH 10, at 15 V/cm for 6 hr [6]. The disaccharide had a green color with a mixture of aniline, diphenylamine and orthophosphoric acid spray reagent [7], characteristic of a 1 → 6 linkage. Pg 2 was identified as pelargonidin 3-rutinoside. A rutinoside previously has been identified from Campanulaceae [8].

Products formed from the H₂O₂ oxidation of the

Table 1. Properties of the pelargonidin glycosides formed from partial acid hydrolysis of the deacylated anthocyanin from flowers of bellflower (*Campanula* sp. cv Rose)

Pg* Glycoside	Color* in UV	$R_f (\times 100)^\dagger$				λ_{\max} in 1% HCl–MeOH	$\frac{E_{440}}{E_{\text{vis max}}}$ (%)	$\frac{E_{\text{UV max}}}{E_{\text{vis max}}}$ (%)
		1% HCl	HOAc–HCl	BAW	BuHCl			
Pg 1	OY	40	72	21	12	269 (sh), 279, 501	41	69
Pg 2	OR	23	54	33	45	269, 508	39	62
Pg 3	OR	10	38	34	41	269, 507	39	58
Pg 4	OR	10	38	34	53	269, 510	40	56
Pg Glycoside		Products of partial and complete acid hydrolysis					Identified as	
Pg 1		Pg 3-glucoside, Pg 7-glucoside, Pg and glucose					Pg 3,7-diglucoside	
Pg 2		Pg 3-glucoside, Pg, glucose and rhamnose					Pg 3-rutinoside	
Pg 3		Pg and glucose					Pg 3-glucoside	
Pg 4		Pg and glucose					Pg 7-glucoside	

* Abbreviations: Pg = pelargonidin; O = orange; Y = yellow; R = red.

† HOAc–HCl = HOAc–HCl–H₂O (15:3:82); BAW = *n*-BuOH–HOAc–H₂O (6:1:2); BuHCl = *n*-BuOH–2N HCl (1:1, organic phase).

isolated anthocyanin were resolved by TLC with BAW [9, 10]. The disaccharide liberated from the C-3 position yielded *p*-hydroxybenzoic acid on alkaline hydrolysis and glucose and rhamnose on acid hydrolysis. The other oxidative products yielded no acyl moiety on alkaline hydrolysis and only glucose on acid hydrolysis, confirming that *p*-hydroxybenzoic acids were attached to the sugars at the C-3 position [11]. All the above criteria indicated that the anthocyanin isolated from the biennial bellflower (*Campanula* sp. cv Rose) was pelargonidin 3-di (*p*-hydroxybenzoyl) rutinoside-7-glucoside.

A 10^{-3} M solution of the isolated anthocyanin in tris acid malonate buffer, pH 6.47, had visible λ_{\max} at 493, 529 and 565 nm. Although the shape of the visible absorption spectra of the isolated anthocyanin and epidermal cells were similar, the λ_{\max} differed by 2–6 nm. The isolated anthocyanin was unstable and lost 80% of the intensity of the absorption spectrum in the visible region within 30 min. The usual flavonoid copigments were not detected in flowers of Rose bellflower but they did contain several compounds that had blue fluorescence in UV. None of these compounds was chlorogenic acid but their absorption spectra were characteristic of caffeic acid esters [12, 13]. Sufficient amounts were not available for complete identification or for a study of their copigment effects.

EXPERIMENTAL

Plant material. Seeds of a biennial bellflower (*Campanula* sp. cv Rose) were obtained from the Geo. W. Park Seed Co., Inc., Greenwood, South Carolina 29646, U.S.A.

Authentic compound. Pelargonidin 3-sophoroside-7-glucoside was obtained from petals of *Papaver orientale* [5].

Isolation and identification. Flowers were harvested, dried at 40° in a forced-draft oven, then ground to pass a 40-mesh screen. Ground tissue was thoroughly extracted with hot (*ca* 60°) citrate-Pi buffer, pH 3.0. The extract was filtered with the aid of celite and the anthocyanin absorbed on a 25 × 400 mm column of purified insoluble PVP [14]. All solvents used with PVP contained 5 ml 2 N HCl/l. The column was washed with *ca* 100 ml of H₂O and the anthocyanin was eluted with 30% aq. MeOH. The alcohol was evapd from the eluant at 40° with

red. pres. and the pH of the aq. residue adjusted to 3.0. The isolation on PVP was repeated and the anthocyanin eluted with H₂O. The isolated anthocyanin was further purified by passage through Sephadex LH-20 with 0.5% HCl in 70% MeOH. The aglycone, sugars and the intermediate products of controlled acid hydrolysis were identified chromatographically by the usual methods [15]. The acylated disaccharide at the C-3 position was obtained by treatment with H₂O₂ [16] and the acyl moiety after alkaline hydrolysis was identified by chromatography and by the mass spectral fragmentation pattern.

Acknowledgements—We thank Dr. R. M. Horowitz for the electrophoresis data, Dr. J. R. Plimmer for the MS data, and Ms. P. S. Budin for her competent technical assistance.

REFERENCES

1. Harborne, J. B. (1963) in *Chemical Plant Taxonomy* (Swain, T., ed.) pp. 359–388. Academic Press, New York.
2. Timberlake, C. F. and Bridle, P. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.) pp. 231–254. Academic Press, New York.
3. Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*, p. 17. Academic Press, New York.
4. Asen, S., Stewart, R. N. and Norris, K. H. (1975) *Phytochemistry* **14**, 2677.
5. Harborne, J. B. (1963) *Phytochemistry* **2**, 85.
6. Williams, C. A. and Harborne, J. B. (1971) *Phytochemistry* **10**, 1059.
7. Schwimmer, S. and Bevenue, A. (1956) *Science* **123**, 543.
8. Saito, N. and Osawa, Y. (1971) *Phytochemistry* **10**, 445.
9. Hrazdina, G. (1970) *Phytochemistry* **9**, 1647.
10. Hrazdina, G. and Franzese, A. J. (1974) *Phytochemistry* **13**, 231.
11. Harborne, J. B. (1964) *Phytochemistry* **3**, 151.
12. Harborne, J. B. and Corner, J. J. (1966) *Biochem. J.* **81**, 242.
13. Jurd, L. (1957) *Arch. Biochem. Biophys.* **66**, 284.
14. Andersen, R. A. and Sowers, J. A. (1968) *Phytochemistry* **7**, 293.
15. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
16. Chandler, B. V. and Harper, K. A. (1961) *Aust. J. Chem.* **14**, 586.