Pergamon

0031-9422(95)00371-1

Phytochemistry, Vol. 40, No. 3, pp. 939–940, 1995

0031-9422/95 $9.50 + 0.00

PELARGONIDIN 3-GLUCOSIDE-5-ACETYLGLUCOSIDE IN *VERBENA* FLOWERS

KENJIRO TOKI, NORIO SAITO,* NORIHIKO TERAHARA† and TOSHIO HONDA‡

Laboratory of Floriculture, Minami-Kyushu University, Takanabe, Miyazaki 884, Japan; *Chemical Laboratory, Meiji-Gakuin University, Totsuka, Yokohama 224, Japan; †Department of Food Science and Technology, Minami-Kyushu University, Takanabe, Miyazaki 884, Japan; ‡Institute of Medicinal Chemistry, Hoshi University, Shinagawa, Tokyo 142, Japan

(*Received* 21 *Feburary* 1995)

Key Word Index—*Verbena hybrida*; Verbenaceae; flower colour; acylated anthocyanin; pelargonidin 3-glucoside-5-(6-acetylglucoside).

Abstract—A new acylated anthocyanin was isolated from deep pink flowers of *Verbena hybrida* cv 'Tropic' as a minor anthocyanin and identified as pelargonidin 3-*O*-β-D-glucoside-5-*O*-(6-*O*-(acetyl)-β-D-glucoside) with chromatographic and spectral methods.

INTRODUCTION

In previous reports we found the presence of 10 anthocyanins acylated with aliphatic acids (acetic and/or malonic) in the several coloured forms of *Verbena hybrida* [1–4]. In further studies a new acetylated anthocyanin was found in the deep pink flowers of this species along with three known anthocyanins, and its structure was identified.

RESULTS AND DISCUSSION

We isolated four anthocyanins as orange–red powder from the dark pink flowers of *Verbena* cv 'Tropic' by a procedure similar to that described previously [2, 4]. The main anthocyanin **1** and two-minor ones, **2** and **3** were identified as the 3,5-diacetylglucoside, 3-acetylglucoside-5-glucoside and 3,5-diglucoside of pelargonidin, respectively, by comparison with authentic specimens [2, 4]. The other minor anthocyanin **4** was a new compound (Table 1; Fig. 1), which on acid hydrolysis gave pelargonidin and glucose. Alkaline hydrolysis of this pigment gave pelargonidin 3,5-diglucoside. The FAB-mass spectrum of **4** showed the $[M]^+$ *m/z* 637, which was composed of one molecule of pelargonidin 3,5-diglucoside and acetic acid. However, **4** was clearly different from pelargonidin 3-acetylglucoside-5-glucoside **3** on TLC and HPLC (Table 1). Moreover, on H_2O_2 degradation **4** did not produce acetylglucose [2, 3], but only glucose. Therefore, **4** must be pelargonidin 3-glucoside-5-acetylglucoside.

The detailed structure was elucidated by ^{1}H NMR spectrum analysis (Experimental). The proton signals of **4** were mainly assigned by ^{1}H–^{1}H COSY [4]. The characteristic signals in the low field region were assigned to pelargonidin nucleus protons. In the sugar region 5.33–3.22 ppm, as the anomeric proton signals of the two glucoses (A and B, Fig. 1) were shifted to lower field (δ5.33 and 5.17) with a large coupling constant (J = 8 Hz), the glycosylated sugars were shown to be the β-anomers. The large coupling constant of sugar ring protons (J = 8–12 Hz) showed that the glucosyl residues are in the pyranose form. The low field shift of C-6 methylene protons (δ4.34 and 4.13) of 5-glucose indicated that the OH-6 of glucose B was acylated. The methyl proton of acetic acid in **4** was assigned at δ2.00. Therefore, the new pigment **4** is pelargonidin 3-*O*-β-D-glucopyranoside-5-*O*-(6-*O*-(acetyl)-β-D-glucopyranoside).

EXPERIMENTAL

Materials. Flower petals of *Verbena hybrida* cv 'Tropic' were collected in the garden of Minami-Kyushu University and dried at 45°.

Extraction and isolation. The dried petals (30 g) were extracted with 2% HCO_2H at room temp. The filtered extract was adsorbed on Diaion HP-20 column, washed with *ca* 0.5% HCO_2H and then eluted with 2% HCO_2H in 70% MeOH. After concn, the eluate was fractionated by Sephadex LH-20 CC using HCO_2H–MeOH–H_2O (1:12:24). The orange–red frs were further purified with PC (*n*-BuOH–HOAc–H_2O, 4:1:2 and 15% HOAc) and HPLC. Prep. HPLC was performed on a Hitachi 6200 system, using an Intertsil ODS-2 (20 × 250 mm) column and HCO_2H solvent system. Pigment **1** (98 mg), **2** (17 mg), **3** (10 mg) and **4** (15 mg) were obtained as organge–red powder.

Analysis. Characterization of **4** was carried out with UV-VIS, FAB-MS and ^{1}H NMR, TLC and HPLC

Table 1. Chromatographic and spectral data for *Verbena* anthocyanins

Anthocyanins*	BAW	R_f values (×100)† BuH	1% HCl	HAc–HCl	R_t (min)	In 0.1% HCl–MeOH λ_{max}(nm)	E_{440}/E_{VIS}	FAB-MS $[M]^+$
Verbena pigments								
Pg3aG5aG (**1**)	61	46	37	69	30.9	269,508	18	679
Pg3aG5G (**2**)	50	29	35	64	21.0	269,508	18	637
Pg3G5G (**3**)	39	12	23	45	11.8	269,508	19	595
Pg3G5aG (**4**)	51	27	27	54	20.2	269,508	18	637

*Pg3aG5aG, pelargonidin 3,5-diacetylglucoside; Pg3aG5G, pelargonidin 3-acetylglucoside-5-glucoside; Pg3G5G, pelargonidin 3,5-diglucoside; Pg3G5aG, pelargonidin 3-glucoside-5-acetylglucoside.

†For key to abbreviations, see Experimental.

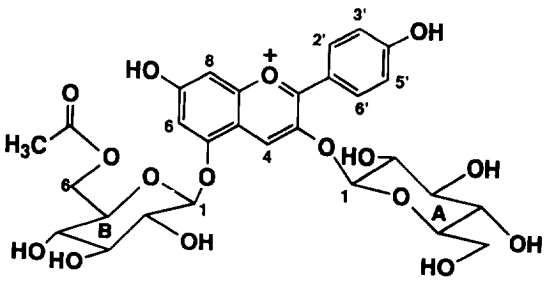

Fig. 1. *Verbena* anthocyanin **4**.

[2–4]. This compound was further analysed by TLC after alkaline deacylation, acid hydrolysis and H_2O_2 degradation [1–5]. TLC was carried out on microcrystalline cellulose (Avicel SF, Funakoshi) using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5), BuOH (*n*-BuOH–2 M HCl, 1:1), 1% HCl and HOAc–HCl (HOAc–HCl–H_2O, 15:3:82) for anthocyanins, BAW, IPB (*iso*-PrOH–*n*-BuOH–H_2O, 7:1:2), PhOH (PhOH–H_2O, 4:1) and IPW (*iso*-PrOH–H_2O, 4:1) for sugars. HPLC was run on a Inertsil ODS-2 column (4.6 × 250 nm) at 35° with a flow rate of 0.8 ml min^{-1}, monitoring at 520 nm. Solvent systems used were as follows: linear gradient elution for 40 min from 25 to 85% solvent B (1.5% H_3PO_4, 20% HOAc, 25% MeCN) in solvent A (1.5% H_3PO_4). ^{1}H NMR spectrum of anthocyanin **4** was obtained with a JEOL JNM-GX 400 spectrometer and a sample was measured in 10% TFA-*d*–90% DMSO-d_6.

Three other anthocyanins **1**–**3** were identified by direct comparison with authentic specimens [5]. FAB-mass spectra of the four anthocyanins were recorded on JEOL JMS SX-102A (positive mode in Magic Bullet, negative mode in glycerol).

Pelargonidin 3-glucoside-5-acetylglucoside. ^{1}H NMR (400 MHz; TFA-*d*-DMSO-d_6, 1:9 at 25°, standard TMS); δ pelargonidin, 8.99 (*s*, H-4), 6.95 (*br s*, H-6), 7.16 (*br s*, H-8), 8.64 (*d*, J = 9 Hz, H-2′, 6′), 7.07 (*d*, J = 9 Hz, H-3′, 5′); 3-glucose (A), 5.33 (*d*, J = 8 Hz, H-1), 3.52 (*t*, J = 8 Hz, H-2), 3.44 (*t*, J = 8 Hz, H-3), 3.22 (*t*, J = 8 Hz, H-4), 3.49 (*m*, H-5), 3.74 (*m*, H-6a), 4.09 (*m*, H-6b); 5-glucose (B), 5.17 (*d*, J = 8 Hz, H-1), 3.52 (*t*, J = 8 Hz, H-2), 3.38 (*t*, J = 8 Hz, H-3), 3.25 (*t*, J = 9 Hz, H-4), 3.74 (*m*, H-5), 4.13 (*dd*, J = 8, 11 Hz, H-6b), 4.34 (*br d*, J = 12 Hz, H-6a); HOAc 2.00 (*s*, Me).

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