Table 2. ^{13}C NMR data homisoflavanones **1**, **2**, **4** and **5** in CD_3OD*

C	1	5	2	4	C	1	5	2	4
2	70.5	70.6	69.0	70.5	1′	130.7	130.0	127.8	131.0
3	†	48.5	128.6	†	2′	117.2	131.1	118.6	117.2
4	201.4	200.9	187.3	200.1	3′	146.4	116.4	148.5[d]	146.4
4a	104.9	104.8	107.7	103.2	4′	145.1	157.0	146.7[d]	145.1
5	148.6[a]	149.2	158.9	158.2	5′	116.5	116.4	116.8	116.5
6	134.4[b]	135.1	93.9	93.5	6′	121.5	131.1	124.9	121.5
7	151.3	151.2	158.3	158.0	6-OMe	—	61.4	—	—
8	132.7[b]	131.4	127.7	127.6	7-OMe	61.6[c]	61.6	56.6	56.7
8a	147.6[a]	146.4	149.1	149.3	8-OMe	61.9[c]	—	—	—
9	33.2	32.6	139.0	33.0					

*Chemical shifts are given in δ (ppm) relative to TMS.
†Buried in solvent signals.
[a–d]Interchangeable values.

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CYANIDIN 3-MALONYLGLUCOSIDE IN TWO *ECHINACEA* SPECIES

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Key Word Index—*Echinacea*; Compositae; malonated anthocyanins.

Abstract—The major anthocyanins of two *Echinacea* species, *E. purpurea* and *E. pallida* have been identified as cyanidin 3-*O*-(β-D-glucopyranoside) and cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside) by NMR.

INTRODUCTION

Recently, the occurrence of several malonylated anthocyanins has been reported in numerous plants, especially in Compositae [1]. Our interest in *Echinacea* species [2] was an opportunity to isolate and identify the major anthocyanins from two of them, *E. pallida* Nutt and *E. purpurea* (L.) Moensch, 3-*O*-(β-D-glucopyranosyl) and 3-*O*-(6-*O*-malonyl-β-D-glucopyranosyl) cyanidin were detected in these two plants.

RESULTS AND DISCUSSION

Anthocyanins were extracted from dry *Echinacea* flowers by mild extraction with acetic acid–methanol–water

and further purified by column chromatography over Sephadex LH 20, by ion exchange chromatography (DEAE Cellulose) and HPLC. Two major pigments were isolated from *E. purpurea* and identified as 3-*O*-(β-D-glucopyranosyl) cyanidin (**1**) and 3-*O*-(6-*O*-malonyl-β-D-glucopyranosyl) cyanidin (**2**) respectively. Identification of **1** was confirmed by its ^{1}H NMR spectrum and TLC by comparison with an authentic sample. Compound **2** was identified by ^{1}H NMR spectrometry. The ^{1}H NMR spectrum was measured in 90% DMSO-d_6–10% TFA following Bridle *et al.* [3]. Under these conditions **2** showed two peaks at δ3.35 and 3.36 which can be assigned to the AB system of the two malonyl–CH_2–. It should be noticed that these signals slowly decrease and finally disappeared in agreement with the ability of these protons to be exchanged in acidic conditions (TFA-*d*). The two signals at δ4.44 (*d*, $J = 11.2$ Hz) and 4.11 (*dd*, $J = 7.4$ Hz; 11.2 Hz) were assigned to the C-6 methylene group of the sugar, showing that the malonyl moiety acylates the hydroxyl group at C-6. The anomeric proton observed at δ5.37 (*d*, $J = 8$ Hz) and the other signals of the sugar assigned upon a 2D homonuclear shift correlated (COSY) spectrum indicated a β-D-glucopyranosyl moiety. All these results are in good agreement with previously reported data [1, 3].

Using TLC R_f values and HPLC retention times, we compared the anthocyanins from *E. pallida* with those identified from *E. purpurea* and showed that **1** and **2** are also present in *E. pallida*. Callus cultures and suspension cultures which produce anthocyanins were derived from the stem of *E. purpurea*. From the suspension cultures, three anthocyanins were extracted, **1** and two other acylated cyanidin glycosides, the structure of which are not yet clearly elucidated. Further investigations are in progress to identify them and to optimize their production.

EXPERIMENTAL

^{1}H NMR spectra were measured at 400 MHz in 90% DMSO-d_6–10% TFA-*d* solutions. The protonated part of the solvent was used as int. standard (DMSO: δ2.50 relatively to TMS). HPLC analysis were performed using Spectra Physics SP 8800 ternary HPLC pump, a Rheodyne injector and a SP 8450 UV/Vis. detector.

Plant material. The extraction of the pigments was carried out on *Echinacea purpurea* (L.) Moensch and *E. pallida* Nutt, cultivated and collected in Germany in 1983 (Bayerische Landesanstalt für Bodenkultur and Pflanzenbau, Sachgebiet PZ 3.3 Vöttingerstrasse 38, D-8050 Freising) and dried at 40°.

Extraction and isolation. Dried and finely powdered flowers (200 g) were extracted with MeOH–H_2O–HOAc (10:9:1) (2 × 700 ml) at 4° for 24 hr. The extracts were combined and MeOH evapd (30°, under red. pres.). The aq. soln was successively extracted with petrol bp 40–60° (3 × 200 ml), $CHCl_3$ (4 × 200 ml) and EtOAc (4 × 200 ml). The anthocyanins were isolated from aqueous solution.

After concn, the extract was fractionated over Sephadex LH 20 (column: 5.2 × 35 cm) using MeOH–H_2O–AcOH (10:9:1). The fractions containing anthocyanins were combined and further purified over DEAE Cellulose DE 52 (Whatmann) (column: 5.2 × 20 cm) using H_2O as solvent (500–700 ml) then MeOH–AcOH–H_2O (4:3:13) to recover the anthocyanins. The different products were finally separated and purified with prep. HPLC (Lichrospher RP 18, 7 μm, 250 × 10 mm; H_2O–MeOH–AcOH gradient).

Compound **1**: ^{1}H NMR (10% v/v TFA–90% DMSO-d_6) δ: 8.88 (1H, *s*, H-4), 8.23 (1H, *dd*, $J = 2.2$, 8.6 Hz, H-6'), 8.00 (1H, *d*, $J = 2.2$ Hz, H-2'), 7.02 (1H, *d*, $J = 8$ Hz, H-5'), 6.90 (1H, *br s*, H-8) 6.69 (1H, *d*, $J = 2$ Hz, H-6). Glucose moiety: 5.34 (1H, *d*, $J = 7.8$ Hz, H-1), 3.71 (1H, *d*, $J = 10.3$ Hz, H-6_b), 3.49 (3H, *m*, H-2, H-5, H-6_a), 3.37 (1H, *dd*, $J = 9.2$, 9.2 Hz, H-3), 3.22 (1H, *dd*, $J =$ 9.2, 9.2 Hz, H-4).

Compound **2**. ^{1}H NMR (10% v/v TFA–90%DMSO-d_6) δ: 8.80 (1H, *s*, H-4), 8.21 (1H, *dd*, $J = 2$, 8.7 Hz, H-6'), 7.99 (1H, *d*, $J = 2$ Hz, H-2'), 7.02 (1H, *d*, $J = 8.7$ Hz, H-5'), 6.89 (1H, *d*, $J = 2$ Hz, H-8), 6.72 (1H, *d*, $J = 2$ Hz, H-6). Glucose moiety: 5.37 (1H, *d*, $J = 8$ Hz, H-1), 4.44 (1H, *d*, $J = 11.2$ Hz, H-6_b), 4.11 (1H, *dd*, $J = 7.4$, 11.2 Hz, H-6_a), 3.81 (1H, *dd*, $J = 7.4$, 9.2 Hz, H-5), 3.52 (1H, *dd*, $J = 8$, 8.8 Hz, H-2), 3.40 (1H, *dd*, $J = 8.8$, 9.2 Hz, H-3), 3.23 (1H, *dd*, $J = 9.2$, 9.2 Hz, H-4), 3.35, 3.36 (2H, malonyl–CH_2–).

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