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CYANIDIN 3-(6-SUCCINYL GLUCOSIDE)-5-GLUCOSIDE FROM FLOWERS OF SEVEN *CENTAUREA* SPECIES

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Key Word Index—*Centaurea*, Compositae, anthocyanin, cyanidin 3-*O*-(6-*O*-succinyl-β-D-glucoside)-5-*O*-β-D-glucoside

Abstract—Extraction, purification and analysis of the anthocyanin pigments of ten taxa of the genus *Centaurea* yielded cyanidin 3-*O*-(6-*O*-succinyl-β-D-glucoside)-5-*O*-β-D-glucoside

INTRODUCTION

The classical identification of the floral pigment of blue cornflower, *Centaurea cyanus* L, was determined as cyanidin 3,5-*O*-β-D-diglucoside [3] This work was repeated later by two teams in Japan with more up-to-date methods [1, 2] and the structure was revised as cyanidin 3-*O*-(6-*O*-succinyl-β-D-glucoside)-5-β-D-glucoside We have now found that a range of other *Centaurea* species also contain this succinate ester of cyanin

RESULTS

The pigments were isolated from the flowers of the various *Centaurea* species (see Experimental) using very mild acid conditions to avoid hydrolysis of the labile ester linkage They were then compared directly with cyanidin 3-(6-succinylglucoside)-5-glucoside isolated from *Centaurea cyanus* Identity was established by (1) cochromatography in seven solvents, (2) detection of glucose, cyanidin and succinic acid on hydrolysis, and (3) detection of all expected intermediates on controlled acid hydrolysis All seven species (and subspecies) contained one and the same pigment Thus, the succinate ester of cyanin seems to be characteristic in the genus

EXPERIMENTAL

Plant material Dried inflorescences of plants were used from the following taxa *Centaurea cyanus* L, *C cyanus* f *plena* Latour, *C grinensis* Reuter subsp *fritschii* Soó, *C jacea* L, *C macroptilon* Borb subsp *oxylepis* Soó, *C micranthos* Gmel, *C pannonica* (Heuff) Simk, *C pannonica* var *semifimbriata* Soó et Jáv, *C sadlerana* Janka, *C spinulosa* Rochel, *C spinulosa* var *hajdonicalis* Wagn The plants were identified taxonomically by the authors

Extraction and purification of the anthocyanin The inflorescences of plants were extracted in two steps at first with MeOH 1% HOAc and 0 033% HCl (at 22°, for 20 min) and then residue was re-extracted with 1% HOAc in MeOH–Me_2CO (1 1) The resulted extracts were concentrated (at 40°, *in vacuo*) and were purified by column chromatography (Sephadex LH-20 in 2 5 × 70 cm column) The adsorbed materials were eluted with MeOH–Me_2CO (1 1) containing 0 1% HOAc The eluates were collected and subjected to prep TLC on silica gel plates (Merck 5745) and developed with EtOAc–HCO_2H–H_2O (14 3 3) The bands were resolved with MeOH and concentrated to small volume before further procedures

Analysis of anthocyanins by TLC The concentrated solutions of anthocyanins were surveyed on cellulose plates (Merck 5716) in HOAc–HCl–H_2O (3 1 8), BuOH–HOAc–H_2O (4 1 2), BuOH–HCl–H_2O (7 2 5), H_2O–HCl–HCO_2H (8 4 1) and BuOH–HOAc–H_2O (6 1 2) and on silica gel plates (Merck 5721) in EtOAc–HCO_2H–H_2O (14 3 3) and BuOH–HCO_2H–H_2O (17 1 2) The anthocyanin solutions from several plants were applied side by side on the one plate with different solvents alternately

Determination of organic acid The acyl group of the anthocyanins was determined using comparison with authentic markers, succinic acid and malonic acid on cellulose plates (Merck 5716) with EtOAc–HOAc–H_2O (3 1 1) hydrolysis the purified anthocyanins were dissolved in 1 ml MeOH containing 1% HCl and reaction mixture was kept at 25° for 24 hr For the detection of spots the bromocresol green reagent was used [1]

Controlled acid hydrolysis The samples of anthocyanins were dissolved in MeOH and acidified with 2 N HCl The reaction mixture was heated in a boiling water bath for 40 min and sampled at 5 min intervals Each sample was spotted on cellulose thin layer plates (Merck 5716) with *n*-BuOH–H_2O–HOAc (6 1 2)

Chromatographic analysis of anthocyanidin The samples of pure anthocyanins obtained after PTLC were hydrolysed in MeOH–2 N HCl (at 100° for 1 hr) The resulted aglycone was extracted with *n*-amyl alcohol and identified on cellulose thin layer plates (Polygram Cell 300, Macherey-Nagel) against authentic cyanidin in HCO_2H–HCl–H_2O (10 1 3) and on silica gel plates (Merck 5721) in EtOAc–HCO_2H–H_2O (85 6 9)

The identification of sugar After hydrolysis the aqueous lower phasis of the reaction mixture was concentrated (at 60°, *in vacuo*) and sugars were identified on silica gel thin layer plates (Merck 5721) in EtOAc–HCO_2H–H_2O (8 1 1) Authentic samples of arabinose, galactose and glucose were used as standards

The chromatograms after drying were sprayed with the Lisboa reagent (0 5 ml anisaldehyde–50 ml HOAc–1 ml H_2SO_4) and visualized at 100° for 10 min

Spectral analysis After purification, hydrolysis and chromatographic treatments of pigments the present purified anthocyanins

and anthocyanidins were extracted respectively in MeOH containing 0 01% cc HCl and centrifuged and their absorbance measured The spectral values were compared with lit data [1, 2]

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REFERENCES

1 Takeda, K and Tominaga, S (1983) *Bot Mag Tokyo* **96**, 359
2 Tamura, H, Kondo, T, Kato, Y and Goto, T (1983) *Tetrahedron Letters* **24**, 5749
3 Willstätter, R and Everest, A E (1913) *Justus Liebigs Ann Chem* **401**, 189

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FLAVONOID AGLYCONES IN THE RESIN OF *HAZARDIA SQUARROSA* VAR. *GRINDELIOIDES**

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Key Word Index—*Hazardia squarrosa*, Asteraceae, leaf resin, 34 flavonoid aglycones

Abstract—Thirty three flavonoid aglycones have been identified from the external stem and leaf resin of *Hazardia squarrosa* var *grindelioides* by TLC co-chromatography with authentic samples The occurrence of compounds with oxygenation at C-6 and C-5′, which are also found in the genus *Haplopappus*, supports the previously believed close relationship between these two genera

INTRODUCTION

Hazardia (Compositae) is a genus of 13 species found in the western United States and adjacent Mexico [1] As part of ongoing work on the chemistry and systematics of *Haplopappus* and its segregate genera [1–9], this study was undertaken to initiate the elucidation of aglycones of the genus, beginning with the most widespread taxon, *H squarrosa* (Hooker & Arnott) Greene var *grindelioides* (DC) Clark The flavonoid glycoside profiles of the entire genus have been published [2], but the nature of the aglycones that are usually found in the leaf and stem exudates of these mostly resinous plants have remained undetermined on this point

RESULTS AND DISCUSSION

The following 33 known compounds were identified from the leaf and stem washings of the sample plant material the flavones apigenin, luteolin, luteolin 7- and 3′-monomethyl ethers, 6-methoxyluteolin, scutellarein 6-methyl and 6,4′-dimethyl ethers, the flavonols kaempferol, kaempferol 7- and 4′-monomethyl ethers, kaempferol 3,4′- and 7,4′-dimethyl ethers, kaempferol 3,7,4′-trimethyl ether, 6-hydroxykaempferol 3,6,4′-trimethyl ether, quercetin, quercetin 3-, 3′- and 7-monomethyl ethers, quercetin 3,7-, 3,3′- and 7,3′-dimethyl ethers, quercetin 3,7,3′-, 3,3′,4′- and 7,3′,4′-trimethyl ethers, quercetin 3,7,3′,4′-tetramethyl ether, quercetagetin 6-methyl ether, quercetagetin 3,6- and 6,3′-dimethyl ethers, quercetagetin 3,6,4′-trimethyl ether, the flavanone eriodictyol and its 7-monomethyl and 7,3′-dimethyl ethers, and dihydrotricetin 7,3′-dimethyl ether (5,4′,5′-trihydroxy, 7,3′-di-*O*-methylflavanone) It should also be noted that 13 additional compounds were observed which behaved chromatographically as flavonoids However, these compounds could not be isolated for further identification due to their low quantities, and they could not be co-chromatographed with known standards with any degree of confidence

In summary, the identifiable aglycones of *H squarrosa* var *grindelioides* are all oxygenated at positions 5,7 and 4′, with additional oxygenation occurring variably at C-3 (22 compounds), C-6 (8 compounds), C-3′ (23 compounds), and C-5′ (1 compound) A preponderance of structures (28 compounds) are methylated, varying from one to four methyl groups, thereby accounting for their distribution in the lipophilic external resin

The number of different structures is relatively high for flavonoid aglycones from a single taxon Although none of the other 12 species of *Hazardia* has been examined thoroughly for their aglycone profiles, species of related genera have been studied None of these, including species

*Part 2 in the series "Flavonoids of *Hazardia*" For Part 1 see ref [2]