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SHORT COMMUNICATION

4,6,4′-TRIHYDROXYAURONE AND OTHER FLAVONOIDS FROM *LIMONIUM*

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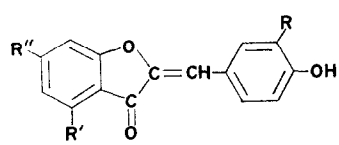
Key Word Index—*Limonium*; Plumbaginaceae; flavonoids; 4,6,4′-trihydroxyaurone; auresidin; glucosides.

Abstract—A new aurone isolated from flowers of *Limonium* cv. 'Gold Coast' was characterized by co-chromatography, absorption spectra, and the mass spectral fragmentation pattern as 4,6,4′-trihydroxyaurone (I). Other yellow pigments present were 4,6,3′,4′-tetrahydroxyaurone (II) (aureusidin), and its 4-glucoside (III) (cernuoside) and 6-glucoside (IV) (aureusin) as well as the 3,4,2′,4′,6′-pentahydroxychalcone (V). Other flavonoids isolated were apigenin, luteolin, isoorientin, quercetin, quercitrin and myricetin 3′-rhamnoside.

INTRODUCTION

MICROSCOPIC examination of the flowers of *Limonium* cv. 'Gold Coast' (Plumbaginaceae) revealed that the yellow pigments were present in the vacuoles of the epidermal cells and that there was no evidence of chromoplasts in the cytoplasm. Therefore, the yellow pigments were presumably flavonoids and not carotenoids.

Aurones with the hydroxylation patterns of luteolin and tricetin are known but the apigenin analogue has yet to be discovered.[1] We now report the isolation of the apigenin analogue 4,6,4′-trihydroxyaurone (I) and other flavonoids from flowers of *Limonium* cv. 'Gold Coast'.



(I) R = H, R′ = R″ = OH
(II) R = R′ = R″ = OH
(III) R = R″ = OH, R′ = *O*-glucose
(IV) R = R′ = OH, R″ = *O*-glucose

RESULTS AND DISCUSSION

Compounds eluted from polyvinylpyrrolidone were separated into 4 fractions.

Fraction 1. Three flavones and a flavone glycoside were isolated. The flavones were chromatographically and spectrophotometrically the same as authentic samples of apigenin, luteolin, and isoorientin.[2] A luteolin glycoside also was present but the glycosidic pattern was not established.

[1] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
[2] T. A. GEISSMAN and J. B. HARBORNE, *J. Am. Chem. Soc.* **78**, 832 (1956).

Fraction 2. A flavonol glycoside was isolated which on acid hydrolysis yielded quercetin and rhamnose and was chromatographically and spectrophotometrically the same as an authentic sample of quercitrin. Two aurones also were isolated. They remained as two distinct bands on cellulose plates when developed with PrOH, BAW and H_2O.* Absorption maxima for each yellow band were identical and they were λ_{max}^{EtOH} 398 nm, λ_{max}^{NaOEt} 455 nm, $\lambda_{max}^{EtOH/AlCl_3}$ 395 nm, and $\lambda_{max}^{EtOH/H_3BO_3}$ 402 nm. Since the λ_{max}^{EtOH} showed little or no change with the addition of $AlCl_3$ or H_3BO_3 these compounds are presumably 4-glycosides[1] without an *o*-dihydroxyl group. One yellow band reverted to the other during the final purification in PFW. This suggests the possibility of geometrical isomers with the conversion of the less stable form in an acid medium. The single yellow compound was acid hydrolyzed and yielded glucose and a second spot thought to be galactose. No further attempt was made to determine the glycosidic pattern because of the lack of material. The aglycone was chromatographically and spectrophotometrically[2] the same as a synthetic sample of 4,6,4′-trihydroxyaurone (I). R_f values were 0·75 in BAW, 0·10 in 30% HOAc, 0·62 in PhOH, and 0·51 in Forestal. Absorption maxima were λ_{max}^{EtOH} 225, 245†, 345†, 392 nm, λ_{max}^{NaOEt} 360, 444 nm, $\lambda_{max}^{EtOH/AlCl_3}$ 260†, 350, 390, 451 nm, and $\lambda_{max}^{EtOH/H_3BO_3}$ 340, 395 nm. Examination of the mass spectral fragmentation pattern showed that (above 60 mass units) the base peak corresponded to the molecular ion at *m/e* 270. Principal fragments were observed at *m/e* 269, 253, 242, 153, and 152. The spectra of authentic 4,6,4′-trihydroxyaurone (I), and the compound isolated from *Limonium* cv. 'Gold Coast' were identical.

Fraction 3. The major yellow pigment was isolated from this fraction. The pigment was aureusidin 4-glucoside (III) (cernuoside) which previously was isolated from *Limonium bonduelli*.[3] R_f values were 0·36 in BAW, 0·19 in 30% HOAc, 0·48 in PhOH, and 0·54 in Forestal. Absorption maxima were λ_{max}^{EtOH} 406 nm, λ_{max}^{NaOEt} 458 nm, $\lambda_{max}^{EtOH/AlCl_3}$ 404 nm, and $\lambda_{max}^{EtOH/H_3BO_3}$ 430 nm. Little or no change in the λ_{max}^{EtOH} with the addition of $AlCl_3$ confirmed that this compound was a 4-glucoside. Acid hydrolysis yielded glucose and an aglycone chromatographically and spectrophotometrically the same as an authentic sample of aureusidin (II). Also isolated were two minor yellow pigments which were chromatographically and spectrophotometrically[1] the same as authentic samples of aureusidin (II) and aureusidin 6-glucoside (IV) (aureusin). Confirmation of the 6-glucoside was obtained by measurement of the large bathochromic shift of the λ_{max}^{EtOH} (*ca.* 70 nm) with the addition of $AlCl_3$[2] and the identification of glucose and aureusidin (II) after acid hydrolysis. A flavonol and a flavonol glycoside also were isolated. The flavonol was chromatographically and spectrophotometrically the same as an authentic sample of quercetin.[1] The flavonol glycoside was identified as myricetin 3′-rhamnoside. R_fs were 0·54 in BAW, 0·45 in PhOH, 0·16 in H_2O, and 0·43 in 15% HOAc. The absorption maxima were λ_{max}^{EtOH} 252, 353 nm, λ_{max}^{NaOEt} unstable, $\lambda_{max}^{EtOH/NaOAc}$ 266, 378 nm, $\lambda_{max}^{EtOH/AlCl_3}$ 271, 310†, 360†, 424 nm, and $\lambda_{max}^{EtOH/H_3BO_3}$ 256, 377 nm. Acid hydrolysis yielded rhamnose and an aglycone chromatographically and spectrophotometrically the same as an authentic sample of myricetin.[1]

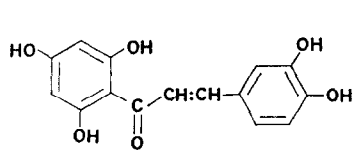
Fraction 4. This fraction contained only one yellow pigment which was identified as 3,4,2′,4′,6′-pentahydroxychalcone (V). R_fs were: 0·75 in BAW, 0·12 in PhOH, and 0·27 in Forestal. Absorption maxima were: λ_{max}^{EtOH} 378 nm, λ_{max}^{NaOEt} 325, 441 nm, $\lambda_{max}^{EtOH/AlCl_3}$ 311, 415 nm, and $\lambda_{max}^{EtOH/H_3BO_3}$ 283, 330, 430 nm. Acid hydrolysis yielded no sugar and the chalcone isomerised to a flavanone chromatographically and spectrophotometrically the

* See Experimental for key to solvent abbreviations.

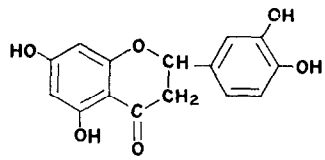
† Inflection.

[3] J. B. HARBORNE, *Phytochem.* **5**, 111 (1966).

same as an authentic sample of 5,7,3′,4′-tetrahydroxyflavanone (VI) (eriodictyol). R_fs were: 0·96 in BAW, 0·12 in H_2O, 0·62 in 30% HOAc, 0·27 in PrOH, and 0·80 in PFW. Absorption maxima were: λ_{max}^{EtOH} 289, 325† nm, λ_{max}^{NaOEt} 245, 326 nm, $\lambda_{max}^{EtOH/NaOAc}$ 290, 326 nm, $\lambda_{max}^{EtOH/AlCl_3}$ 310, 378 nm, and $\lambda_{max}^{EtOH/H_3BO_3}$ 290, 325 nm.



(V)



(VI)

EXPERIMENTAL

Authentic pigments. Eriodictyol (VI) and 4,6,4′-trihydroxyaurone (I) were kindly supplied by Geissman and Harborne. Aureusin (IV) was isolated from *Antirrhinum majus* cv. 'Yellow Rocket'.[4]

Plant material. Plants of *Limonium* cv. 'Gold Coast' were grown from seed obtained from G. J. Ball, Inc., West Chicago, Ill., 60185, U.S.A. Plants were air-dried, the petals of the small flowers harvested, and then ground to pass a 40-mesh screen.

Isolation of flavonoids. Pigments were extracted from the dried tissue with boiling MeOH. The volume of the extract was reduced. The concentrated extract was then absorbed on purified polyvinylpyrrolidone,[5] and eluted with MeOH. Four fractions were obtained and the compounds present in each fraction were resolved and purified by preparative TLC on 2 mm-layers of microcrystalline cellulose. The solvents were: H_2O; 15% aq. HOAc (15% HOAc); *n*-BuOH–HOAc–H_2O (6:1:2) (BAW); *iso*PrOH–HCO_2H–H_2O (2:5:5) (PFW); *iso*PrOH–H_2O (11:39) (PrOH); Me_2CO–HOAc–H_2O (4:1:5); and Et_2O–HOAc–H_2O (10:4:2). Additional solvents used for R_fs were phenol: H_2O (73:27, w/v) (PhOH); HOAc–HCl–H_2O (30:3:10) (Forestal); and 30% aq. HOAc.

Hydrolysis. Purified compounds were hydrolyzed in boiling 2 N HCl–EtOH (1:1) for 1 hr under reflux. Aglycones were either extracted with EtOAc or precipitated in the cold and the sugar in the aqueous phase was determined by methods previously described.[6]

Identification of aurones. Aurone pigments were identified by co-chromatography on microcrystalline cellulose plates 250 μ thick and spectral properties in direct comparison with authentic pigments. The mass spectral fragmentation pattern of 4,6,4′-trihydroxyaurone was determined with a Perkin–Elmer GC 270 mass spectrometer (solids probe at 140° and 80 eV).

Identification of chalcone. Acid hydrolysis yielded a flavanone which was identified by co-chromatography on microcrystalline cellulose plates 250 μ thick, reaction with $FeCl_3$–$K_3Fe(CN)_6$ and comparison of its spectral properties with those of eriodictyol (VI).

Identification of other flavonoids. The aglycones obtained from acid hydrolysis were identified by co-chromatography on microcrystalline cellulose plates 250 μ thick and spectral properties in direct comparison with authentic compounds.

Acknowledgements—We express appreciation to Drs. T. A. Geissman and J. B. Harborne for samples of 4,6,4′-trihydroxyaurone (I) and eriodictyol (VI) and to Miss P. S. Budin for her competent technical assistance.

[4] E. C. Jorgensen and T. A. Geissman, *Arch. Biochem. Biophys.* **54,** 72 (1955).
[5] H. A. Anderson and J. A. Sowers, *Phytochem.* **7,** 293 (1968).
[6] S. Asen and P. S. Budin, *Phytochem.* **5,** 1257 (1966).