5,7,3′,4′,5′-Pentahydroxyflavanone in the Bracts of *Helichrysum bracteatum*

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Z. Naturforsch. **38 c**, 891 – 893 (1983); received July 15, 1983

Helichrysum, Compositae, Naringenin, Eriodictyol, 5,7,3′,4′,5′-Pentahydroxyflavanone

Chromatographic investigations on *Helichrysum bracteatum* revealed the presence of three flavanones in the bracts. Two of them were the well known flavanones naringenin and eriodictyol. The third flavanone was identified as the hitherto in nature unknown 5,7,3′,4′,5′-pentahydroxyflavanone. This was accomplished by chromatographic and spectrophotometric comparison with an authentic sample prepared from 3,4,5,2′,4′,6′-hexahydroxychalcone-2′-glucoside. Moreover, precursor experiments with suitable white flowering mutants of *Matthiola incana* and *Antirrhinum majus* also confirmed that the third compound was identical with 5,7,3′,4′,5′-pentahydroxyflavanone.

Introduction

In 1965, it has been shown that the intensive yellow colour of the bracts of *Helichrysum bracteatum* is caused by the presence of 3,4,2′,4′,6′-pentahydroxychalcone-2′-glucoside, 3,4,5,2′,4′,6′-hexahydroxychalcone-2′-glucoside and its related auron bractein [1, 2]. These compounds were isolated from a yellow flowering strain of *Helichrysum*. During the isolation of these compounds we found out that besides the already well known flavanones naringenin and eriodictyol a further flavanone occurs in the bracts. The latter flavanone was identified as 5,7,3′,4′,5′-pentahydroxyflavanone.

Materials and Methods

The investigation included the commercial strains "Gelbe Kugel", "Bronzekugel" and "Rote Kugel" (Weigelt and Co., Walluf, Germany) of *Helichrysum bracteatum*.

Fresh bracts were ground in a mortar and pestle in ethyl acetate or ether. After filtration and concentration under reduced pressure the extracts were separated on Whatman 3 MM with CAW (Chloroform/acetic acid/water, 10:9:1). Flavanones were detected by reduction with borohydride and subsequent exposure to HCl fumes [3] on small strips of the chromatogram. The parts containing flavanones were eluted with methanol. Further purification was achieved by successive chromatography on paper 2043b (Schleicher & Schüll, Dassel, Germany) using BAW (*n*-Butanol/acetic acid/water, 4:1:5) and 15% acetic acid.

R_f-values were determined on 0.1 mm cellulose TLC plates (Schleicher & Schüll) in Forestal (acetic acid/HCl/water, 30:3:10), TBA (*tert*-butanol/acetic acid/water, 3:1:1) and in the solvent systems mentioned above. Spectral analysis was performed according to ref. [4]. Authentic samples of naringenin, eriodictyol, 3,4,5,2′,4′,6′-hexahydroxychalcone-2′-glucoside, dihydrokaempferol, dihydroquercetin and dihydromyricetin were available from our laboratory collection. 5,7,3′,4′,5′-pentahydroxyflavanone was prepared by hydrolysis of 3,4,5,2′,4′,6′-hexahydroxychalcone-2′-glucoside with β-glucosidase (Serva). The flavanone formed by spontaneous cyclisation of the deglucosylated chalcone was extracted with ether and purified chromatographically. The precursor experiments were performed according to ref. [5] using method 2 for the administration of the precursors to the flowers.

Results and Discussion

After separation of an ethyl acetate extract of bracts from the strain "Gelbe Kugel" with CAW, the borohydride-HCl test [3] revealed the presence of at least three compounds with colour reactions typical for flavanones. Compound **I** (R_f 0.89, red), compound **II** (R_f 0.63, lilac) and compound **III** (R_f 0.32, blue) were further purified chromatographically using BAW and 15% acetic acid as solvents. Compound **I** and **II** proved to be the well

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0341-0382/84/1100-0891 $ 01.30/0

known flavanones naringenin and eriodictyol, respectively (Table I). Compound **III** showed spectral data similar to naringenin and eriodictyol. The peak of **III** (λ_{max} 288 nm, methanol) exhibited bathochromatic shifts both in the presence of sodium acetate and aluminium chloride, indicating free hydroxyl groups at position 5 and 7. Moreover, in the presence of sodium hydroxide a bathochromatic shift of 33 nm was observed, which is typical for 5,7-dihydroxyflavanones [4]. The R_f-values in different solvents also suggest that compound **III** is an aglycone (Table I). Furthermore, treatment with 2 N HCl at 100 °C for 30 min or with β-glucosidase did not influence the R_f-values and spectral data of compound **III**.

Comparing compound **III** with naringenin and eriodictyol (Table I) in respect to R_f-values in five solvent systems, to the spectral data and to the colour reaction after treatment with borohydride and subsequent exposure to HCl fumes, this comparison revealed that compound **III** is most likely 5,7,3′,4′,5′-pentahydroxyflavanone. To confirm this assumption, 3,4,5,2′,4′,6′-hexahydroxychalcone-2′-glucoside isolated from the yellow flowering strain of *Helichrysum* (Gelbe Kugel) [1] was hydrolysed with β-glucosidase. This treatment yielded authentic 5,7,3′,4′,5′-pentahydroxyflavanone by spontaneous cyclisation of the chalcone aglycone, formed by action of β-glucosidase. Both 5,7,3′,4′,5′-pentahydroxyflavanone prepared from the chalcone and compound **III** were found to be identical with respect to R_f-values, spectral data and colour reaction (Table I).

Precursor experiments on suitable acyanic mutants of *Matthiola incana* and *Antirrhinum majus* also confirmed the identity of compound **III** with 5,7,3′,4′,5′-pentahydroxyflavanone (Table II). In flowers of the *Matthiola* mutant, anthocyanin synthesis can be initiated by the administration of flavanones and dihydroflavonols, whereas in flowers of the *Antirrhinum* mutant only the administration of dihydroflavonols is effective [6, 7]. In both cases, the anthocyanins formed reveal the B-ring substitution pattern of the precursor administered (Table II). Both 5,7,3′,4′,5′-pentahydroxyflavanone and compound **III** initiated the formation of delphinidin derivatives in acyanic flowers of *Matthiola*, thus confirming the 3′,4′,5′-hydroxylation pattern of compound **III**. Furthermore, neither the authentic flavanones nor compound **III** were found to be con-

Table I. R_f-values, spectral data and colour after borohydride reduction of compound III in comparison with authentic flavanones.

Compound	R_f (×100) in					Spectral maxima [nm] in				Colour after reduction
	30% HOAc[a]	Forestal	CAW	BAW	TBA	MeOH[a]	+NaOH	+NaOAc[a]	+$AlCl_3$	
I (Naringenin)	65	91	85	95	92	288	323	323	312	red
II (Eriodictyol)	59	83	61	90	87	289	324	324	311	lilac
III 5,7,3′,4′,5′-pentahydroxyflavanone	51	74	28	84	76	288	321	323	307	blue
	52	74	29	84	75	288	321	322	307	blue

[a] HOAc = acetic acid; MeOH = methanol; NaOAc = sodium acetate.

Table II. Anthocyanidin types formed in flowers of acyanic mutants of *Matthiola incana* and *Antirrhinum majus* after administration of authentic precursors and compound III.

Precursor	B-ring hydroxylation pattern	*M. incana* Mutant *ffbb*	*A. majus* Mutant *inc eos*
Naringenin	4′-OH	pelargonidin	–
Eriodictyol	3′,4′-OH	cyanidin	–
5,7,3′,4′,5′-Pentahydroxyflavanone	3′,4′,5′-OH	delphinidin	–
Compound **III**	3′,4′,5′-OH	delphinidin	–
Dihydrokaempferol	4′-OH	pelargonidin	pelargonidin
Dihydroquercetin	3′,4′-OH	cyanidin	cyanidin
Dihydromyricetin	3′,4′,5′-OH	delphinidin	delphinidin

verted into anthocyanins in acyanic flowers of *Antirrhinum*, indicating that compound **III** is also a flavanone (Table II).

Naringenin, eriodictyol and compound **III** were also present in ethyl acetate extracts of the strains "Bronzekugel" and "Rote Kugel". Furthermore, the three compounds were found in ether extracts of fresh bracts that were prepared under liquid nitrogen, and can therefore not have been produced during the extraction procedure by the corresponding chalcone glucosides.

Up to now, 5,7,3′,4′,5′-pentahydroxyflavanone was not yet found as aglycone in nature. But very recently two flavanone glycosides – probably based on 5,7,3′,4′,5′-pentahydroxyflavanone – were reported to be present in a special white mutant of *Petunia hybrida* [8]. A chromatographic (TLC, HPLC) comparison of 5,7,3′,4′,5′-pentahydroxyflavanone isolated from *Helichrysum* with the aglycone of the flavanone glycosides isolated from *Petunia* showed that both compounds are identical (Schram, personal communication).

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