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

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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 spontaneous cyclisation of the deglucosylated chalcone 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 (Rf 0.89, red), compound II (Rf 0.63, lilac) and compound III (Rf 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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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.

Rf-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.

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known flavanones naringenin and eriodictyol, respectively (Table I). Compound III showed spectral data similar to naringenin and eriodictyol. The peak of III (λ<sub>max</sub> 288 nm, methanol) exhibited bathochromic 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 bathochromic shift of 33 nm was observed, which is typical for 5,7-dihydroxyflavanones [4]. The R<sub>f</sub>-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<sub>f</sub>-values and spectral data of compound III.

Comparing compound III with naringenin and eriodictyol (Table I) in respect to R<sub>f</sub>-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<sub>f</sub>-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>
<thead>
<tr>
<th>Compound</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; (×100) in Forestal</th>
<th>R&lt;sub&gt;f&lt;/sub&gt; (×100) in BAW</th>
<th>TBA</th>
<th>MeOH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>+ NaOAc&lt;sup&gt;a&lt;/sup&gt;</th>
<th>+ AlCl&lt;sub&gt;3&lt;/sub&gt;</th>
<th>+ NaOH</th>
<th>Spectral maxima (nm)</th>
<th>Colour after reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Naringenin)</td>
<td>65</td>
<td>85</td>
<td>95</td>
<td>87</td>
<td>76</td>
<td>75</td>
<td>312</td>
<td>311</td>
<td>blue</td>
</tr>
<tr>
<td>(Eriodictyol)</td>
<td>99</td>
<td>62</td>
<td>28</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>322</td>
<td>322</td>
<td>blue</td>
</tr>
<tr>
<td>III (5,7,3',4',5'-pentahydroxyflavanone)</td>
<td>51</td>
<td>52</td>
<td>74</td>
<td>74</td>
<td>74</td>
<td>74</td>
<td>307</td>
<td>307</td>
<td>blue</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>B-ring hydroxylation pattern</th>
<th><em>M. incana</em> Mutant <em>ffbb</em></th>
<th><em>A. majus</em> Mutant <em>inc eos</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Naringenin</td>
<td>4'-OH</td>
<td>pelargonidin</td>
<td>—</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>3',4'-OH</td>
<td>cyanidin</td>
<td>—</td>
</tr>
<tr>
<td>5,7,3',4',5'-Pentahydroxyflavanone</td>
<td>3',4',5'-OH</td>
<td>delphinidin</td>
<td>—</td>
</tr>
<tr>
<td>Compound III</td>
<td>3',4',5'-OH</td>
<td>delphinidin</td>
<td>pelargonidin</td>
</tr>
<tr>
<td>Dihydrokaempferol</td>
<td>4'-OH</td>
<td>pelargonidin</td>
<td>cyanidin</td>
</tr>
<tr>
<td>Dihydroquercetin</td>
<td>3',4'-OH</td>
<td>cyanidin</td>
<td>cyanidin</td>
</tr>
<tr>
<td>Dihydromyricetin</td>
<td>3',4',5'-OH</td>
<td>delphinidin</td>
<td>delphinidin</td>
</tr>
</tbody>
</table>

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).