FLAVONOIDS FROM *TEUCRIUM FRUTICANS* L.

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(Received: March 1, 2000. Accepted: March 30, 2001)

ABSTRACT

From aerial parts of *Teucrium fruticans* L. three flavonoids were isolated and identified as 5-hydroxy-6, 7, 3', 4'-tetramethoxyflavone, 5, 4'-dihydroxy-6, 7, 3'-trimethoxyflavone (cirsilineol) and 5, 4'-dihydroxy-6, 7-dimethoxyflavone (cirsimaritin). The former compound was found to be a predominant flavone aglycone constituent of the plant material. This is the first report on the isolation of flavonoids from the plant.

KEY WORDS: *Teucrium fruticans*, Lamiaceae, flavonoid compounds, flavones.

INTRODUCTION

The genus *Teucrium* (Lamiaceae) comprises some 300 species of which 49 species are distributed in Europe, mainly in the Mediterranean basin. A previous comparative TLC study (Harborne et al. 1986) on flavonoids of 42 European *Teucrium* species revealed the widespread occurrence of free flavone aglycones accumulated externally at leaf and stem surfaces. All the excreted flavonoids showed the same substitution pattern in the A-ring (5-hydroxy-6, 7-dimethoxyflavones) and different substitutions in the B-ring. Moreover, a correlation between the preferred habitat (xeric, alpine) and the production of the excreted flavonoids has been noted, indicating that these compounds may provide resistance to damaging UV-B radiation. Increases in the synthesis of flavonoids at both the leaf surface and in the epidermal cells have been reported in other plant species subjected artificially to UV-B radiation (Harborne and Williams 2000). In addition, the flavonoid aglycones are useful chemotaxonomic markers within the genus *Teucrium* and other genera of the Lamiaceae (Tomas-Barberan and Wollenweber 1990). For this reason, we have examined *Teucrium fruticans* L., a species originating from Sicily, for its free flavonoid aglycone composition. The plant reportedly contains several neoclerodane diterpenoids (Savona et al. 1978a and 1978b; Bruno et al. 1992) and steryl glucoside esters (Fontana et al. 1999). However, the isolation of the flavonoids from the plant extract has not been described, so far.

MATERIAL AND METHODS

General

Merck silica gel (Art. 7754) was used for column chromatography. Petrol refers to b.p. 40-70°C. Semi-preparative RP-HPLC was performed on a Delta-Pak C-18 column (25 mm × 100 mm) using methanol-0.025% H₃PO₄ (2 : 1) as eluent at a flow rate of 5 ml min⁻¹. ¹H NMR spectra were recorded at 500.13 MHz with TMS as internal standard.

Plant material

Aerial parts of *T. fruticans* were collected during flowering on hills around Palermo (Sicily, Italy) in May 1998 and a voucher specimen was deposited in the herbarium of the Botanical Garden in Palermo.

Extraction and isolation of flavonoids 1-3

The air-dried plant material (1 kg) was finely ground and exhaustively extracted with acetone at room temperature. The residue (75 g) obtained after evaporation of the solvent *in vacuo* was chromatographed on a silica gel column using a petrol-ethyl acetate gradient solvent system. Fractions eluted with petrol-ethyl acetate (1 : 1) mixture which contained flavonoid compounds, as shown by TLC, were combined and concentrated, and then treated with hot ethyl acetate. The ethyl acetate soluble part of the fractions yielded pure **1** (147 mg). The residue (19.0 mg) was subjected to semi-preparative RP-HPLC to give an additional amount of **1** (15.5 mg) and a mixture (1.5 mg) of **1**, **2** and **3**.

in a ratio *ca.* 2 : 5 : 1, respectively (by analysis of integrals in the high resolution ^1H NMR spectrum).

5-Hydroxy-6, 7, 3', 4'-tetramethoxyflavone (1)

^1H NMR ($\text{DMSO}-d_6$) δ : 7.06 (s, H-3 or H-8), 6.99 (s, H-3 or H-8), 7.60 (d, $J = 2.2$ Hz, H-2'), 7.14 (d, $J = 8.6$ Hz, H-5'), 7.73 (dd, $J = 8.6$ and 2.2 Hz, H-6'), 3.74 (3H, s, 6-OCH₃), 3.86 (3H, s, 3'-OCH₃ or 4'-OCH₃), 3.89 (3H, s, 3'-OCH₃ or 4'-OCH₃), 3.94 (3H, s, 7-OCH₃), 12.91 (s, 5-OH).

5, 4'-Dihydroxy-6, 7, 3'-trimethoxyflavone (cirsilineol, 2)

^1H NMR ($\text{DMSO}-d_6$) δ : 6.99 (s, H-3 or H-8), 6.97 (s, H-3 or H-8), 7.60 (d, $J = 2.0$ Hz, H-2'), 6.94 (d, $J = 8.3$ Hz, H-5'), 7.62 (dd, $J = 8.3$ and 2.0 Hz, H-6'), 3.73 (3H, s, 6-OCH₃), 3.90 (3H, s, 3'-OCH₃), 3.94 (3H, s, 7-OCH₃), 12.95 (s, 5-OH).

5, 4'-Dihydroxy-6,7-dimethoxyflavone (cirsimaritin, 3)

^1H NMR ($\text{DMSO}-d_6$) δ : 6.95 (s, H-3 or H-8), 6.87 (s, H-3 or H-8), 7.98 (2H, d, $J = 8.8$ Hz, H-2' and H-6'), 6.93 (2H, d, $J = 8.8$ Hz, H-3' and H-5'), 3.73 (3H, s, 6-OCH₃), 3.93 (3H, s, 7-OCH₃), 10.40 (s, 4'-OH), 12.94 (s, 5-OH).

RESULTS AND DISCUSSION

The acetone extract from the aerial parts of *T. fruticans* was separated by column chromatography on silica gel followed by semi-preparative RP-HPLC to afford compound **1** as a predominant flavonoid constituent, along with compounds **2** and **3** in trace amounts (Fig. 1). Compound **1** was identified as 5-hydroxy-6, 7, 3', 4'-tetramethoxyflavone by

Fig. 1. Chemical structures of flavonoids from *Teucrium fruticans* L.

comparison of its physical (melting points of **1** and its acetyl derivative) and ^1H NMR spectral data in CDCl_3 with those of the flavone isolated previously from *Teucrium pseudochamaepitys* L. in the laboratory in Palermo (Savona et al. 1979). Compounds **2** and **3** were identified as 5,4'-dihydroxy-6, 7, 3'-trimethoxyflavone (cirsilineol) and 5, 4'-dihydroxy-6, 7-dimethoxyflavone (cirsimaritin), respectively, on the basis of direct comparison of their high resolution ^1H NMR spectra with each other and with that of **1**, all recorded in $\text{DMSO}-d_6$. Furthermore, the spectral data of the three compounds, given in the experimental part, were in accordance with those reported previously (Von Brieskorn and Riedel 1977; Martinez et al. 1987). In

the above-mentioned chemotaxonomic study (Harborne et al. 1986) on flavonoids of different *Teucrium* species, the free flavone aglycones present in the aerial parts of the plants as excretion compounds were analysed by TLC of extracts obtained by rinsing a small leafy stem of each plant in chloroform. The analysis revealed that compound **1** was frequent in species of three out of six *Teucrium* sections, i.e. *Teucrium*, *Stachybotrys* and *Chamaedrys*. In the study, a sample of *T. fruticans* (*Teucrium* section) originated from Spain was also examined and characterized by the presence of compound **1** accompanied by 5, 3', 4'-trihydroxy-6, 7-dimethoxyflavone (cirsiliol). The results of our study indicate that cirsiliol is absent from *T. fruticans* collected in Italy and the flavone aglycone profile of the plant is predominated by compound **1** with traces of compounds **2** and **3**. Thus, the highly methoxylated flavone **1** can be easily isolated from the plant material.

Most of the flavonoid aglycones occurring in plants of the Lamiaceae family are accumulated externally and the species from (semi-)arid and alpine habitats, e.g. *Teucrium*, *Sideritis*, *Salvia* and *Thymus*, being those which generally produce these compounds (Tomas-Barberan and Wollenweber 1990).

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FLAWONOIDY *TEUCRIUM FRUTICANS* L.

STRESZCZENIE

Z nadziemnych części *Teucrium fruticans* L. wyodrębniono i zidentyfikowano trzy flawonoidy: 5-hydroksy-6, 7, 3', 4'-tetrametoksyflawon, 5, 4'-dihydroksy-6, 7, 3'-trimetoksyflawon (cirsilineol) i 5, 4'-dihydroksy-6,7-dimetoksyflawon (cirsimarytyna). Pierwszy z wymienionych flawonów jest dominującym składnikiem aglikonów flawonoidowych występujących w badanym materiale roślinnym. Jest to pierwsze doniesienie o izolacji flawonoidów z tego gatunku.

SŁOWA KLUCZOWE: *Teucrium fruticans*, związki flawonoidowe, flawony.