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Bioactive flavonoids of *Tanacetum parthenium* revisited

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Bio-guided fractionation of an extract from *Tanacetum parthenium* showing activity as mitotic blocker allowed the isolation and identification of santin **3**, jaceidin **2** and centaureidin **1**. The latter two closely related flavonols, which, to the best of our knowledge, are isolated here together for the first time, form a mixture difficult to resolve and which is probably the reason for the confusion in the literature regarding their occurrence. Centaureidin **1** had an IC50 of 1 μM while jaceidin **2** and santin **3** were 200 times less active.

Keywords: *Tanacetum parthenium*; Flavonols; Compositae; Centaureidin; Jaceidin; Santin; Mitotic blockers

1. Introduction

In the course of screening various plant extracts for new mitotic blockers we discovered significant activity in an extract of feverfew, *Tanacetum parthenium* (L.) Schultz Bip., a plant widely used in herbal medicine for the treatment of migraine and arthritis (Johnson et al., 1985, Murphy et al., 1988, Hoult et al., 1995, Knight, 1995). The activity was located in the fractions of medium polarity especially in the ethyl acetate extracts and fractions thereof. A bio-guided purification of the extracts led to the isolation of some lipophilic flavonols. A preliminary investigation of the literature surfaced a series of papers authored by Professor J. B. Harborne and co-workers, which indicated there were some difficulties concerning the original identification of these compounds (Williams et al., 1995, 1999a,b). Given the interesting activity of the extract, it was decided to start new investigations on the nature of these bioactive flavonols from *Tanacetum parthenium*.

2. Results and discussion

2.1. Bioguided fractionation

During the preliminary screening, using a versatile whole-cell immunodetection assay to identify compounds that cause mitotic arrest (Stockwell et al., 1999), activity was found in two fractions: the crude ethyl acetate extract from the plant and a fraction obtained by silica gel chromatography of this extract eluted with chloroform. No activity was found in the more polar chromatographic fractions eluted with pure methanol and with mixtures of chloroform and methanol. The active fractions contained as a major compound, the known sesquiterpene lactone parthenolide (Bohlmann and Zdero, 1982), which however was found only weakly active in the test. Parthenolide was first separated by a liquid/liquid extraction between methanol and hexane; after elimination of the methanol, the extract was partitioned between chloroform and water. An aliquot of this chloroform extract was subjected to medium pressure liquid chromatography with chloroform as the eluent. The activity was concentrated in fractions which showed the typical UV spectra of flavonols (maxima at 258, 270 and 351 nm). Final purification was

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achieved by semi-prep. HPLC (Lichrospher 100 RP 18, 5 μm) and yielded a compound showing a single peak on HPLC with a retention time of 35 min and a single spot on TLC. The mass spectrum of this compound showed a molecular ion at m/z 359.1 $[M-H]^-$ for a $C_{18}H_{16}O_8$ composition (negative electrospray ionization).

Given the previous studies on the plant, it was assumed that this compound was centaureidin **1**, also known as quercetagetin 3,6,4′-trimethylether (Williams et al., 1999a, b). Interestingly also, centaureidin **1** is a long known compound from *Centaurea* and *Tanacetum* species (Abad et al., 1993) which has been found to be cytotoxic via inhibition of tubulin polymerisation (Beutler et al., 1993). Because of the past inconclusive NMR spectroscopic data on *Tanacetum parthenium* flavonols, we set to study in detail the NMR spectra of the molecule. To our surprise, it was found that the 500 MHz ^1H NMR spectrum of the active compound showed a series of small peaks and of shoulders on some peaks suggesting the presence of an impurity. Horizontal TLC with multiple developments allowed the observation of another spot superimposed with the spot of centaureidin (**1**). Preparative thin layer chromatography on HPTLC plates allowed full separation of the compounds after 12 plate migrations and afforded besides pure **1**, compound **2** which proved to be an isomer of **1**.

2.2. Flavonoid identification

The identity of **1** with centaureidin was established by ^1H and ^{13}C NMR spectroscopic analyses, whose assignments were facilitated by COSY, HSQC and HMBC experiments. Compound **2** had a similar mass spectrum (negative ESI: m/z 359.1 $[M-H]^-$ for $C_{18}H_{16}O_8$) and its NMR spectra showed identical signals at slightly different chemical shifts. The main and most significant differences between the two products were observed in the classical or in the acquisition delayed COSY experiments which showed long range couplings between some of the methoxy and the neighbouring aromatic protons. Thus in **1** correlation between, a methoxy and a large doublet at δ 7.05 ($J = 8.5$ Hz) was observed, i.e. between H-5′ and OMe- 4′. For minor compound **2**, a similar correlation was observed with a sharp aromatic doublet at δ 7.68 ($J = 2$ Hz). The rest of the spectra being of similar nature, it was deduced that **2** was the position isomer of **1** in the substitution of ring B, that is to say, 3,6,3′-trimethyl quercetagetin also known as jaceidin. The same deductions could be made on the basis of HMBC long range correlation assignments. It is worth noting that the observation or not of interproton long range correlations between the methoxy and the adjacent aromatic protons provide a simple solution and a sensitive method to locate methoxy in ring A or B, which seemed to have been a problem in the first investigations on the flavonols from this plant (Williams et al., 1995, 1999a,b).

The presence of closely related flavonol isomers such as centaureidin and jaceidin in other plant species may well have gone undetected in the past. The present results highlight the necessity for more modern techniques e.g. HPTLC and advanced NMR spectroscopic studies for the isolation and identification of the constituents in such mixtures. It is also possible in feverfew that in the same plant and depending on collection conditions, the amount of each compound varies, giving rise to minute differences in UV spectral behaviour, a criterion widely used and developed by Professor Harborne to identify flavonoids and which unfortunately finds its limitations in the rare cases of co-occurrence of closely related compounds.

2.3. Biological activity

Mitotic arrest evaluation of the pure products **1**–**3** was carried out and the IC_{50}s determined. As regards biological activities, once the products were separated and proven to be pure, IC_{50} were determined. In accordance with the known cytotoxic properties of **1**, we obtained IC_{50} of **1** μM for **1** and of 200 μM for **2**. A similar value (220 μM) was obtained for the related santin **3**, another flavonol from the plant (first improperly named tanetin). These results clearly show that subtle differences in substitution (**1** vs. **2**) can induce considerable differences in biological properties and that not all flavonoids have the same activity (Zhu et al., 1997).

3. Experimental

General experimental procedures: analytical HPLC: Merck Lachrom, DAD, column chromosep SS 150 mm, OmniSpher 5C18 Varian; semi prep HPLC Waters 600 with RT250-25 HIBAR, Lichrospher 100 RP 18 column. Mass spectrometry: Bruker ion trap Esquire with electrospray ion source. NMR: Bruker Avance 500 MHz; standard Bruker pulse programs were used for 2D experiments.

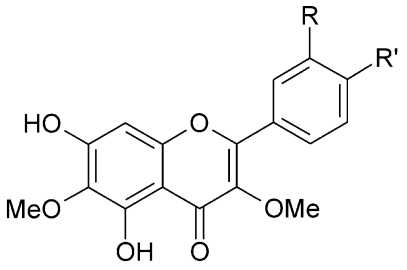
Plant material: *Tanacetum parthenium* was purchased from Florina (Valanjou, France). A voucher specimen verified by one of us (B.D.) was deposited in the Pierre Fabre Research Institute's specimen collection under No. V100278.

Extraction and isolation: Finely ground aerial parts of *Tanacetum parthenium* (1 kg) were macerated in ethyl acetate (10 l) at room temperature for 24 h. After filtration and evaporation of the solvent, 24 g of extract were obtained; a second maceration of the cake with the same solvent (10 l) gave another 10.2 g of extract. The combined extracts were dissolved in MeOH (500 ml) and extracted with hexane (4×250 ml). Then MeOH was removed in vacuo and the residue was extracted between

water (500 ml) and chloroform (2×300 ml). Evaporation of the chloroform yielded 18.8 g of extract. An aliquote (15 g) of the extract was adsorbed on silica gel and placed on a Büchi MPLC system (460×55 mm) eluted with chloroform (7 l). A total of 55 fractions (125 ml each) were collected and pooled according to composition. Activity was concentrated in fr. 31–45 (1.25 g). Santin **3** was in fr. 25–30; it was purified by crystallization from water/acetonitrile (yield: 112 mg). The fractions containing **1** and **2** were further purified by HPLC using a C_{18} column and a mixture of water and acetonitrile as eluent (7/3). A sample (10 mg) of the mixture was deposited on an HPTLC 60F254 Merck plate and eluted 12 times with $CHCl_3$.

Centaureidin (**1**): slower moving spot (R_f 0.14). ^{1}H NMR ($CDCl_3/CD_3OD$, 500 MHz): 6.4 (*s*, H-8), 7.63 (*d*, $J=2.2$ Hz, H-2′), 7.05 ($J=8.5$ Hz, H-5′), 7.65 (*dd*, $J=8.5$, 2.2 Hz, H-6′), 3.78 (*s*, 3H, 3-OMe), 3.88 (*s*, 3H, 6-OMe), 3.96 (*s*, 3H, 4′-OMe).

^{13}C NMR ($CDCl_3/CD_3OD$, 125 MHz): 156.2 (C-2), 138.2 (C-3), 178.8 (C-4), 152.5 (C-5), 131.4 (C-6), 158.2[a] (C-7), 94.1 (C-8), 152.2 (assignments may be interchanged) (C-9), 104.9 (C-10), 121.4 (C-1′), 114.8 (C-2′), 146.1 (C-3′), 150.2 (C-4′), 110.9 (C-5′), 120.9 (C-6′), 59.6 (3-OCH_3), 59.9 (6-OCH_3), 55.7 (4′-OCH_3).



1 R = OH, R' = OMe, centaureidin
2 R = OMe, R' = OH, jaceidin
3 R = H, R' = OMe, santin

Jaceidin (**2**): faster moving spot (R_f 0.17). ^{1}H NMR ($CDCl_3/CD_3OD$, 500 MHz): 6.35 (*s*, H-8), 7.68 (*d*, J = 2 Hz, H-2′). 6.95 (*d*, $J=8.5$ Hz, H-5′), 7.63 (*dd*, $J=8.5$, 2 Hz, H-6′), 3.76 (*s*, 3H, 3-OMe), 3.87 (*s*, 3H, 6-OMe), 3.94 (*s*, 3H, 4′-OMe).

^{13}C NMR ($CDCl_3/CD_3OD$, 125 MHz): 156.5 (C-2), 138.2 (C-3), 178.8 (C-4), 152.5 (C-5), 131.4 (C-6), 158.2 (assignments may be interchanged) (C-7), 94.1 (C-8), 152.2 (assignments may be interchanged) (C-9), 104.9 (C-10), 121.4 (C-1′), 111.4 (C-2′), 147.7 (C-3′), 149.7 (C-4′), 115.1 (C-5′), 122.4 (C-6′), 59.6 (3-OCH_3), 59.9 (6-OCH_3), 55.4 (3′-OCH_3).

Biological activity: mitotic arrest evaluation was performed according to Stockwell et al. (1999).

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