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# A SESQUITERPENE COUMARIN ETHER FROM TRANSFORMED ROOTS OF *TANACETUM PARTHENIUM*

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**Key Word Index**—*Tanacetum parthenium*; Asteraceae; transformed roots; *Agrobacterium rhizogenes*; coumarins; sesquiterpene coumarin ether.

**Abstract**—The roots of *Tanacetum parthenium* transformed with *Agrobacterium rhizogenes* afforded, in addition to the known coumarin isofraxidin, a new isofraxidin drimenyl ether which was characterized as 9-epipectachol B by spectral methods. © 1997 Elsevier Science Ltd

## INTRODUCTION

In our previous paper [1], we reported on the occurrence and concentrations of spiroketalenolether acetylenes in the roots of *Tanacetum parthenium* (L.) Sch. Bip. transformed with *Agrobacterium rhizogenes* LBA 9402. In the course of our investigation, it became apparent that, besides significant quantities of the acetylenic metabolites, the extract of the transformed roots contained smaller amounts of other UV-visible compounds. Two of them were isolated and found to be the known coumarin isofraxidin (**1**) and a new isofraxidin drimenyl ether (**2**). The structure and relative configuration of the new compound have now been established by spectroscopic techniques (NMR and MS), as described below.

## RESULTS AND DISCUSSION

The transformed roots of *T. parthenium* were extracted with hexane followed by a mixture of hexane–acetone (1:1). The latter extract was separated by semi-preparative HPLC to afford sufficient quantities of the coumarins **1** and **2** for spectral analysis. Isofraxidin (**1**) was readily identified by comparison of its $^1$H NMR and mass spectral data with those reported in the literature [2]. The compound was previously isolated from callus and cell suspensions of *T. parthenium* and was also found in a detectable amount (HPLC) in the roots of the field grown plants [2].

The overall spectral data of **2** strongly suggested that the compound was closely related to the isofraxidin ethers with *trans*-fused bicyclic sesquiterpene moieties of the drimenol type, reported earlier from some Asteraceae–Anthemideae species [3–7]. Apart from the signals characteristic for isofraxidin protons, the $^1$H NMR spectrum of **2** (Table 1) contained signals of three tertiary methyls, an exocyclic methylene, a methylene bearing an ether oxygen and a methine bearing hydroxyl group. In particular, the signals were very similar to those of pectachol B (**3**), which had been reported from *Brocchia cinerea* [6]. Similarly to pectachol B, the hydroxyl group at C-3 in **2** was proved to be equatorially oriented, because H-3 appeared as a double doublet ($J$ = 11.6 and 4.4 Hz, $W_{1/2} > 15$ Hz). The corresponding proton geminal to the axial hydroxyl group would result in the relatively narrow signal ($W_{1/2}$ *ca* 7–9 Hz). The changed configuration at C-9, in comparison to that of **3**, was clear from the chemical shifts of the >CH—CH$_2$—OAr proton signals. The AB of an ABX system at $\delta$ 4.25 and $\delta$ 4.21 ($\Delta\delta_{AB} < 0.1$ ppm) was typical for an equatorial configuration of $-CH_2OAr$ group in **2**. In pectachol B and comparable compounds $\Delta\delta_{AB}$ for axial $-CH_2OAr$ is generally >0.2 ppm [4–6]. The assignments of almost all signals in the $^1$H NMR spectrum of **2** were confirmed by $^1$H–$^1$H COSY correlations (Table 2) and the splitting patterns of the sesquiterpene ring proton signals were supported by comparison with the more detailed spectra of other drimenyl derivatives with the same structure and stereochemistry [8, 9]. Therefore, the structure of **2** was elucidated to be 9-epipectachol B or its optical isomer. The compound is reported with the same absolute configuration as pectachol B. However, in plants belonging to the Asteraceae–Anthemideae the enantiomeric series of isofraxidin ethers have been found, even within the same species [4, 6].

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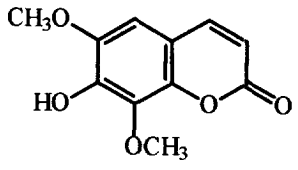

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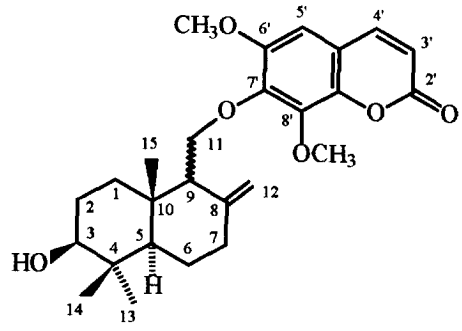

**2** 9αH
**3** 9βH

Table 1. $^1$H NMR spectral data of compound **2** (500.13 MHz, $CDCl_3$, TMS as internal standard, δ-values)

| H | | H | |
|---|---|---|---|
| 1a | 1.82 *ddd* | 11a | 4.25 *dd* |
| 1b | 1.64–1.74 *m* | 11b | 4.21 *dd* |
| 2a | 1.64–1.74 *m* | 12a | 5.03 *d br* |
| 2b | 1.59 *dddd* | 12b | 5.00 *d br* |
| 3 | 3.30 *dd* | 13 | 1.02 *s* |
| 5 | 1.19 *dd* | 14 | 0.80 *s* |
| 6a | 1.77 *dddd* | 15 | 0.76 *s* |
| 6b | 1.44 *dddd* | 3′ | 6.34 *d* |
| 7a | 2.48 *ddd* | 4′ | 7.60 *d* |
| 7b | 2.12 *ddd br* | 5′ | 6.65 *s* |
| 9 | 2.26 *dd br* | 6′-OMe | 3.87 *s* |
| | | 8′-OMe | 3.99 *s* |

*J* (Hz): 1a, 1b = 13.0; 1a, 2a = 1a, 2b = 3.4; 1b, 2b = 13.1; 2a, 2b = 13.1; 2a, 3 = 4.4; 2b, 3 = 11.6; 5, 6a = 2.6; 5, 6b = 12.5; 6a, 6b = 13.0; 6a, 7a = 2.4; 6a, 7b = 5.2; 6b, 7a = 4.2; 6b, 7b = 13.0; 7a, 7b = 13.1; 9, 11a = 9.4, 9, 11b = 3.9; 11a, 11b = 9.4; 12a, 12b = 1.3; 3′, 4′ = 9.5.

Table 2. $^1$H–$^1$H COSY correlations of compound **2**

| H | Correlated H |
|---|---|
| 3 | H-2a, H-2b |
| 5 | H-6a, H-6b |
| 6a | H-5, H-6b, H-7a, H-7b |
| 6b | H-5, H-6a, H-7a, H-7b |
| 7a | H-6a, H-6b, H-7b |
| 7b | H-6a, H-6b, H-7a, H-12b |
| 9 | H-11a,b, H-12a |
| 11a,b | H-9 |
| 12a | H-9 |
| 12b | H-7b |
| 3′ | H-4′ |
| 4′ | H-3′ |

## EXPERIMENTAL

*Plant material.* Roots of *T. parthenium* transformed with *Agrobacterium rhizogenes* LBA 9402 were obtained and cultured as described elsewhere [10].

*Extraction and isolation of compounds* **1** *and* **2**. Lyophilized plant material (23 g) was extracted with hexane followed by hexane–$Me_2CO$ (1:1) mixt. at room temp. and the solvents were evapd under red. pres., giving 0.8 g and 0.5 g of residues, respectively. Each residue was dissolved in 30 ml of warm MeOH and left to stand overnight at 4°. After filtration and evapn of the solvent, each residue was redissolved in 3 ml MeCN and filtered through a membrane filter (0.22 μm) before subjection to semiprep. HPLC. The HPLC sepn was performed on a Prep Pak C 18 cartridge column (particle size 10 μm, 25 × 100 mm) coupled to a UV photodiode array detector, using MeCN–$H_2O$ (9:11) mixt. at flow rate 10 ml min$^{-1}$. In addition to acetylenes present in both extracts [1], the hexane–$Me_2CO$ extract yielded noncrystalline coumarin derivatives **1** (2.8 mg, $R_t$ = 5.5 min) and **2** (3.3 mg, $R_t$ = 52 min) that possessed the same UV absorption maxima (207, 226, 294 and 340 nm) and base peaks at *m/z* 222 (isofraxidin) in their EI mass spectra. A molecular ion peak at *m/z* 442 (0.7%) and other peaks in the EI MS spectrum of **2** were of low abundances.

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