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2-Hydroxy-4-methoxy-trans-cinnamic Acid as a Precursor of Herniarin in *Artemisia dracunculus**

Short Communication

Otmar Hofer[a], **Géza Szabó**[b], and **Harald Greger**[b]

[a] Institute of Organic Chemistry, University of Vienna, A-1090 Wien, Austria.
[b] Institute of Botany, University of Vienna, A-1030 Wien, Austria

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The isolation of (*E*)-2-hydroxy-4-methoxycinnamic acid (**1**) from *Artemisia dracunculus* L. supports strongly the assumption that this compound is an intermediate in the biosynthesis of 7-methoxycoumarin (herniarin, **3**). The structure of the UV-unstable compound **1** was derived from ^{1}H-NMR data and by comparison of the stable dimethylated derivative with synthetic (*E*)-2,4-dimethoxycinnamic acid methyl ester (**2**).

(Keywords: Coumarins; Biosynthesis; Herniarin; Artemisia dracunculus; Compositae)

2-Hydroxy-4-methoxy-trans-zimtsäure als Vorläufer von Herniarin in Artemisia dracunculus (Kurze Mitteilung)

Die Isolierung von 2-Hydroxy-4-methoxy-*trans*-zimtsäure (**1**) aus *Artemisia dracunculus* L. stützt die Annahme, daß diese Verbindung eine Zwischenstufe bei der Biosynthese von 7-Methoxycumarin (Herniarin, **3**) darstellt. Die Strukturaufklärung der UV-instabilen Verbindung **1** erfolgte mittels ^{1}H-NMR und durch Vergleich des stabilen methylierten Derivates **2** mit synthetischem (*E*)-2,4-Dimethoxyzimtsäuremethylester.

A thorough study by *Brown* on the biosynthesis of coumarin and herniarin [1] showed that 2-hydroxy-4-methoxy-*trans*-cinnamic acid (**1**) may be an important intermediate in the biosynthetic route to *trans*- and *cis*-2-glucosyloxy-4-methoxycinnamic acid (*trans*- and *cis-GMC*), the direct precursors of herniarin [1].

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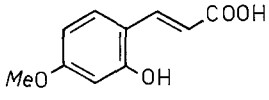
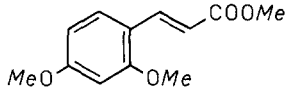
* Dedicated to Prof. Dr. *Kurt L. Komarek* on the occasion of his 60th birthday.

However, although most of the intermediates postulated in [1] have been shown to be precursors of herniarin or "bound herniarin" (*cis-GMC*), there was no direct proof for **1** as a key intermediate. In spite of some efforts, compound **1** could not be detected in the extracts of *Lavandula officinalis* Chaix investigated by *Brown* [1] in 1963. In a later paper (1965) *Brown* reported the detection of traces of the glucoside *trans-GMC* [2]. High turnover rates or unknown derivatives of **1** (for instance esters) were made responsible for the failure to detect compound **1** as a naturally occurring constituent. Free **1** was reported only once by *Khan* et al. (1979) as a natural constituent form *Tamarix dioica* Roxb., a medical plant of India [3].

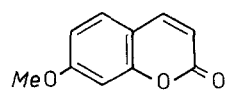
In the latter paper the structure elucidation was described without any details on NMR (NMR was only mentioned, no data were given). The decision between possible isomers (2-hydroxy-4-methoxy- or 4-hydroxy-2-methoxy-cinnamic acid) was based on a colour test for unsubstituted aromatic CH para to a phenolic group (*Gibbs* reaction [4]). The most characteristic and well known light sensitivity of **1** [5, 6] leading to herniarin (**3**) was not mentioned in Ref. [3].



1



2



3

We have now isolated **1** during our current comparative investigations on secondary constituents of the genus *Artemisia* (*Asteraceae-Anthemideae*). A methanolic extract of the leaves of *Artemisia dracunculus* L. afforded a very polar compound which was rather sensitive during thin layer chromatography: in the presence of light this compound was transformed into a very much less polar product which exhibited the characteristic fluorescence of herniarin under the UV lamp. Careful chromatography avoiding UV irradiation gave enough pure material for an unambiguous structure elucidation.

Compound **1** showed the typical ^{1}H-NMR resonance pattern for a 1,2,4-trisubstituted benzene derivative: a broad singlet (H3) and a AB system with one sharp doublet (H6) and one broad doublet (H5) with a small *meta* coupling). A further AB system in the olefinic range of the spectrum with a coupling constant of 16 Hz indicated a *trans* substituted double bond in the main side chain. A further substituent was a methoxy group, no other resonances could be detected in CD_3OD (solubility in $CDCl_3$ < 0.2 mg/ml).

These data and the behaviour during the TLC purification procedure [partical transformation of **1** to herniarin (**3**) via photo-isomerization of the *trans* double bond to *cis* and ring closure to the cyclic lactone (*Rf* value and UV identical with authentic **3**)] suggested strongly the structure of a *trans* cinnamic acid derivative with *o*-OH and *p*-O*Me* for compound **1**. Independent evidence for the positions of OH and O*Me* in the aromatic ring was provided by the methylation of **1** with diazomethane to the corresponding 2,4-dimethoxycinnamic acid methyl ester (**2**) and comparison with a synthetic product obtained by *Knoevenagel*-condensation of 2,4-dimethoxybenzaldehyde with malonic acid followed by esterification with diazomethane; both products were identical (^{1}H-NMR in $CDCl_3$ and CD_3OD).

The presence of compound **1** as a potential precursor of herniarin (**3**) in *A. dracunculus* is in contrast to the investigations of *Brown* [1, 2] in the case of *Lavandula officinalis*, where a comparable isolation procedure (boiling aqueous alcohol) did not yield **1** as a naturally occurring intermediate. However, appreciable amounts of **1** in *A. dracunculus* indicate the importance of this compound in the biosynthetic pathway to herniarin in this species.

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Experimental

Plant material was grown under field conditions in the Botanical Garden of the University of Vienna (AR-312; achenes from USSR, Uzbek. SSR, Taškent); voucher specimen deposited at the herbarium of the Institute of Botany, University of Vienna (WU).

Instruments: M.p.: *Kofler* micro-hotstage (uncorrected); UV: Perkin-Elmer Lambda 7; NMR: Bruker WM 250 MHz; MS: Varian MAT CH-7.

Air dried leaves (70 g) of *A. dracunculus* were extracted with boiling *Me*OH for 45 min. The concentrated extract was treated with hot H_2O to remove chlorophyll and then partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ fraction contained the coumarins (e.g. ca. 25 mg herniarin [7, 8]) and other less polar components, A second extraction with ethyl acetate gave a fraction containing polar products. TLC of this *Et*O*Ac* fraction on 1 mm thick layers of silica gel GF 254 (Merck) avoiding UV-irradiation and using Et_2O/*Et*O*Ac* (9 : 1) as solvent afforded 10 mg of **1**.

(E)-2-Hydroxy-4-methoxy-cinnamic Acid (**1**)

Colourless crystals; m.p. 185—192 °C (decomp.), Ref. [9] 195–198 °C (decomp.). ^{1}H-NMR (CD_3OD, δ/ppm): 7.75 (d, 1 H, J = 16 Hz, olefin. H), 7.35 (d, 1 H, J = 9 Hz, aromat. H 6), 6.40 (d, 1 H, J = 16 Hz, olefin. H), 6.34 (s, 1 H, aromat. H 3), 6.32 (br. d, 1 H, J = 9 Hz, aromat. H 5), 3.75 (s, 3 H, O*Me*4). The solubility in $CDCl_3$ is very poor (< 0.1 mg in a 0.5 ml NMR sample).

(E)-2,4-Dimethoxy-cinnamic Acid Methyl Ester (**2**) *from* **1**

Compound **1** was treated with CH_2N_2/Et_2O giving the dimethoxymethylester **2**; m.p. 83–86 °C after TLC purification, Ref. [9, 10] 87 °C. ^{1}H-NMR ($CDCl_3$, δ/ppm): 7.92 (d, 1 H, J = 16 Hz, olefin. H), 7.45 (d, 1 H, J = 9 Hz, aromat. H 6), 6.51 (dd, 1 H, J = 9 and 2.5 Hz, aromat. H 5), 6.46 (d, 1 H, J = 2.5 Hz, aromat H 3), 6.45 (d, 1 H, J = 16 Hz, olefin. H), 3.88 (s, 3 H, O*Me*), 3.85 (s, 3 H, O*Me*), 3.79 (s, 3 H, O*Me*). ^{1}H-NMR (CD_3OD, δ/ppm): 7.92 (d, olefin. H), 7.52 (d, H 6), 6.59 (br. s, H 3), 6.57 (dd, H 5), 6.44 (d, olefin. H), 3.90 (s, O*Me*), 3.85 (s, O*Me*), 3.76 (s, O*Me*). MS (70 eV, 60 °C): *m/e* 222 (75% r.I., M^+), 191 (100), 176 (28), 149 (45), 148 (30), 133 (20), 121 (21). UV (*Et*OH): λ 327 nm (ε = 20 500), 292 (15 800), 239 (12 300), 216 (12 000).

2 *from 2-Hydroxy-4-methoxybenzaldehyde*

2 was prepared from 2-hydroxy-4-methoxybenzaldehyde (Janssen Chimica, 2340 Beerse, Belgium) by methylation (CH_2N_2) to 2,4-dimethoxybenzaldehyde, followed by *Knoevenagel*-condensation [malonic acid, pyridine (+ piperidine), 6 h, 105 °C] and esterification (CH_2N_2) of the resulting cinnamic acid. This product gave identical NMR spectra ($CDCl_3$, CD_3OD) compared to the data of the methylation product of **1**.

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