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ANTIFUNGAL STRESS COMPOUNDS FROM *VICIA CRACCA*

MUSTAFA M. SALEH*† and KARL-WERNER GLOMBITZA‡

†Pharmaceutical Science Department, National Research Centre, Dokki Cairo; ‡Institut für Pharmazentische Biologie, Friedrich-Wilhelms-Universität, S3115 Bonn, Germany

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Key Word Index—*Vicia cracca*; Leguminosae; antifungal stress compounds.

Abstract—From the leaflets of *Vicia cracca* two new antifungal stress compounds, 2-methoxy-6-[2-(4-methoxyphenyl)ethenyl]pyran-4-one and 2-methoxy-6-[2-(phenyl)ethenyl]pyran-4-one were isolated. Their structures were elucidated by spectroscopic means. Copyright © 1997 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Surveys of the Vicieae have revealed that the plant parts have the capacity to produce antifungal stress compounds, such as isoflavonoid phytoalexins [1–3], furanoacetylenes [4] and, rarely, a limited range of other biologically active compounds [5, 6].

Antifungal stress compound formation in response to treatment with chemical reagents has been studied and a wide range of compounds has been found to be effective, including the salts of several heavy metals. Mercuric and cupric chlorides were the most effective and were not phytotoxic [7].

RESULTS AND DISCUSSION

Two compounds were isolated. Compound **1** was assigned the molecular formula $C_{15}H_{14}O_4$ from its high resolution EI-mass spectrum (*m/z* (rel. int.): 258.089 (100, $[M]^+$), 230 (32), 215 (9), 202 (5) and 187 (43)). The spectral data show the presence of an aromatic system and two methoxyl groups. The coupling constant ($J = 16$ Hz) for the doublet at δ 6.33 in the ^{1}H NMR spectrum (Table 1) revealed the presence of two *trans*-ethylenic protons at C-7 and C-8. The symmetric system for the aromatic carbon atoms in the ^{13}C NMR (Table 2) (C-11, C-13 and C-10, C-14) shows that one of the methoxyl groups should be attached to the *para* position, with the ethylenic group of the benzene ring being a methoxyl *trans*-styryl derivative. The high chemical shift value for the carbonyl carbon C-4 at δ 171.5 indicates a γ-pyrone system, rather than an α-pyrone, which is found around δ 165. The doublet in the ^{1}H NMR spectrum at δ 6.85 ($J = 2$ Hz) indicates that the γ-pyrone ring contains two *meta* protons. This is confirmed by the low ^{13}C NMR values for the carbon attached to these protons (C-3 and C-5). Therefore, the other methoxyl group should be placed α to the heteroatom. The above data show that **1** is a 2-methoxy-γ-pyrone-*p*-methoxystyryl derivative. Comparison of the data obtained with those reported in the literature shows that the data for the styryl part

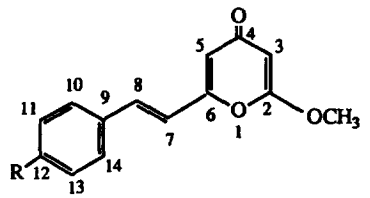
Table 1. ^{1}H NMR spectral data of **1** and **2** (400 MHz, $CDCl_3$)

H	1	2
3	5.40 (*d*, $J = 2$ Hz)	5.45 (*d*, $J = 2$ Hz)
5	5.85 (*d*, $J = 2$ Hz)	5.92 (*d*, $J = 2$ Hz)
7	6.35 (*d*, $J = 16$ Hz)	6.55 (*d*, $J = 16$ Hz)
8	7.41 (*d*, $J = 16$ Hz)	7.45 (*d*, $J = 16$ Hz)
10, 14	7.39 (*d*, $J = 9$ Hz)	7.40 (*m*)
11, 13	6.85 (*d*, $J = 9$ Hz)	7.35 (*m*)
15	3.75 (*s*)	3.78 (*s*)
16	3.74 (*s*)	—

Table 2. ^{13}C NMR spectral data of **1**, **2** and yangonin (75 MHz, $CDCl_3$)

C	1	2	Yangonin [8]
2	164.0	164.0	162.0
3	88.1	88.5	116.7
4	171.5	171.8	144.3
5	100.3	101.8	106.8
6	160.1	158.2	153.3
7	116.1	118.5	116.6
8	135.4	135.8	135.4
9	128.2	135.5	128.2
10	129.0	129.5	129.0
11	114.2	127.8	114.5
12	189.0	129.8	159.2
13	114.2	127.8	114.5
14	129.0	129.5	129.0
15	55.0	56.0	55.4
16	55.8	—	55.8

*Author to whom correspondence should be addressed.



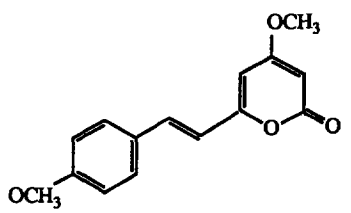
1 R = OCH_3

2 R = H

Table 3. R_f values (Et_2O–n-hexane, 3 : 1) and colour reactions (with 10% H_2SO_4) of antifungal compounds from *Vicia cracca* on silica gel TLC plates

Compound	R_f	Colour
A	0.19	Violet-pink
B	0.28	Violet
C	0.40	Orange
D*	0.57	Pink
E	0.69	Faint pink
F†	0.88	Pink

*Compound **1**.
†Compound **2**.



Yangonin

of the molecule are in agreement with those published for yangonin [8].

Compound **2** gave a high resolution EI-mass spectrum *m/z* (rel. int.): 228.08 (100, $[M]^+$, $C_{14}H_{12}O_3$), 215.07 (2), 200.09 (49), 157.07 (32), 141.07 (93) and 128.06 (24). The $[M]^+$ shows that **2** contains one less OCH_3 group than **1**. The ^1H and ^{13}C NMR data also confirm this, and show that the missing methoxyl group was that of the benzene ring due to the loss of the symmetrical carbon atoms and protons. Therefore, **1** is the methoxy derivative of **2**.

EXPERIMENTAL

Spectroscopy. MS were measured at 70 eV. ^1H and ^{13}C NMR of $CDCl_3$ solutions, at 400 and 90 MHz, respectively, are relative to TMS.

Plant material. Leaflets of *Vicia cracca* L. (3–4 weeks old) were detached from the plant and rinsed in dist. H_2O. They were then placed abaxial surface downwards onto a perforated shelf 2 cm away from the bottom of polyethylene boxes. The bottom of the boxes were lined with filter paper moistened with dist. H_2O to maintain high humidity. The boxes were divided into 7 groups and treated as follows. (1) $HgCl_2$ 10^{-2} M; (2) $CuCl_2$ 3×10^{-2} M; (3) spore suspension of *Heminthosporium turcicum* (5×10^4 spores ml^{-1}) in 0.05% aq. Tween-20; (4) spore suspension of *Penicillium digitatum* (5×10^4 spores ml^{-1}) in 0.05% aq. Tween-20; (5) irradiation with UV light (366 nm) for 60 min before incubation; (6) injury by pricking the leaflets on the abaxial surface, using pins fixed to an eraser; (7) control (treated with water and Tween-20). The boxes were then incubated at 25° for 48 hr. The inoculated area of the leaflets, i.e. the area beneath the droplets, was removed. Leaflet discs were then extracted with 95% EtOH (2 ml g^{-1} fr. wt) and the extract evapd to dryness *in vacuo* at 50°. The dry residue was washed with $CHCl_3$ and the $CHCl_3$ extract partitioned $\times 3$ with an equal vol. of 0.2 N NaOH. The NaOH fr. was acidified to pH 3, then partitioned $\times 3$ each with one half its vol. of $CHCl_3$. The combined final $CHCl_3$ extract was evapd *in vacuo* and the residue was dissolved in a small vol. of 95% EtOH. Silica gel TLC plates were spotted with equivalent volume of the phenolic extracts of the different treatments and developed in the solvent system Et_2O–n-hexane (3 : 1). After development, the plates were sprayed with 10% H_2SO_4. No additional components appeared in any of the treatments other than those present in the control, except for the two pink spots present in the $HgCl_2$ treatment.

The developed plates were sprayed with a spore suspension of *P. digitatum* [9]. The phenolic extracts of all treatments showed the same inhibition zones, except in the case of $HgCl_2$, which had two additional inhibition zones. The spots that correspond to the inhibition zones are listed in Table 3. After developing on silica gel plates, bands were extracted separately with $CHCl_3$–MeOH (1 : 1), then centrifuged. Extracts were filtered, then evapd to dryness and dissolved in a small vol. of EtOH. TLC purification was repeated to yield compounds **1** and **2**.

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