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PHYTOTOXINS FROM *ALTERNARIA HELIANTHI*: RADICININ, AND THE STRUCTURES OF DEOXYRADICINOL AND RADIANTHIN

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Key Word Index—*Alternaria helianthi*; Dematiaceae; phytotoxin; sunflower; radicinin; deoxyradicinol; radianthin.

Abstract—A novel compound, radianthin, with phytotoxic activity was isolated from liquid cultures of *Alternaria helianthi* and identified as a pyrone related to radicinin. A second metabolite was identified as radicinin itself while deoxyradicinol is described for the first time as a natural product.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oil seed crop grown extensively in many areas of the world, while *Alternaria helianthi* (Hansf.) Tubaki and Nishihara [1] is an aggressive pathogen of this plant [2]. Two phytotoxic derivatives of radicinin (1) produced by this fungus have already been described as deoxyradicinin (2) and 3-epideoxyradicinol (3) [3, 4]. Here we report the identification of three additional phytotoxic products of *A. helianthi* as radicinin itself (1) [5], and the novel metabolites deoxyradicinol (4) and radianthin (5). Radicinin was previously known as a metabolite of the closely related species *A. chrysanthemi*, Simmons and Crosier [6] and other organisms [7]. Deoxyradicinol (4) is described here as a natural product for the first time.

RESULTS AND DISCUSSION

In addition to the major phytotoxic metabolite deoxyradicinin (2) [3] and the less abundant 3-epideoxyradicinol (3) [4], three minor phytotoxic derivatives were also isolated from liquid cultures of *A. helianthi*. Two of these were identified as radicinin (1) and deoxyradicinol (4) by direct comparison with authentic material. The former, previously known metabolite (1), was produced only in relatively trace amounts by *A. helianthi* (*ca* 3 mg/l of culture filtrate) as compared with the yield obtained from the closely related fungal pathogen *A. chrysanthemi* [6]. Radicinin obtained from *A. helianthi* was apparently optically pure as judged from an examination of its ^{1}H NMR spectrum after the addition of the chiral solvating reagent (*S*)−(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol (ratio of chiral solvating agent to radicinin 68:1) [8, 9]. Also, upon addition of the above chiral solvating reagent (18:1) to an equimolar mixture (4 mg) of 1 *ex A. helianthi* and authentic radicinin isolated from *A. chrysanthemi*, no additional multiplicity in the ^{1}H NMR signals was observed, indicating that the (4*S*,5*S*)-configuration [6, 7] is the same in each case. For biogenetic and chiroptical reasons, we assume deoxyradicinol (4) and 3-epideoxyradicinol (3) have the same (5*S*)-configuration. Deoxyradicinin, however, was isolated as a mixture of enantiomers. The spectral data (MS, UV, ^{1}H and ^{13}C NMR) of deoxyradicinol (4), which also occurred in small amounts (*ca* 3 mg/l), were identical with those of the major reduction product (4) obtained upon sodium borohydride reduction of deoxyradicinin (2) [4]. Previously the reduction of this compound, when performed in ethanol at ambient temperature, gave as major products deoxyradicinol (4) and its epimer (3) in a 4:1 ratio. When the reaction was performed at lower temperature using ethyl acetate as solvent [10], deoxyradicinol became an even more predominant component (ratio of 4 to 3 was *ca* 10:1). This supports the proposed *cis* relationship between the C-5 methyl and C-3 hydroxyl groups of 4 since attack of the metal hydride from the less hindered side is expected to predominate [4]. Further support was found in the ^{13}C NMR spectra of 4 and its epimer 3. The chemical shift differences between C-3, C-4 and C-5 in 4 and 3 (2.4, 0.2, and 3.1 ppm, respectively) were similar to corresponding differences between *cis* and *trans* 3-methylcyclohexanol (4.0, 2.8, and 5.1 ppm for C-1, C-2 and C-3, respectively) [11]. The optical activity ($[\alpha]_D^{20}$ + 134.6°) of natural 4 was considerably higher than that of synthetic deoxyradicinol (4) ($[\alpha]_D^{20}$ + 9.2°) indicating an enantiomeric ratio (3*R*,5*S*:3*S*,5*R*) of 54:46 for the latter. The optical purity of the starting material, deoxyradicinin (2), was examined by ^{1}H NMR in the presence of (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol [8, 9] as chiral solvating agent. This revealed an enantiomeric ratio (5*S*:5*R*) of 56:44. A second sample of 2 isolated on a different occasion was determined to possess an enantiomeric ratio (5*S*:5*R*) of 66:34 by the same technique. This variation in enantiomeric distribution suggests an acyclic precursor (6) which could undergo competitive enzymatic and non-enzymatic intramolecular Michael-type addition to give deoxyradicinin (2). The net result is a product 2 whose optical purity is determined by the extent of the enzymatic reaction. So far, attempts to isolate 6 have not been successful. The agreement in sign of the optical activities

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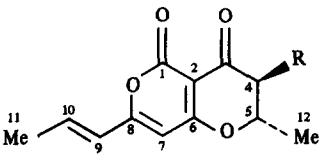

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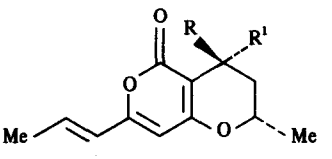
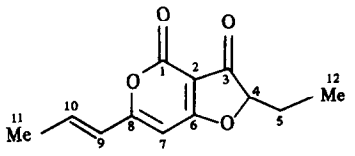
2 R = H



3 R = OH, R¹ = H
4 R = H, R¹ = OH



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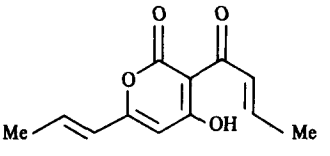

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of natural and synthetic deoxyradicinol (**4**) reveals that **4** and the more abundant enantiomer of deoxyradicinin possess the *S* configuration at C-5. It is conceivable that the two enantiomers of deoxyradicinin (**2**) may possess quite different biological activities. If one enantiomer possessed significantly less phytotoxic activity than the other, discrepancies in the results of bioassays performed using different samples of deoxyradicinin might well result.

Small amounts of a fifth metabolite, designated radianthin, $C_{12}H_{12}O_4$ [*m/z* 220.0734], exhibited a UV spectrum whose pattern was similar to that of deoxyradicinin (**2**), but with a hypsochromic shift of 10 nm in its λ_{max} (330 nm), diagnostic of a change in ring-size from a six- to five-membered enone system [12]. Radianthin revealed the characteristic set of ^{1}H NMR signals of the (2*E*)-2-butenylidyne moiety of the radicinin-derived metabolites. The presence of this moiety, and a CH_3–CH_2–CH–O– moiety was substantiated by spin–spin decoupling experiments. These features, together with a one-proton singlet at δ6.07, typical of H-7 in radicinin (**1**) and related compounds [3, 5, 7], indicated **5** as the structure for radianthin. This structure was supported by signals at δ8.6 (C-12), 18.9 (C-11), 24.5 (C-5), 96.8 (C-7), 123.0 (C-9), 141.8 (C-10) in its ^{13}C NMR spectrum. Due to shortage of material the remaining resonances (long relaxation times) could not be determined with certainty. The base peak in the mass spectrum of **5** at *m/z* 192.0431 $[M-C_2H_4]^+$ may be rationalized as loss of ethylene from the C_2 side-chain in a McLafferty rearrangement.

When droplets (10 μl) containing from 1–10 μg of radianthin were applied to adaxial leaf surfaces of sunflower, dark brown necrotic spots became evident within 24 hours of treatment. An investigation of the relative toxicities of compounds **1–5** employing a more refined and sensitive bioassay technique is in progress.

EXPERIMENTAL

^{1}H and ^{13}C NMR: 300 and 75.4 MHz, respectively, $CDCl_3$; UV: MeOH; EIMS: direct inlet probe at 70 eV; CIMS: isobutane.

Fungal culture. A. helianthi isolate 'C', kindly provided by Dr. N. V. Rama Raje Urs, Dahlgren & Co., Crookston, MN, was maintained on V-8 juice agar. Routinely liquid shake culture was performed in modified Czapek-Dox broth [6]. Batches (300 ml each) in 1 l. conical flasks were inoculated with homogenized mycelial suspension (3 ml) and incubated at 28° on a gyratory shaker operating at 170 rpm for 15 days.

Isolation of toxins **1–4**. Culture filtrates were extracted with $CHCl_3$ as previously described [3]. TLC of $CHCl_3$ extracts (silica gel, $CHCl_3$–MeOH, 50:1) afforded **1** and **2**, R_f 0.34 and 0.24. Similarly TLC (silica gel, Et_2O) afforded **3** and **4**, R_f 0.39 and 0.48. Yields of **1**, **2**, **3** and **4** obtained per l. of culture filtrate were *ca* 3, 40, 10 and 3 mg, respectively. All four compounds were visualized as quenching bands under short λ UV light. The sensitivity of visualization was increased, however, after spraying chromatograms with 50% aq. H_2SO_4 followed by gentle heating to give long λ UV blue fluorescence of **1**, **2** and **5** and yellow fluorescence of **3** and **4**. Compound **3** was further purified by HPLC, reversed phase C18 Radialpak column (Waters), H_2O–MeCN, 9:5; 1.4 ml/min, detection 225 nm, retention time 4.0 min.

Isolation of radianthin (**5**). $CHCl_3$ extracts of *A. helianthi* culture filtrate (1 l.) were subjected to preparative HPLC: reversed phase C_{18} μBondapak (7.8 × 300 mm, Waters), H_2O–MeCN, 9:5; 2.2 ml/min, detection 225 nm, *ca* 4 mg per injection volume of 75 μl. This gave **5** (*ca* 3 mg), R_t 19.9 min, together with **3**, **1**, **2**, and **4**, R_ts 8.6, 11.4, 13.4 and 16.8 min, respectively.

Radicinin (**1**). ^{1}H NMR and MS of **1** were in agreement with those of authentic radicinin *ex A. chrysanthemi* and published data [5]; **1** also co-chromatographed with authentic radicinin.

Deoxyradicinin (**2**). $[\alpha]_D^{20}$ + 4.8° ($CHCl_3$; *c* 0.42); ^{13}C NMR: see Table 1. Other spectral and physical properties have been reported previously [3]. The sample had an enantiomeric ratio of 56:44 (see Results).

3-Epideoxyradicinol (**3**). $[\alpha]_D^{20}$ − 84.7° ($CHCl_3$; *c* 1.2); ^{13}C NMR: see Table 1. Other spectral and physical properties have been published earlier [4].

Deoxyradicinol (**4**). MS and UV as previously reported for **3** and **4** [4]. $[\alpha]_D^{20}$ + 134.6° ($CHCl_3$; *c* 0.37); ^{1}H NMR: δ1.44 (3H, *d*, *J* = 6.5 Hz, H-12), 1.78 (1H, *m*, *J* = 9.7, 11.3 and 13.7 Hz, H-4). 1.89 (3H, *dd*, *J* = 1.6 and 6.9 Hz, H-11), 2.31 (1H, *m*, *J* = 2.0, 6.8 and 13.7 Hz, H-4), 4.28 (1H, *m*, *J* = 2.0, 6.4 and 11.3 Hz, H-5), 4.45 (OH), 4.84 (1H, *dd*, *J* = 6.7 and 9.7 Hz, H-3), 5.73 (1H, *s*, H-7), 5.94 (1H, *dd*, *J* = 1.7 and 15.6 Hz, H-9), 6.69 (1H, *dq*, *J* = 7.0 and 15.5 Hz, H-10); ^{13}C NMR: see Table 1.

Correlation of deoxyradicinol (**4**) *and deoxyradicinin* (**2**). $NaBH_4$ (3.3 mg) was added to a chilled (0°) soln of de-

Table 1. ^{13}C NMR data of deoxyradicinol (4), 3-epideoxyradicinol (3) and deoxyradicinin (2).

C*	4†	3†	2
1	164.2	164.4	157.2
2	102.4	102.0	100.1
3	61.2	58.8	186.4
4	36.5	36.3	43.7
5	73.7	70.6	76.5
6	165.6	166.1	175.9
7	99.2	99.2	98.1
8	158.3	158.4	163.4
9	122.6	122.7	122.6
10	135.1	135.0	139.9
11	18.4‡	18.4‡	18.6
12	20.7‡	20.3‡	20.3

*The assignments are based on selective ^1H decoupling experiments (for 2), the multiplicities observed in the off-resonance ^1H decoupled spectra of 2 and synthetic 4, ^{13}C-labelling experiments (3 and 4), and by analogy with the assignments of radicinin (1) [13]. Some of the assignments of 2 given in [3] have been revised.

†Isolated from *A. helianthi* grown in the presence of a 1:1 mixture of $^{13}CH_3COONa$ and $CH_3{}^{13}COONa$, both 99% enriched.

‡Assignments may be reversed.

oxyradicinin (2), (13 mg, enantiomeric ratio 56:44, see above) in EtOAc (10 ml). The reaction mixture was stirred and the reduction monitored by TLC. Additional $NaBH_4$ (6.6 mg) was added after 13 min. The reduction was complete after a total of 17 min. The mixture was transferred to a separatory funnel containing H_2O (4 ml) and the product extracted with EtOAc (40 + 20 ml). The extract was dried over $MgSO_4$ concentrated *in vacuo*, and the resulting gum subjected to prep TLC (silica gel, Et_2O–hexane, 3:1) to afford one major and one minor band, R_f 0.6 and 0.4, respectively. Deoxyradicinol (4, 6.5 mg, 50%) was obtained from the major band as a colourless gum. UV and MS: as for lit. [4]; $[\alpha]_D$ +9.2° ($CHCl_3$; *c* 0.47); ^1H NMR as above for natural 4; ^{13}C NMR: see Table 1. The epimeric alcohol 3 was a minor component of the reaction. The 10:1 ratio of the reduction products 4 and 3 was determined by ^1H NMR.

Radianthin (5). 1.6 mg, solidified on standing at +5°. $[\alpha]_D^{20}$ 0° ($CHCl_3$; *c* 0.15); TLC (silica gel, Et_2O) R_f 0.85; UV λ_{max} nm: 216, 232, 268, 278 and 330; EIMS, *m/z* (rel. int.): 220.0734 [calc. for $C_{12}H_{12}O_4$: 220.0736] (26), 192.0431 [calc. for $C_{10}H_8O_4$: 192.0432] (95), 131 (25), 119 (29), 69 (100); CIMS, *m/z* (rel. int.): 221 (87), 192 (100), 149 (60), 69 (70); ^1H NMR: δ1.04 (3H, *t*, *J* = 7.5 Hz, H-12), 1.83–1.95 (1H, seven lines separated by *ca* 7.7 Hz, H-5), 1.99 (3H, *dd*, *J* = 1.8 and 6.9 Hz, H-11), 2.05–2.15 (1H, *m*, H-5), 4.69 (1H, *dd*, *J* = 4.4 and 7.3 Hz, H-4), 6.09 (1H, *s*, H-7), 6.14 (1H, *d* with broad lines, *J* = 16.4 Hz, H-9), 7.04 (1H, *dq*, *J* = 6.9 and 15.5 Hz, H-10). Irradiations at δ1.99, 6.14, 7.04 and δ1.04, 1.88, 2.11, 4.69, respectively, established the presence of (2*E*)-2-butenylidyne and propylidene moieties which were not mutually spin-spin coupled. ^{13}C NMR: δ8.6 (C-12), 18.9 (C-11), 24.5 (C-5), 96.8 (C-7), 123.0 (C-9), 141.8 (C-10); shortage of material prevented determination of the remaining resonances with certainty.

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