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3-EPIDEOXYRADICINOL AND THE BIOSYNTHESIS OF DEOXYRADICININ

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Key Word Index—*Alternaria helianthi*; Hyphomycetes; *Helianthus annuus*; Compositae; deoxyradicinol; deoxyradicinin; phytotoxin; sunflower; biosynthesis.

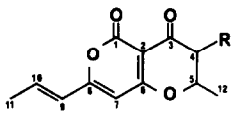
Abstract—A novel phytotoxic compound, in addition to the known phytotoxin deoxyradicinin, has been isolated from *Alternaria helianthi* and its structure elucidated as 3-epideoxyradicinol. Its epimer is also briefly described. The polyacetate biosynthetic origin of deoxyradicinin has been demonstrated.

INTRODUCTION

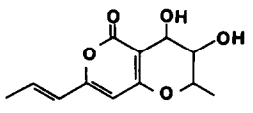
One of the most important diseases of sunflower [*Helianthus annuus* L. (Compositae)], an extremely important source of edible vegetable oil, is that caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara (seedling blight and leaf spot) [1, 2]. In a recent publication [3] we described the production of a major phytotoxic metabolite by this pathogenic fungus when grown in liquid culture and its identification as deoxyradicinin (**1**). Production of the congener, radicinin (**2**) together with its reduction product radicinol (**3**) by the closely related species *A. chrysanthemi* has also been described recently [4]. Here we report the occurrence of a second phytotoxic component of *A. helianthi* culture filtrates and its identification as the novel metabolite 3-epideoxyradicinol (**4**).

RESULTS AND DISCUSSION

Culture filtrates of *A. helianthi* were obtained as for *A. chrysanthemi* [4] and the known phytotoxin **1** was readily crystallized from chloroform extracts of this liquid as previously described [3]. TLC (silica gel, *n*-hexane–Me_2CO, 1:1) of the resultant mother liquor, or of chloroform extracts of mature culture filtrate, resulted in the isolation of a short wavelength UV-quenching component (R_f 0.42). This was further purified and subsequently identified as the novel phytotoxin 3-epideoxyradicinol (**4**).



1 R = H
2 R = OH



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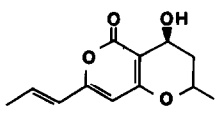

4

High resolution mass spectrometry of **4** (M^+ = 222.0889) gave its molecular formula as $C_{12}H_{14}O_4$, thus it has two additional hydrogens as compared with deoxyradicinin (**1**). The UV spectrum of **4** was almost indistinguishable from that of radicinol (**3**) recorded under identical conditions. The ^1H NMR spectrum was also entirely consistent with structure **4**, the proton at C-3 giving rise to a doublet of doublets at δ4.70. Confirmation of the identification of the metabolite as 3-epideoxyradicinol was obtained by its successful derivation from **1**. Upon reduction of **1** with sodium borohydride in ethanol two major reaction products were formed which were readily separable by TLC. The less abundant of these was identical with **4** obtained from *A. helianthi*. The other product was identified as deoxyradicinol on the basis of its MS and UV spectral data which was identical to that of **4** within the limits of experimental error. Analogous reaction products have been observed when radicinin **1** is treated with sodium borohydride [5]. Molecular models indicate (if we assume the C-5 stereochemistry of radicinin [4]) the conformation of the 2H-4-pyrone ring of deoxyradicinin to be as depicted in (a), and that the major reduction product would possess a 3α-hydroxyl on the basis of the relative ease with which borohydride would attack the unhindered β-surface. The less abundant epimer would then have the stereochemical configuration at C-3 as given for **4**. Supporting evidence for this is provided by the ^1H NMR data. The combined features of an unfavourable steric interaction and a favourable H-bonding forces the 3α epimer into conformer (b). Then 3α-hydroxyl is indicated for the more abundant product from the relatively large coupling constants for $J_{3\beta,4\beta}$ (7 Hz) and $J_{3\beta,4\alpha}$ (10 Hz) (cf. $J_{3\alpha,4\beta} = J_{3\alpha,4\alpha} = 2$ Hz for the natural product). The conformation in both compounds is the same as revealed by the identical pattern of coupling between the C-5 proton and the C-4 methylene protons in both C-3 epimers. It is of interest to note that the proposed stereochemistry for **4** at C-3 is contrary to that assigned to radicinol [5].

3-Epideoxyradicinol (**4**) was a product of all five isolates of *A. helianthi* previously examined for the production of **1** [3] and was also produced by one additional isolate

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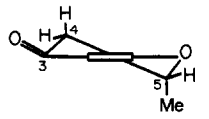
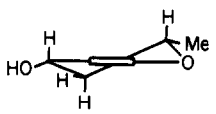


(a)



(b)

obtained from Australia. In each case **4** was considerably less abundant than its oxidized derivative (**1**), yields obtained from three–four week cultures being *ca* 10 mg/l of culture filtrate. A third (minor, unidentified) phytotoxic metabolite was also detected in culture filtrates of *A. helianthi*, as a short wavelength UV-quenching band after TLC of chloroform extracts (silica gel, $CHCl_3$–EtOH, 75:4, R_f 0.80). The UV absorption spectrum of this compound was similar to those of **1** and **2** (λ_{max} 343 nm) suggesting that it too may possess a radicinin-like structure, while at the same time indicating its non-identity with a putative toxic metabolite of *A. helianthi* reported by Bhaskaran and Kandaswamy (λ_{max} 260–265 nm) [6].

Specific labelling of deoxyradicinin with either Na acetate-1-^{13}C or -2-^{13}C and subsequent ^{13}C NMR analysis of the two differentially labeled samples of toxin confirmed the polyacetate origin of **1** as previously postulated for this metabolite [3]. Thus, in the ^{13}C NMR spectrum of **1** obtained from sodium acetate-1-^{13}C signals assigned to carbons 1, 3, 5, 6, 8 and 10 are enhanced and become evident while no peaks due to natural abundance are discernible. Similarly in the case of **1** which incorporated sodium acetate-2-^{13}C the ^{13}C NMR spectrum showed signals attributable to alternate carbon atoms, 2, 4, 9, 11 and 12. However, the signal at δ100.3 assigned to C-7 [3] was not discernible. This is presumably due to the very weak signal attributable to C-7 recorded in the natural abundance ^{13}C NMR spectrum of **1** [unpublished result].

The production of deoxyradicinol as well as deoxyradicinin by *A. helianthi* is of chemical, taxonomic and phytopathological interest in that an analogous situation exists in the morphologically indistinguishable [7] and pathologically similar species *A. chrysanthemi* [1] which produces radicinol in addition to radicinin [4]. However, it is readily apparent that the amounts of **1** and **4** produced by *A. helianthi* are at least one order of magnitude less than the (exceptionally high) yields of *A. chrysanthemi* metabolites **2** and **3**, when the two fungi are cultured under the same conditions [3, 4].

Application of deoxyradicinol (10 μg) over needle punctures on sunflower leaves (cv. Polestar) resulted in small necrotic spots within 24 hr; these later enlarged and became surrounded by chlorotic halos. These symptoms are typical of the leaf spot phase of the disease of *H. annuus* caused by *A. helianthi*. **1**, but not **4**, was identified as a constituent of *A. helianthi*-induced sunflower leaf lesions. Details of the occurrence of deoxyradicinin as a vivotoxin together with other biological aspects of **1** and **4** will be reported elsewhere.

EXPERIMENTAL

Fungal cultures and isolation of toxin **4**. Five of the isolates of *A. helianthi* used were from sources given previously [3]. The sixth isolate was acquired from Dr. J. K. Kochman, Department of Primary Industries, Toowoomba, Australia. Cultures were maintained on potato dextrose agar or V-8 juice agar and cultured in modified Czapek-Dox liquid medium as previously described [4]. TLC (silica gel, *n*-hexane–Me_2CO, 1:1) of $CHCl_3$ extracts of culture filtrate gave **4**, R_f 0.42, further purified by TLC in $CHCl_3$–EtOH, 75:4 R_f 0.33 or in *n*-pentane–Et_2O–HOAc, 10:60:2, R_f 0.30.

3-Epideoxyradicinol (**4**). Colourless crystals from EtOH, decomp. < 100°. λ_{max}^{EtOH} nm (log ε): 225 (4.48), 262 (3.47), 272 (3.50), 318 (3.97); cf. authentic **2** (from *A. chrysanthemi* [4]) λ_{max}^{EtOH} nm (log ε): 225 (4.51), 262 (3.38), 271 (3.40), 319 (4.04). $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2360, 1700, 1580, 1218. EIMS (solid probe) 65 eV, *m/z* (rel. int.): 222 $[M]^+$ (41), 221 (21), 205 (18), 189 (20), 152 (100), 137 (8), 111 (22). ^{1}H NMR (250 MHz, $CDCl_3$, TMS as int. standard): δ6.67 *dq* (*J* = 7, 15 Hz, H-10), 5.94 *dq* (*J* = 2, 15 Hz, H-9), 5.73 *s* (H-7), 4.70 *dd* (*J* = 2, 4 Hz, H-3), 4.36 *m* (H-5), 2.04–2.10 *dt* (*J* = 2, 12 Hz, H-4β), 1.88 *dd* (*J* = 2, 7 Hz, H-11), 1.60–1.72 *m* (H-4α), 1.41 *d* (*J* = 6 Hz, H-12).

Reduction of deoxyradicinin (**1**). Compound **1** (25 mg) in EtOH (7.5 ml) was treated with $NaBH_4$ (5 mg) under N_2 in darkness for 3 hr. Solvent was removed and the EtOH soluble residue subjected to TLC (silica gel, *n*-hexane–Me_2CO, 2:1) to afford 3-epideoxyradicinol **4** (1 mg) at R_f 0.27 together with its epimer (4 mg) at R_f 0.38. Deoxyradicinol dried as a colourless gum; UV and MS as for **4**. $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3000, 1695, 1578, 1218. ^{1}H NMR: δ6.64 *dq* (*J* = 7, 15 Hz, H-10), 5.90 *dq* (*J* = 2, 15 Hz, H-9), 5.70 *s* (H-7), 4.79 *dd* (*J* = 7, 10 Hz, H-3), 4.25 *m* (H-5), 2.30 *m* (*J* = 2, 7, 12 Hz, H-4β), 1.85 *dd* (*J* = 2, 7 Hz, H-11), 1.57–1.80 *m* (H-4α), 1.39 *d* (*J* = 6 Hz, H-12).

Biosynthesis of **1**. Two 2 l. flasks containing 1 l. of modified Czapek-Dox liquid medium were inoculated with *A. helianthi* and the fungus cultured under the conditions previously decribed [4] for 14 days. NaOAc-1-^{13}C (1 g) was aseptically added to one culture vessel; the other flask received 1 g of NaOAc-2-^{13}C. After incubation for a further 14 days crystalline **1** was prepared from each culture as previously described [3] and each sample subjected separately to ^{13}C NMR analysis (250 MHz, $CDCl_3$, TMS as int. standard).

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