concd. It showed D-glucose (PC; *n*-BuOH–AcOH–H_2O, 4 : 1 : 5; R_f 0.16).

Permethylate of saponin **1**. Compound **1** (1 g) was permethylated to give a crude product (**2**) which was purified by CC (C_6H_6–EtOAc, 1:1) and crystallized from MeOH as colourless silky needles, mp 177–179°, $[\alpha]_D^{20} - 75.0°$ ($CHCl_3$, $c = 1.0$); IR ν_{max}^{KBr} cm^{-1}: no OH; EIMS (probe) 70 eV, *m/z* (rel. int.): 868 $[M]^+$ (6), 633 (12), 414 (7), 400 (10), 399 (15), 398 (75), 253 (6), 219 (20), 187 (100). (Found: C, 64.80; H, 9.15. $C_{47}H_{80}O_{14}$ requires: C, 64.97; H, 9.21%).

Hydrolysis of **2**. Compound **2** (150 mg) was hydrolysed with 7% H_2SO_4 as usual, the ppt was filtered, crystallized with $CHCl_3$ and identified as chlorogenin. The aq. hydrolysate was neutralized (Ag_2CO_3), filtered and concentrated to provide 2, 3, 4, 6-tetra-*O*-methyl-D-glucose (PC; *n*-BuOH–EtOH–H_2O, 5 : 1 : 4; R_G 1.0, authentic sample run in parallel).

Methanolysis of **2**. Compound **2** (150 mg) in NHCl–MeOH (25 ml) was refluxed for 4 hr, neutralized (Ag_2CO_3), filtered and the filtrate was concentrated and purified by CC (C_6H_6–Me_2CO, 10 : 1) to give methyl-2, 3, 4, 6-tetra-*O*-methyl-D-glucopyranoside (10 mg): EIMS (probe) 70 eV, *m/z* (rel. int.): 219 (0.04), 205 (0.08), 187 (0.35), 175 (3.1), 149 (7.0), 145 (1.8), 131 (2.5), 127 (9.0), 104 (45), 88 (100), 75 (25), 73 (10), 71 (10), 45 (20).

Enzymatic hydrolysis of **1**. Compound **1** (50 mg) in H_2O (10 ml) was incubated with β-glucosidase (Sigma) at 37° for 7 hr. The hydrolysate was filtered. The ppt. was identified as chlorogenin. The aq. phase contained D-glucose only (PC, as above).

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DEOXYRADICININ, A NOVEL PHYTOTOXIN FROM *ALTERNARIA HELIANTHI*

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Key Word Index—*Alternaria helianthi*; Dematiaceae; *Helianthus annuus*; Compositae; sunflower; phytotoxin; deoxyradicinin.

Abstract—A novel major metabolite which possesses phytotoxic and antifungal properties has been isolated from culture filtrates of *Alternaria helianthi* and its structure elucidated as deoxyradicinin.

INTRODUCTION

Alternaria helianthi (Hansf.) Tubaki and Nishihara (Dematiaceae) is a phytopathogenic fungus causing seedling blight and leaf spot of sunflower [*Helianthus annuus* L. (Compositae)] [1], a crop plant of considerable economic importance [2]. In a previous paper the isolation and identification of the phytotoxic pyranopyrone radicinin from *A. chrysanthemi*, a pathogen of *Leucanthemum maximum* (Ram.) DC. (shasta daisy), was described [3]. *A. helianthi* and *A. chrysanthemi* are reported to be morphologically indistinguishable [4] and are pathologically similar in that both species

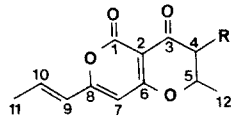
Table 1. UV and IR spectral data of 1 and 2

	1	2
UV λ_{max}^{EtOH}, nm (log ϵ)	222(4.21)	221(4.31)
	269(3.72)	269(3.72)
	281sh(3.62)	280sh(3.62)
	343(4.25)	343(4.33)
IR ν_{max}^{KBr}, cm^{-1}	—	3455(OH)
	3083(=CH)	3090
	1761(C=O)	1760
	1666(C=O)	1656
	1615(C=C)	1599
	1526	1516

are pathogenic to Japanese chrysanthemum, though only *A. helianthi* will infect sunflowers [1]. A causal role for radicinin in the pathogenicity of *A. chrysanthemi* has already been postulated [3]. Therefore we considered that it was worthwhile to determine whether *A. helianthi* resembled *A. chrysanthemi* in the character of radicinin production.

RESULTS AND DISCUSSION

Isolates of *A. helianthi* were cultured under the conditions previously described for *A. chrysanthemi* [3]. The resulting culture filtrates of this fungus contained a novel phytotoxic compound which was isolated and characterized as an analogue of radicinin. It is assigned structure **1** on the basis of the data presented below. The UV spectrum of **1** was virtually identical with that of radicinin (**2**) [3], indicating a close structural relationship and an identical chromophore for the two compounds (Table 1). High resolution mass spectrometry (M^+ 220.0736) gave the molecular formula of **1** as $C_{12}H_{12}O_4$, which is equivalent to one oxygen atom less than is present in the radicinin molecule. In contrast to radicinin, **1** did not form an acetate upon treatment with acetic anhydride in pyridine, strongly suggesting that the hydroxyl function of radicinin was absent from the novel analogue. This was confirmed by an examination of the IR spectra of the two metabolites, an absorbance peak due to hydroxyl stretching present in the spectrum of radicinin at 3455 cm^{-1} being absent from that of **1** (Table 1). Identification of the novel phytotoxin as 4-deoxyradicinin (**1**) was confirmed by NMR spectroscopy. Thus chemical shifts in both ^1H and ^{13}C NMR spectra of **1** and **2** were in close agreement, with the accountable differences outlined below. In the ^1H NMR spectrum of radicinin a one-



1 R=H
2 R=OH
3 R=OAc

Table 2. NMR chemical shifts of deoxyradicinin (**1**), radicinin (**2**) and acetylradicinin (**3**)*

	^1H NMR			^{13}C NMR	
	1	2	3	1	2
1	—	—	—	157.1	156.0†
2	—	—	—	98.0	97.8†
3	—	—	—	186.2	188.9
4	2.65 *m*	3.99 *d*	5.22 *d*	43.9	72.3
5	4.74 *ddq*	4.36 *dq*	4.70 *dq*	76.5	80.3
6	—	—	—	175.9	176.7
7	5.84 *s*	5.86 *s*	5.83 *s*	100.3	98.2
8	—	—	—	163.7	164.7
9	6.02 *d*	6.05 *d*	6.02*d*	122.9	122.9
10	6.90 *dq*	6.97 *dq*	6.94 *dq*	139.9	141.3
11	1.95 *d*	1.97 *d*	1.94 *d*	20.2	18.9
12	1.54 *d*	1.66 *d*	1.53 *d*	18.5	18.3
OH	—	3.85 *s*	—	—	—
OAc	—	—	2.19 *s*	—	—

*δ values (TMS reference) in $CDCl_3$.
†Lit. values [5].

proton doublet centred at δ 3.99 and assigned to H-4 moved downfield to δ 5.22 in acetylradicinin (**3**). In contrast, the ^1H NMR spectrum of **1** showed a complex multiplet (centred) at δ 2.65 as the AB part of an ABX system. These signals, which jointly integrated as two protons, are readily assignable to the two non-equivalent protons at C-4 of deoxyradicinin. Similarly, in the ^{13}C NMR spectrum of radicinin the signal at *ca* δ 72 attributable to C-4 [5] moved upfield to 43.9 in the spectrum of deoxyradicinin (Table 2).

All five isolates of *A. helianthi* examined in this study produced deoxyradicinin and in each case it was the major constituent (*ca* 40% by wt) of chloroform extracts of culture filtrates harvested at age 18 days. Yields of deoxyradicinin obtained from the various isolates were broadly similar being of the order of 60 mg/l., an order of magnitude less than the yield of radicinin obtained from *A. chrysanthemi* [3]. It should also be noted, however, that mycelial growth of *A. helianthi* under the cultural conditions utilized was relatively meagre (*ca* 0.4 g dry wt/l. of mature culture medium). In a preliminary bioassay against two members of the Compositae, namely *Cirsium arvense* (L.) Scop. (Canada thistle) and *H. annuus* (cv Giant Gray Stripe), deoxyradicinin (330 ppm) expressed phytotoxicity in the forms of necrosis and loss of turgor in both species tested within 24 hr of treatment. However, the antibiotic activity of deoxyradicinin is not restricted to phytotoxicity since, like its oxygenated derivative **2**, **1** also suppresses fungal growth (*Cladosporium* sp.) in the standard TLC bioassay of Homans and Fuchs [6]. More detailed studies on some of the biological effects of deoxyradicinin are currently in progress. The results of an investigation of the relative sensitivity of the suscepts of the two pathogens to deoxyradicinin and its C-4 hydroxylation product may be of particular interest from the phytopathological viewpoint. The oxygenation pattern of **1** is consistent with a polyacetate origin for this metabolite,

as has already been demonstrated for radicinin [7], with the oxygenation of 1 representing a terminal stage in the biosynthesis of radicinin. However, the latter compound was not detected in *A. helianthi* culture filtrates harvested after 18 days nor in the filtrate of cultures incubated for an additional 2 days, although a second metabolite, which is presumed to be a derivative of 1, was present in 20-day-old culture filtrates in relatively small amounts. The identity of this compound will be investigated in due course.

EXPERIMENTAL

Fungal isolates and cultures. Isolates of *A. helianthi* were kindly supplied by Dr. P. E. Lipps, Ohio Agricultural Research and Development Centre (three isolates) and by Dr. N. V. Rama Raje Urs, Dahlgren & Co., Crookston, MN (two isolates). Cultures were maintained on potato dextrose agar and cultured as described previously [3], routinely for a period of 18 days.

Isolation of deoxyradicinin. Mature culture filtrate was extracted with $CHCl_3$ (3 × 1/3 vol.) and the $CHCl_3$ phase dried in vacuo at 40°. The residue was washed with a min. vol. of C_6H_6 to remove pigment(s) and dissolved in hot 95% EtOH; deoxyradicinin crystallized on cooling and evapn of solvent, mp 178–181°. Recrystallization from MeOH gave colourless prisms, mp 183–185°. Yields of deoxyradicinin in culture filtrates were determined by UV absorption of 1 (log ε, 343 nm = 4.25) purified by TLC (Si gel, $CHCl_3$–EtOH, 75:4) of a portion of the $CHCl_3$ extract. Deoxyradicinin was detected as a short UV λ quenching band, R_f 0.47.

Deoxyradicinin (1) UV, IR and NMR, (see Tables 1 and 2). EIMS (solid probe) 65 eV, *m/z* (rel. int.): 220 $[M]^+$ (36), 205 $[M-Me]^+$ (7), 179 (11), 178 (38), 110 (71), 69 (100).

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