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TRANS–TRANS-3,11-TRIDECADIENE-5,7,9-TRIYNE-1,2-DIOL, AN ANTIFUNGAL POLYACETYLENE FROM DISEASED SAFFLOWER *(CARTHAMUS TINCTORIUS)*

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Abstract—Safflower plants (*Carthamus tinctorius* L.) were wound-inoculated with *Phytophthora drechsleri* Tucker, a fungus which incites a root and stem-rot disease of cultivated safflower. Extracts of diseased hypocotyls were highly toxic to the linear growth of the pathogen. The major toxic compound was isolated from the extracts and identified with the aid of u.v., i.r. and mass spectroscopy as *trans–trans*-3,11-tridecadiene-5,7,9-triyne-1,2-diol.

INTRODUCTION

A ROOT and stem rot incited by *Phytophthora drechsleri* Tucker is one of the major diseases of cultivated safflower (*Carthamus tinctorius* L.)[1,2] The development of varieties with moderate root rot resistance and desirable agronomic characteristics has been accomplished,[3] but higher levels of resistance are needed for heavy, flood-irrigated soils.

The highest known level of resistance to *P. drechsleri* was found in a safflower introduction selected at Biggs, California.[4] In a study of the nature of disease resistance in the Biggs selection,[5] the extracts of hypocotyls infected with *P. drechsleri* were highly fungitoxic to the pathogen as compared with the antifungal activities of extracts from non-infected hypocotyls. The identification of the major antifungal compound in these extracts is reported here.

RESULTS AND DISCUSSION

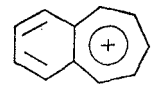
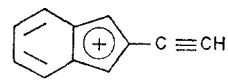
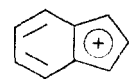
The u.v. spectrum with λ_{max} (ϵ) at 354 (19,000), 330 (29,200), 309 (23,000), 290 (13,600), 269 (61,600), *255* (75,000), *246* (73,200), *235* (51,200), *225 (35,800)* and 215 nm (34,300) indicated the antifungal substance was a polyacetylene with an ene-triyne-ene chromophore.[6] The i.r. spectrum indicated the presence of aliphatic hydroxyl (3600, 3430, 1050 cm^{-1}), *trans* ethylenic linkage (1620, 946 cm^{-1}) and asymetrically disubstituted acetylenic linkage (2190, 2170 cm^{-1}). Absorption characteristic of *cis* ethylenic linkage (690 cm^{-1}) was not observed.

The mass spectrum indicated the antifungal substance was a single compound. High resolution mass measurements showed that the compound had an empirical formula of $C_{13}H_{12}O_2$ (Table 1). A peak in the mass spectrum at *m/e* 182 indicated the loss of water to give an ion of low intensity. The peak at *m/e* 169 showed the loss of $-CH_2OH$ to give an

[1] P. F. KNOWLES, *Advan. Agron.* 10, 289 (1958).
[2] D. C. ERWIN, *Phytoputhol. 42, 32* (1952).
[3] C. A. THOMAS, D. D. RUBIS and D. S. BLACK, *Phytopatthol. 50,* 129 (1960).
[4] C. A. THOMAS and J. M. KLISIEWICZ, *Phytopathol. 53,368* (1963).
[5] C. A. THOMAS and E. H. ALLEN, *Phytopathol.* 60,261 (1970).
[6] F. BOHLMANN, H. BORNOWSKI and C. ARNDT, *Fortschr. Chem. Forsch. 4,* 138 (1962).

oxonium ion (Table 1).[7] The peak at *m/e* 139 indicated the loss of $-CH(OH)CH_2OH$ and a metastable peak was observed for this process. Aplin and Safe[8] reported that polyacetylenes containing either the ene-diyne-diene or triyne-diene chromophores have common fragments in mass spectrometry. The ion intensities of several peaks in the mass spectrum for all *trans*-2,4,10-dodecatriene-6,8-diyne-1-ol (C_{12}) are very similar to the intensities for the same peaks for the antifungal compound (C_{13}), *m/e* (intensity for C_{12}; intensity for C_{13}): 141 (29;12), 115 (86;100), 89 (15;15), 63 (21;25), 51 (18;14). Aplin and Safe[8] presented evidence that ions *m/e* 141 and *m/e* 115 are, respectively, the benztropylium cation and the indenyl cation; we propose structures for ions *m/e* 141, *m/e* 139 and *m/e* 115 in the mass spectrum of the antifungal compound by analogy to the structures proposed by these authors (Table 1).

TABLE 1. PROPOSED STRUCNRES AND RELATIVE INTENSITIES OF THE MORE PROMINENT PEAKS IN THE MASS SPECTRUM OF THE ANTIFUNGAL COMPOUND FROM *Carthamus tinctorius*

Observed mass	Intensity*	Probable composition (mass)		Proposed structure?
200.0858	*28*	$C_{13}H_{12}O_2$	*(200.0837)*	$[CH_3-CH=CH-(C\equiv C)_3-CH=CH-CH(OH)CH_2OH]^+$
182.0749	**4**	$C_{13}H_{10}O$	(182·0731)	$[CH_3-CH=CH-(C\equiv C)_3-CH=CH-CH=CHOH]^+$
169.0660	**93**	$C_{12}H_9O$	**(169.0653)**	$CH_3-CH=CH-(C\equiv C)_3-CH=CH-CH=\overset{+}{O}H$
141.0713	**19**	$C_{11}H_9$	(141·0704)	
139.0556	**33**	$C_{11}H_7$	(139·0548)	
115.0555	**100**	C_9H_7	(115·0548)	

*** Relative to *m/e* 115·0555 = 100.**

† H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, p. 176, Holden-Day, San Francisco (1964); R. T. APLIN and S. SAFE, *Chem. Commun.* 140 (1967).

These data show that the antifungal compound is *trans–trans*-3,11-tridecadiene-5,7,9-triyne-1,2-diol (first structure in Table 1), a polyacetylene previously isolated from *Centaurea ruthenica* L.[9] and C. *tinctorius*.[10] Bohlmann and Herbst[11] proved the structure of this polyacetylene by synthesis and gave the following data for the synthetic compound: mol. wt. 200.2 ($C_{13}H_{12}O_2$); u.v. λ_{max} (E): 354 (20,200), 330 (27,600), 308.5 (21,000), 290 (12,200), 269 (60,000), 255 (76,000), 245 (72,700), 235.5 nm (51,500); i.r. ($CHCl_3$): -OH (3540,3400 cm^{-1}), $-C\equiv C-$ (2180,2160 cm-'), -CH====CH-(1610,945 cm^{-1}). For the

[7] **H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, p. 176, Holden-Day, San Francisco (1964).**
[8] **R. T. APLIN and S. SAFE, *Chem. Commun. 140 (1967).***
[9] **F. BOHLMANN, S. POSTULKA and J. RUHNKE, Chem. *Ber.* 91, 1642 (1958).**
[10] **F. BOHLMANN, S. KÖHN and C. ARNDT, *Chem. Ber. 99, 3433 (1966).***
[11] **F. BOHLMANN and P. HERBST, *Chem. Ber. 92,* 1319 (1959).**

naturally occurring compound, Bohlmann *et al.*[9,10] reported i.r. data ($CHCl_3$) as -OH (3610,3290,1048 cm^{-1}), $-C\equiv C-$ (2190,2170 cm^{-1}), $-CH\equiv CH-$ (1630,953 cm^{-1}) and u.v. data as λ_{max} (ϵ): 353 (19,100), 328.5 (26,800), 307.5 (20,000), 289 (10,700), 267 (61,600), 254 (76,000), 245 (70,500), 235 nm (46,400). In order to simplify referral to this antifungal polyacetylene, we propose the name safynol.

Polyacetylenes are thermally unstable and readily undergo changes in their chemical structure in the presence of light[12] and oxygen.[13] The surfaces of polyacetylene crystals rapidly become green, blue, and red.[12] Also, Bu' Lock[12] emphasizes that "crystallization, even at low temperatures, is of limited use, since it may lead to considerable losses, and must be regarded as a desirable rather than indispensable stage in purification". We found safynol to be stable if stored in the dark at — 18" at less than 200 μg/ml absolute ethanol. In air, safynol forms an alcohol soluble, ether insoluble, red resin which is not fungitoxic. Celmer and Solomons[13] have shown that the oxygen content of the red resin formed from mycomycin is higher than expected from polymerization of the parent polyacetylene. This indicates peroxide formation. Polyacetylenes may have been missed in other disease resistance studies due to their destruction in the usual methods of extraction and bioassay.

The concentration of safynol increased 20 fold in infected safflower hypocotyls.[5] Safynol inhibited the linear growth of *P. drechsleri 50* per cent at 12 μg/ml and 100 per cent at 30 μg/ml.[5] These figures are probably minimal due to loss of the antifungal compound during assay. The fungal-induced production of safynol is being determined for resistant and susceptible varieties in a breeding program to develop disease-resistant safflower. The results of these studies may help to explain a role of polyacetylenes in safflower.

A preliminary report of the present study has been made.[14] While the present paper was in press, a report appeared concerning an identified antifungal monoacetylenic compound named wyerone acid which accumulates in *Botrytis* infected broad bean.[15]

EXPERIMENTAL

Plant and Fungus Culture

Safflower plants, selection Biggs, were grown in steam-sterilized soil held by porous 20-cm clay pots in the greenhouse. *Phytophthora drechsleri,* isolate 201, was grown on lima-bean agar in Petri dishes at 27".

Inoculation

Inoculum from I-day-old cultures of the fungus was smeared into a 7-mm incision made about 25 per cent of the way through the hypocotyls of 'I-week-old plants. The inoculated area on each hypocotyl was covered with a plastic tube and the plants were held at 30" in the greenhouse.

Extraction

Four days after inoculation, hypocotyl sections were cut at the vertical edges of the necrotic lesions and extracted four times with methanol, 3 ml/g fresh tissue, by disintegration in a blender for 5 min. Tissue fragments were recovered by filtration after each extraction. The combined alcohol extracts were reduced to an alcohol free aqueous solution by distillation *in vacuo* at *28".* The aqueous solutions were extracted with peroxide-free Et_2O. The ether extracts were evaporated to near dryness *in vacuo* at *20"* and the near dry samples were maintained under N_2. All operations were performed in the dark or with as little light as necessary.

Isolation by TLC

The residues were dissolved in Et_2O and streaked on TLC plates. The adsorbant consisted of one part SilicAR (TLC-4GF, Mallinckrodt) mixed with 40 parts Kiesel-Gel (D5, A. H. Thomas). Three TLC solvents

[12] J. D. Bu' Lock, in *Progress in Organic Chemistry* (edited by J. Cook and W. Carruthers), Vol. *6,* p. *86,* Butterworths, London (1964).
[13] W. D. Celmer and I. A. Solomons, *J. Am. Chem. Soc.* 74, 2245 (1952).
[14] C. A. Thomas and E. H. Allen, *Phytopathol. 59, 1053 (1969).*
[15] R. M. Letcher, D. A. Widdowson, B. J. Deverall and J. W. Mansfield, *Phytochem. 9,249* (1970).

were used: (A) C_6H_6–EtOAc–HCO_2H, 75:24: 1, v/v; (B) $CHCl_3$–Me_2CO–HCO_2H, 95:4:1, v/v; and (C) Et_2O–petrol–HCO_2H, 80: 19: 1, v/v. The locations of compounds on the plates were revealed as bands which quenched the fluorescence of the SilicAR under u.v. (254 nm) lamp. An antifungal band at R_f 0·21 (solvent A) was eluted with Et_2O and chromatographed with solvent B. An antifungal band at R_f 0·17 (solvent B) was eluted and chromatographed with solvent C. An antifungal band at R_f 0·50 (solvent C) could not be separated by further TLC using other solvents and was saved for identification. The ether eluates were washed with small portions of water. The antifungal substance was stored in absolute ethanol in the dark at −18°.

Antifungal Assay

Silica gel from quenching as well as non-quenching bands on TLC plates was eluted with Et_2O. Eluates were washed with water and evaporated to dryness. The resulting residues were mixed with sterile, aqueous lima-bean extract, pH 5.7, and tested for toxicity to the radial growth of P. ***drechsleri*** from 5 mm lima-bean-agar discs in Petri dishes held for 24 hr at 25" in the dark.

Spectral Analyses

All spectral analyses were conducted on freshly isolated compound with as little light as necessary. The compound was dissolved in absolute ethanol for u.v. analysis and $CHCl_3$ for i.r. analysis in KBr cells. Elemental compositions of ions were determined by high-resolution measurements in a mass spectrometer with direct insertion probe (60-110" and 70 eV.).

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