

## TWO NOVEL ANTIFUNGAL ALKA-2,4-DIENALS FROM *TRITICUM AESTIVUM*

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**Key Word Index**—*Triticum aestivum*; Gramineae; wheat; dienals; isolation; structure; antifungal compounds; 4-decyl-*E*-pentadienal; 2*E*, 4*Z*-pentadecadienal.

**Abstract**—Two novel antifungal compounds have been isolated from water extracts of the shoots of *Triticum aestivum* cv Maris Huntsman, and characterized as 4-decyl-*E*-pentadienal ( $\alpha$ -triticene) and 2*E*, 4*Z*-pentadecadienal ( $\beta$ -triticene) by <sup>1</sup>H NMR and mass spectrometry.

### INTRODUCTION

Previous studies of water extracts of wheat leaves have established the presence of antifungal compounds in the form of benzoxazolinone derivatives, enzymically released from glucoside precursors on extraction[1,2]. These have been shown to be of dubious importance in the resistance of wheat to infection by fungi, e.g. *Septoria* (*Leptosphaeria*) *nodorum* L.[2,3] and their presence in the mature plant has not been detected[2]. Baker and Smith[2] found additional antifungal compounds at all stages of growth, but these were not characterized and their importance in resistance is not known.

Aliphatic 2,4-dienals have not previously been shown to exhibit antifungal activity in *T. aestivum*, their presence in plants being generally associated with flavours and aromas[4], although 2-hexenal has been shown to have a pronounced antifungal activity in other plants[5]. The present paper describes the isolation and characterization of two new antifungal compounds from water extracts of wheat as C<sub>15</sub> 2,4-dienals.

### RESULTS AND DISCUSSION

Four-week-old *T. aestivum* cv Maris Huntsman shoots were used as a source of the two antifungal compounds. Among ether-soluble compounds in an aqueous extract, two produced antifungal zones when run on TLC, and bioassayed with *Cladosporium cucumerinum*[6]. These compounds were absent from a methanol extract, this indicating enzymic release. Subsequent tests showed that the two compounds were unrelated to the benzoxazolines, giving a negative reaction with ferric chloride, whilst their reaction with *p*-phenylenediamine indicated that a carbonyl group was present.

Examination of the <sup>1</sup>H NMR spectra of the two

compounds gave the data shown in Table 1. The proton assignments for 1 and 2 were made on the basis of the selective decoupling experiments (Table 1). The 360 MHz spectra of 1 and 2 confirmed the presence of an aldehydic proton by a doublet at 89.48/9.58, whilst the magnitude of the coupling between H<sub>b</sub> and H<sub>c</sub> in each compound (15.5 and 14.0 Hz, respectively) confirmed the *trans* stereochemistry of the double bond. In 1, the resonance signals assigned to H<sub>d</sub> and H<sub>e</sub> appeared at 86.4. There was a long range coupling from H<sub>c</sub> to both H<sub>d</sub> and H<sub>e</sub> and also to the terminal methylene of the alkyl chain. The aldehydic proton showed direct coupling to proton H<sub>b</sub>. These data established the site of attachment of the alkyl chain at the C-4 position.

In 2, there was a coupling of 10 Hz between H<sub>c</sub> and H<sub>d</sub>, suggesting that they are on adjacent carbon atoms. Furthermore, H<sub>d</sub> had a 10 Hz coupling to H<sub>e</sub>, indicative of a *cis* configuration about the double bond. The aldehydic proton again showed direct coupling to proton H<sub>b</sub>. This data established the site of attachment of the alkyl chain at the C-5 position.

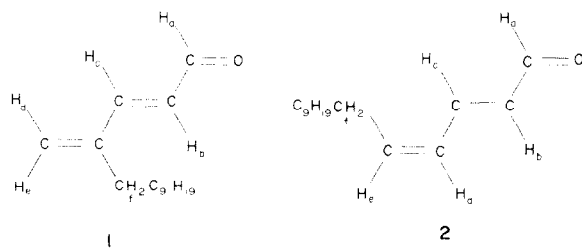
The electron impact mass spectrum of 1 exhibited a M<sup>+</sup> at *m/z* 222 (3%) which suggested 1 was a pentadecadienal, a second peak at *m/z* 81 (85%) corresponding to the 2,4-dienal component, and a typical hydrocarbon fragment series corresponding to the aliphatic C<sub>10</sub> chain. The presence of an aldehyde group was consistent with a substantial peak observed at *m/z* 29 (71%), (corresponding to the <sup>+</sup>CHO and <sup>+</sup>C<sub>2</sub>H<sub>5</sub> fragments), and confirmed by a shift in the M<sup>+</sup> peak from *m/z* 222 to 237 on prior reaction of 1 and 2 with hydroxylamine. The mass spectrum of 2 was essentially identical to that of 1, indicating that both were isomers.

Comparisons of the UV spectra in ethanol of 1 and 2 with those of the benzoxazolinones further indicated the dissimilarity of the compounds to the benzoxazolinones, whilst their UV maxima (at 276 and 278 nm, respectively) supported their mutual similarity, also shown by mass spectrometry and <sup>1</sup>H NMR.

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Table 1. 360 MHz  $^1\text{H}$  NMR proton assignments and decoupling experiments for 1 and 2

Signal	Shift/multiplicity	Coupling (Hz)	Irradiation results	Assignment
Compound 1				
1	9.48, <i>d</i>	$J_{1,2} = 8.4$	2	a
2	6.08, <i>d</i>	$J_{2,3} = 15.5$	1,3	b
3	7.29, <i>m</i>	—	2,4	c
4	6.40, <i>q</i>	—	3,5	d,e
5	2.25, <i>q</i>	—	—	f
Compound 2				
1	9.58 <i>d</i>	$J_{1,2} = 8.0$	2	a
2	6.13 <i>q</i>	$J_{2,3} = 14.0$	1,3	b
3	7.70, <i>q</i>	$J_{3,4} = 10.0$	2,4	c
4	6.32, <i>t</i>	$J_{4,5} = 10.0$	3,5	d
5	6.07, <i>q</i>	—	4,6	e
6	2.40, <i>q</i>	—	5	f



Compounds 1 and 2 are thus 4-decyl-*E*-pentadienal (4-decylpenta-2-*trans*,4-dienal), and 2*E*, 4*Z*-pentadecadienal (pentadeca-2-*trans*,4-*cis*-dienal), respectively, for which the trivial names  $\alpha$ -triticene and  $\beta$  triticene, respectively, are proposed. The importance of these compounds in the resistance of wheat to fungal attack is currently being investigated.

#### EXPERIMENTAL

Seeds of *T. aestivum* L. cv Maris Huntsman were sown in J. Arthur Bowers seed and potting compost, and grown at 19° for 28 days (lighting conditions ca 14 hr photoperiod) then harvested for extraction.

All aerial parts (9.7–10.1 kg, dependent on batch used) were homogenized by mincing the fr. tissue in a hand mincer; after standing at room temp. for 1 hr, the homogenate liquid phase (6.0–6.5 l.) was separated by expression through 4-layers of folded muslin. The solid residue was washed with sufficient MeOH to constitute 25% v/v when added to the aq. phase. Particulate matter was removed by centrifugation at 2000 g for 10 min, the supernatant collected and its polarity enhanced by addition of NaCl to give a final concn of 5% v/v. Partition was effected using serial vols of Et<sub>2</sub>O. The Et<sub>2</sub>O phase was dried (Na<sub>2</sub>SO<sub>4</sub>), then evaporated to dryness under vacuum. The residue was dissolved in CHCl<sub>3</sub> and applied to a Si gel column (10 × 2 cm i.d.). The column was first eluted with 35 ml CHCl<sub>3</sub> to remove excess pigments, then with 70 ml MeOH–CHCl<sub>3</sub> (1:9), the eluate being collected in 5 ml fractions. Samples (10  $\mu$ l) were examined by TLC on Si gel, using C<sub>6</sub>H<sub>6</sub>–HOAc–H<sub>2</sub>O (10:7:3, upper phase) as developing solvent. Location of 1 and 2 was by UV absorption at 254 nm and reaction with *p*-phenylenediamine spray reagent (2% in cold 10% TCA), which gave brown spots. For the bioassay the TLC develop-

ing solvent used was EtOH–cyclohexane (1:19) the bioassay being performed by the *Cladosporium cucumerinum* method[6]. It was found that areas inhibitory to fungal growth corresponded with those giving a brown colour with *p*-phenylenediamine.

The active fractions were combined, evaporated to dryness under vacuum and dissolved in 100  $\mu$ l CHCl<sub>3</sub>. Further fractionation was performed by HPLC, using a Partisil 5 Si column (25 cm × 7.6 mm i.d.) with EtOH–cyclohexane (3:97) as eluant, at 3 ml/min. Detection was by UV absorption at 280 nm. Fractions were manually collected corresponding to eluted peaks having *R*<sub>f</sub>s of 8.5–9 min (1), and 9.5–10 min (2), identification of 1 and 2 being by their UV spectra. Identified fractions were evaporated to dryness under vacuum, then dissolved in 100  $\mu$ l CHCl<sub>3</sub>. Fractionation was again performed by HPLC, using a Spherisorb ODS 5  $\mu$ m column (25 cm × 7.6 mm i.d.) with MeOH–NaPi pH 7, 0.1 M (1:1) as eluant, at 3 ml/min. Fractions corresponding to major peaks having *R*<sub>f</sub>s of 4–6 min, detected as above, were collected, partitioned into 3 × 1/3 their vols of Et<sub>2</sub>O and evaporated to dryness under vacuum. The dried material was dissolved in 100  $\mu$ l CHCl<sub>3</sub> and fractionated again on the Partisil 5 Si column, using the EtOH–cyclohexane system. The collected fractions were evaporated to dryness under vacuum, then dissolved in 500  $\mu$ l EtOH, before drying into vials under dry N<sub>2</sub>; 1 was a pale green liquid, 2 a pale yellow solid. Typical yields were 2.2 mg (1) and 1.5 mg (2) per kg fr. wt.

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