Phytochemistry, Vol. 25, No. 1, pp 241-243, 1986
Printed in Great Britain

0031 9422/86 $3.00 + 0.00

VOLATILES FROM WINTER WHEAT: IDENTIFICATION OF ADDITIONAL COMPOUNDS AND EFFECTS OF TISSUE SOURCE

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(*Revised received* 20 *June* 1985)

Key Word Index—*Triticum aestivum*; Gramineae; wheat; volatiles; pentadecanal; host-parasite interactions.

Abstract—Sixteen volatile compounds have been identified in oil prepared from winter wheat by reduced pressure steam distillation–extraction. Most of these compounds were found in relatively small quatities (0.5% or less) with the exception of pentadecanal. Comparisons were also made of the relative amounts of C_9 alcohols and aldehydes obtained from fresh versus frozen plants, cut versus intact plants and leaves versus culms.

INTRODUCTION

French *et al.* [1, 2] evaluated many synthetic volatiles and found that several compounds stimulate germination of fungal spores associated with wheat rust disease. As part of an investigation of the possible role of volatile compounds in wheat–fungal pathogen interactions, we have isolated and identified compounds which are present in relatively small quantities among the volatiles prepared by distillation–extraction. In a previous study several major volatile compounds from wheat were identified [3]. In the present work, identification of pentadecanal, a relatively major (3.5%) high boiling constituent of wheat, was confirmed by synthesis of an authentic standard and comparison of mass spectral and GC data with those of the plant constituent. Since French and co-workers [1, 2] found that nonanal and 1-nonanol were effective stimulators of spore germination, the effects of tissue source on the relative amounts of these compounds and related C_9 aldehydes and alcohols prepared by distillation extraction of wheat plants were evaluated.

Buttery *et al.* [4] recently examined headspace volatiles from young intact wheat plants as part of investigation of compounds which attract certain insects to undisrupted wheat plants. This contrasts to the situation with fungal pathogens such as rust which disrupt wheat tissue during growth, development and formation of spore pustules. The headspace trapping method employed a porous polymer, Tenax, and a 24 hr sampling period during which an air stream was passed over the tissue to entrain the volatiles. In preliminary tests we found the procedure works well for small amounts of volatiles; however, certain aldehydes, including nonanal, of interest in relation to rust spore germination were apparently oxidized on prolonged exposure to the air stream [unpublished results].

RESULTS AND DISCUSSION

Volatile compounds identified by comparison of mass spectral and gas chromatographic data of the plant constituents with those of authentic samples are listed in Table 1. These compounds were present in relatively small amounts (0.5% or less) in the volatiles isolated from wheat plants and hence were not identified as readily as compounds reported earlier [3]. The C_6 compounds, 3-hexen-1-ol and 3-hexen-1-yl acetate, have characteristic green, grass-like odours and were isolated in small quantities from mature plants (stored frozen prior to analysis) that were used as a source of volatiles for identification as shown in Table 1. In young plants harvested in the autumn the odour of these C_6 compounds was strong and

Table 1. Identification of additional volatile compounds isolated from wheat by distillation–extraction*

Compound	Evidence	Percentage in oil
cis-3-Hexen-1-yl acetate	MS, R_t	trace†
cis-3-Hexen-1-ol	MS, R_t	0.4
trans-2-Octen-1-ol	MS, R_t	0.1
1-Decanol	MS, R_t	0.1
Eugenol	MS, R_t	0.5
Octanal	MS, R_t	trace
Undecanal	MS, R_t	0.3
trans-2-Undecanal	MS, R_t	0.2
Tridecanal	MS, R_t	0.1
Tetradecanal	MS, R_t	0.4
Pentadecanal	MS, R_t	3.5
cis,cis-8,11-Heptadecadienal	MS, R_t‡	0.2
cis,cis,cis-8,11,14-Heptadecatrienal	MS, R_t‡	0.4
Pentadecane	MS, R_t	0.1
Hexadecane	MS, R_t	0.1
2-Octadecanone	MS	0.8

*Harvested May 1983.
†Indicates less than 0.1%.
‡Standard isolated from frozen cucumber fruit.

they apparently make an important contribution to the grass-like odour quality of the plants. Wheat volatiles were examined for 1-octen-3-ol which was a principal component isolated from alfalfa plants by distillation–extraction having a characteristic earthy odour [5]. Although there was GC evidence for a trace amount of this compound (less than 0.1 %) there was not an adequate amount to obtain mass spectral confirmation.

A relatively major compound was isolated which mass and IR spectral data indicated was pentadecanal [3]. An authentic sample of pentadecanal was synthesized by controlled oxidation of 1-pentadecanol using chromic anhydride intercalated in graphite. The synthetic compound yielded a mass spectrum which compared favourably with that of the plant constituent and the synthetic sample co-eluted with the plant component from a capillary GC column permitting confirmation of its identity. This compound along with 8,11-heptadecadienal and 8,11,14-heptadecatrienal probably arise from enzymatic α-oxidation of palmitic, linoleic and linolenic acids, respectively, as described by Galliard and Mathew [6]. Originally, 8,11-heptadecadienal and 8,11,14-heptadecatrienal were isolated from cucumber fruit where they were characterized from their spectral data [7]. These compounds were present in relatively small quantities in wheat (Table 1) and the mass spectral and GC retention data matched those obtained for standards from cucumber fruit.

A high boiling compound (eluting prior to C_{17} aldehydes on the SP 2100 column) was isolated which from its fragmentation pattern indicated that it was an aliphatic methyl ketone exhibiting a characteristic base peak at m/z 58 $[M - MeCOHCH_2]^+$ and a series of ions 14 mu apart. CI mass spectrometry using methane gas showed that the M_r was 268. These data indicated that the compound was 2-octadecanone. Similar fragmentation patterns were found in the literature for other methyl ketones [8]. However, since no authentic standard was available, this identification is considered tentative.

In order to have adequate samples available for analysis as needed for identification studies, wheat plants were stored frozen prior to distillation–extraction. A quantitative comparison of volatiles from fresh and frozen young plants harvested two months after seeding in November 1983 is given in Table 2. The same C_9 compounds that were detected in frozen tissue were also isolated from fresh tissue. An overlapping peak interfered with determinations of the relative quantities of 2-nonen-1-ol. The remaining volatile compounds identified in plants stored frozen were also isolated from fresh wheat by distillation–extraction. The numerical values presented for C_9 compounds in Table 2 represent GC peak areas normalized to a pentadecane internal standard. These values are useful to indicate general trends such as increase or decrease in the amount of a compound. Frozen tissue yielded increased amounts of C_9 volatiles. Earlier work also showed that freezing samples prior to distillation–extraction increased the yield of C_9 compounds from cucurbits [9].

Data from the analysis of C_9 compounds in leaves and culms of wheat plants are presented in Table 2. Mature plants were harvested in late Spring (harvested April 1984; seeded September 1983) when it was practical to separate leaf blades from culms (hollow stems) but prior to the appearance of the inflorescence. Composite values were calculated for whole plants from 54% of leaf values and 46% of culm values. These percentages represent the contribution of the parts to the total aerial weight of the plants at harvest. As in the comparisons of the fresh and frozen plants, all of the C_9 compounds analysed were present in both the leaves and culms of the plants. With regard to the relative amounts of C_9 compounds, nonanal and 1-nonanol were found to predominate in leaves and there were increased proportions of alcohols such as 3,6-nonadien-1-ol in culms. When culms were cut open (split vertically and opened) there was a characteristic odour reminiscent of unsaturated C_9 alchohols such as 3,6-nonadien-1-ol [10]. All other compounds identified in wheat were also found in both leaves and culms.

In Table 2, data are also presented on comparisons of C_9 compounds isolated from intact plants (cut at soil level) and plants cut into 3–6 cm pieces. It was found that C_9 compounds from the intact and cut plant tissue were qualitatively the same except that the peak corresponding

Table 2. Effects of tissue source on relative composition of C_9 compounds from wheat

	Harvested Nov. 1983*		Harvested April 1984†			Harvested April 1985‡	
	Fresh	Frozen	Leaves	Culms	Composite	Intact	Cut
Nonanal	4.4§	10.6	39.2	7.9	22.3	2.1	5.0
trans-2-Nonenal	0.3	1.3	0.2	0.8	0.6	0.1	0.2
trans,cis-2,6-Nonadienal	0.9	4.4	0.4	1.8	1.2	0.1	0.2
1-Nonanol	1.0	2.5	17.7	9.8	13.5	2.2	6.0
cis-3-Nonen-1-ol	0.6	0.9	0.5	11.3	6.4	0.1	1.4
trans,cis-2,6-Nonadien-1-ol	0.4	5.7	0.2	1.1	0.7	—	0.1
cis,cis-3,6-Nonadien-1-ol	1.2	1.3	1.0	19.7	11.1	0.1	2.6

*Immature plants (20–30 cm tall); seeded Sept. 1983; macerated in Waring blender.

†Mature plants (40–50 cm tall); seeded Sept. 1983; cut into 3–6 cm segments.

‡Immature plants (30–50 cm tall); seeded Sept. 1984; cut into 3–6 cm segments or intact.

§Values normalized to added internal standard, pentadecane; mean of two determinations. A value of 1.0 was estimated as equivalent to *ca* 1 ppb yield of compound from plant tissue on a wt per fr. wt basis.

to 2,6-nonadien-1-ol in intact plants was too small for mass spectral confirmation. Generally, there were greater quantities of C_9 compounds in the cut tissue. However, there was a marked increase in 3-nonen-1-ol and 3,6-nonadien-1-ol when the tissue was cut. Disruption of wheat tissue results in increased amounts of C_9 volatiles probably due to greater mixing of enzymes and substrates [11]. Growth and development of fungal pathogens on host plants also cause disruption of plant tissue; however, specific similarities to the physically induced processes remain to be clarified. Generally, the same compounds were obtained by distillation–extraction of all the tissues analysed.

EXPERIMENTAL

Plant material. Winter wheat cultivar 'Arthur 71' grown on a University of Kentucky farm was harvested and stored frozen (−20°) or refrigerated (5°) overnight. Volatile compounds were isolated using the procedure and apparatus (2.8 l. H_2O per kg plant tissue) described for red. pres. steam distillation–extraction [3]. Mature plants stored frozen as described previously [3] were used as a source of tissue for the identification of additional compounds. In expts comparing plant parts, freshly harvested plants were refrigerated overnight after harvest and then leaf blades were removed from culms and tissue was cut into *ca* 3-6 cm segments prior to distillation–extraction. For comparison of intact versus cut wheat, plants were cut at soil level and refrigerated overnight and then used intact or cut into 3-6 cm pieces.

GC and GC/MS. GC separations were carried out using a 1.8 m × 4 mm i.d. column containing 20% SP 2100 on Supelcoport for initial fractionation of the samples and a 30 m × 0.32 mm Supelcowax 10 fused silica capillary column for subsequent high resolution GC analysis. GC/MS analyses using EI-MS were performed as described in ref. [3] and CH_4 was the reagent gas for CI-MS. For comparisons of C_9 compounds, GC peak areas were determined using an electronic integrator and normalized to an int. standard, pentadecane. Fractions of C_9 compounds collected from the SP2100 packed column were washed from U-tubes with 50 μl of Me_2CO containing pentadecane at a concn of 50 ng/μl. One μl portions of these solns were injected into the capillary column for quantification.

Synthesis of pentadecanal. To 25 ml of toluene were added 1.1 g of 1-pentadecanol and 10 g of CrO_3 intercalated in graphite (Alfa Products). The mixture was refluxed for 24 hr according to the general procedure of ref. [12] for selective oxidation of alcohols to aldehydes. After cooling, the mixture was filtered through an ultra-fine sintered glass funnel and subsequently through a Millipore FH 0.5 μm filter to remove graphite insolubles. The reaction mixture which had a slight green colour was purified by micro-distillation on a Vigreaux column under red. pres. (~25 mm Hg) followed by prep. GC on the 20% SP 2100 column described above. There was a *ca* 20% conversion of the alcohol to pentadecanal as shown by GC analysis. The product yielded the following major mass spectral peaks above *m/z* 40: (%) 82 (100), 57 (85), 41 (76), 55 (72), 43 (68), 96 (63), 81 (56), 83 (51), 69 (45), 95 (45), 67 (44), 68 (43), 97 (37), 71 (35) and 44 (30).

Acknowledgements—We are grateful to Pierce Fleming and John Loughrin for assistance and to George Lovelace for obtaining MS data. Bedoukian Research, Inc. supplied synthetic standards. This paper (85-10-3-19) is published with the approval of the Director of the Kentucky Agricultural Experiment Station.

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