Phytochemistry, Vol. 22, No. 11, pp. 2521–2523, 1983.
Printed in Great Britain.

0031–9422/83 $3.00+0.00

VOLATILES FROM THE EPICUTICULAR WAX OF WATERCRESS (*RORIPPA NASTURTIUM-AQUATICUM*)

ROSE-MARIE M. SPENCE and OWEN G. TUCKNOTT

Long Ashton Research Station, University of Brsitol Long Ashton, Bristol BS18 9AF, U.K.

(*Received* 11 *February* 1983)

Key Word Index—*Rorippa nasturtium-aquaticum*; Cruciferae; watercress; volatiles; epicuticular wax.

Abstract—Volatiles from the epicuticular wax of watercress were collected by ether washing and examined using gas chromatographic and mass spectrometric analysis. Cell damage by the solvent resulted in several glucosinolate degradation products being identified. The quantities of most compounds increased as the watercress aged. The epicuticular wax of watercress contains many volatile compounds which are produced within the plant and subsequently trapped in the wax layer where they contribute to the aromas observed at different stages of deterioration of the plant material.

INTRODUCTION

The epicuticular wax has been shown to contribute to the external aroma of fruits [1–3]. The volatiles in the wax of watercress were examined to determine whether the wax traps the volatile compounds released from within the plant [4], thus contributing to the aroma of watercress.

RESULTS AND DISCUSSION

The results shown in Table 1(a) were obtained for bulked concentrates from washings of several mature watercress samples. The removal of epicuticular wax by washing in ether resulted in tissue damage and glucosinolate degradation. The residue after vacuum sublimation consisted of the non-volatile compounds which comprise the wax layer. However, several compounds detected in the wax [5] were also found in the ether extract; namely, naphthalene, phenol, benzaldehyde, benzyl alcohol, 2-phenethyl alcohol, 3-phenylpropionitrile and 2-phenethyl isothiocyanate. The latter are probably glucosinolate degradation products [6] which are both present within the plant and become trapped in the wax. Benzyl isothiocyanate, detected in the wax, was not found in the ether extract, but its corresponding nitrile, phenylacetonitrile, was only present in the ether extract. Phenethyl nitrile was identified in the ether extract and methyl isothiocyanate, not previously reported in the Cruciferae, was also found. Nitriles and isothiocyanates are major contributors to the characteristic flavour and aroma of crushed watercress [7]. They are present in ether washings but not in headspace collections [8], except for a small amount of phenethyl isothiocyanate, because of their low volatility and because they are only released on tissue damage, for example by solvent washing.

Dimethyl sulphone, which was also identified in the headspace collection of volatiles with dimethyl sulphoxide [8, 9] is thought to be an oxidation product of dimethyl sulphide [8] which is important in the flavour of several brassicas. The short chain fatty acids detected may be intermediates in plant metabolism, but the reason for the large amounts of acetic acid in both ether washing and headspace samples is unknown. The possibility of plant microflora producing acetic acid was investigated [10] but no acetic acid producing micro-organisms were identified. The benzene derivatives, which were also identified in the headspace collection samples and in the wax are thought to be genuine volatiles of watercress and not Porapak Q breakdown products [11] because this polymer was not used in the ether washing collection of volatiles.

The results of ether washing samples from young and old cress [Table 1(b) and (c)] show an increase in the relative quantities of the majority of compounds as the watercress ages. Dimethyl sulphone, several hydrocarbons (pentadecane, naphthalene, octadecane and eicosane) and benzenes (ethylbenzene, propylbenzene and 1-ethyl-2-methylbenzene) were only present in the old watercress sample: further work is necessary to determine those new compounds produced as watercress ages and those which only increase in quantity. In the samples from old and young watercress, α-ionone may be responsible for the floral odour observed as watercress deteriorates [12]. β-Ionone was detected in the samples from the headspace collection of watercress volatiles [8] and, together with α-ionone, it may be formed by oxidation of carotenes [13].

This evidence suggests, as observed with plums [3], that the surface lipid layer of watercress [5] acts as a trapping and concentrating medium for the more volatile components released from within the plant. These compounds contribute to the aromas observed at different stages of deterioration of the plant material.

EXPERIMENTAL

Plant material. Watercress was grown in controlled and natural environment watercress bed simulation tanks [7] and stems cut (18–20 cm long) 12–18 weeks from sowing.

Collection procedure. Watercress (75 g), washed in glass distilled water, was placed in a glass funnel (70 × 8 cm diameter) with 200 ml Et_2O (purified as described by Spence and Tucknott [8]) and agitated gently for 30 min to ensure complete immersion of the watercress in the solvent. The Et_2O was run into a 250 ml

Table 1. Compounds identified in samples from ether washing watercress, (a) bulked sample of several washings of mature watercress samples, (b) young watercress, (c) old watercress

Compound	(a) Relative % in bulked sample of several washings of mature watercress samples*	(b) Relative % in sample from young watercress*	(c) Relative % in sample from old watercress*
Pent-3-en-2-one	1.7	—	—
Ethylbenzene	—	—	0.6
Propylbenzene	—	—	0.1
1-Ethyl-2-methylbenzene	—	—	0.1
2-Methyltetrahydrofuran	1.2	—	—
Ethyl acetate	—	1.4	—
Vinylbenzene	2.7	0.6	1.0
Acetic anhydride	0.6	1.2	1.4
Methyl isothiocyanate	0.6	—	0.3
3-Hydroxybutanone	0.2	—	—
4-Hydroxy-4-methylpentan-2-one	1.2	0.6	0.2
1,4-Dichlorobenzene	0.2	—	0.4
Acetic acid	11.3	8.4	3.6
Benzofuran	0.1	—	—
Benzaldehyde	4.2	0.4	0.3
Unknown, base peak *m/z* 43, possible M^+ 180	—	0.3	13.5
2-Hydroxy-2-methylpropanoic acid	0.7	—	—
2-Methylpropanoic acid	—	2.4	0.2
Ethane-1,2-diol diacetate	—	—	tr
Phenylacetonitrile	0.2	—	—
Pentadecane	—	—	0.2
Butanoic acid	0.1	tr	0.1
Possible isothiocyanate, M^+ 157 (3%), base peak *m/z* 43 (100%)†	—	0.1	0.2
Unknown, aromatic base peak *m/z* 91	0.7	0.1	0.2
Ethane-1,2-diol monoacetate	1.0	0.1	0.4
Unknown, base peak *m/z* 83	—	—	0.2
5-Methylhexane3-ol	0.4	—	—
Naphthalene	tr	—	0.1
Pentanoic acid	0.2	—	0.1
Possible isothiocyanate, M^+ 171, base peak *m/z* 43†	—	—	0.1
2-Hydroxymethyl benzoate	—	—	0.1
Possible aromatic aldehyde, M^+ 148 (2%), base peak *m/z* 91 (100%)	7.9	1.0	2.0
Octadecane	—	—	0.3
Hexanoic acid	0.6	0.1	0.6
α-Ionone	—	0.3	tr
Benzyl alcohol	0.8	0.2	0.3
2-Methylbutyl decanoate	0.2	0.2	0.5
2-Phenethyl alcohol	0.2	0.1	—
Unknown, aromatic, base peak *m/z* 91	2.9	0.4	0.6
Benzothiazole	—	0.2	0.1
Unknown, base peak *m/z* 43, possible M^+ 135	—	—	0.2
Heptanoic acid	—	—	0.1
Dimethyl sulphone	0.6	—	tr
Phenol	1.3	0.2	0.3
Unknown, base peak *m/z* 43	—	—	1.0
Unknown, base peak *m/z* 99	—	—	0.6
Octanoic acid	0.1	—	0.2
3-Phenylpropionitrile	14.7	2.5	4.3
Benzyl isothiocyanate	—	0.1	—
Ethyl tetradecanoate	3.3	—	—
Eicosane	—	—	0.5
2-Phenethyl isothiocyanate	27.9	56.1	25.7
Hexadecanoic acid	—	1.0	—
Tetradecanal	—	1.0	—
Diethyl-1,2-benzene dicarboxylate	—	—	1.7

*Order of elution on 56 m × 0.5 mm glass SCOT column coated with Carbowax 20M, sample size 0.5 μl.
†Homologous series increasing by CH_2.
tr, trace.

flask containing 20–25 g dry Na_2SO_4 and stored at $-20°$ overnight. The Et_2O washings were frozen in liquid N_2 before attachment to the sublimation apparatus (lyophilizer) [7]. Volatile material sublimed under vacuum (0.01 mm Hg) condensed on the cold finger and was collected before concn by fractionation on a 40 × 1.5 cm diameter column packed with Fenske helices. Final evaporation was carried out in N_2 and the concentrate (50 μl) stored in an ampoule at $-20°$ until examined. The non-volatile waxes and lipophilic compounds remaining in the first flask were dissolved in Et_2O, transferred to an ampoule and stored at $-20°$ for subsequent use in the epicuticular wax investigation [5].

Gas chromatography. GC analyses were carried out using a Hewlett-Packard 5710 gas chromatograph with a flame ionization detector and fitted with a 56 m × 0.5 mm glass SCOT column coated with Carbowax 20M. The temperature was held at 65° for 2 min, then programmed to 180° at 2°/min, and held isothermal. N_2 carrier-gas flow was 5 ml/min and the injection port and detector were held at 250°.

Gas chromatography/mass spectrometry. Mass spectra were obtained using a Finnigan 4000 coupled gas chromatograph/mass spectrometer with a 2100 data system, operated at 50 eV. Chromatographic column and conditions were the same as described under Gas Chromatography except that He was used as carrier gas.

Identification of compounds. Compounds were identified by comparison of spectra with those present in the Finnigan library (National Bureau of Standards), with spectra in compilations, our own reference spectra and those in literature.

Acknowledgements—Acknowledgement is made to the Ministry of Agriculture, Fisheries and Food for a postgraduate studentship to Rose-Marie M. Spence at Bath University and to the Long Ashton/Meat Research Institute Mass Spectrometry Group for providing the mass spectral facilities.

REFERENCES

1. Croteau, R. and Fagerson, S. (1971) *Phytochemistry* **10**, 3247.
2. Paillard, N. M. M. (1975) Thèse de Doctoral d'état des Sciences naturelle, Université Pierre et Marie Curie, Paris.
3. Ismail, H. M., Brown, G. A., Tucknott, O. G., Holloway, P. J. and Williams, A. A. (1977) *Phytochemistry* **16**, 769.
4. Spence, R-M. M. and Tucknott, O. G. (1983) *J. Sci. Food Agric.* **34**, 412.
5. Spence, R-M. M. and Tucknott, O. G. (1983) *Phytochemistry* **22**, 1753.
6. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1365.
7. Spence, R-M. M. (1980) Ph.D. Thesis, University of Bath.
8. Spence, R-M. M. and Tucknott, O. G. (1983) *J. Sci. Food Agric.* (in press).
9. Spence, R-M. M. and Tucknott, O. G. (1983) *J. Sci. Food Agric.* (in press).
10. Spence, R-M. M. and Tucknott, O. G. (1983) *J. Appl. Bacteriol.* (in press).
11. Williams, A. A. and Lewis, M. J. (1978) International Federation of Fruit Juice Producers Symposium Report, Bern, 183.
12. Spence, R-M. M. (1982) *J. Food Technol.* **17**, 633.
13. Stevens, M. A. (1970) *J. Am. Soc. Hort. Sci.* **95**, 461.