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COMPOUNDS ASSOCIATED WITH THE SURFACE LIPID LAYER OF WATERCRESS

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Key Word Index—*Rorippa nasturtium-aquaticum*; Cruciferae; watercress; surface lipid layer; glucosinolate degradation products; trapped volatiles; plant wax compounds.

Abstract—The composition of the surface layer of watercress leaves, stems and flowers was examined using standard methods of wax analysis and GC/MS. The wax of watercress contains many classes of compounds commonly found in other plant waxes, viz. alkanes, primary alcohols, alcohol acetates, aldehydes and terpenes. The main aliphatic constituents were alkan-1-ols, chiefly hexacosan-1-ol. Many components were identified which were possible lipid degradation products and others were plant volatiles, such as thiocyanates, isothiocyanates, nonanal, dimethyl trisulphide and 3-phenylpropionitrile, trapped in the wax layer. The volatiles comprised *ca* 14% of the total extract. The wax from watercress flowers contained similar compounds to those from the leaves and stems. Several of the sulphur compounds trapped in the wax are reported to be significant in the flavour and aroma of different vegetables, and they probably also contribute to watercress odour, particularly as they accumulate during plant senescence.

INTRODUCTION

Previous work has suggested that the epicuticular wax of fruits and leaves may contribute to their external aromas by trapping volatile compounds [1]. The composition of the epicuticular wax of watercress was examined for compounds comprising the wax layer and for those trapped in the wax which may contribute to the aromas observed as watercress deteriorates [2, 3].

RESULTS AND DISCUSSION

Confirmation of the classes of compounds separated by prep. TLC and homologue identifications were obtained using GC and GC/MS. The results for the whole leaf wax sample in chloroform are given in Table 1. Most of the peaks were mixtures with several unidentified compounds. However, results from the analysis of prep. TLC fractions showed the wax contained hydrocarbons with both odd and even numbers of carbon atoms between C_{19} and C_{35}, with C_{27}, C_{29} and C_{31} occurring in the largest quantities, C_{24} and C_{26-29} primary alcohol acetates and C_{16}, C_{22} and C_{25-31} primary alcohols. Phthalates were detected which may be naturally occurring, although they are a common environmental contaminant. Phenethyl isothiocyanate and other possible thiocyanates and isothiocyanates (base peak *m/z* 101) were also found. The three unidentified compounds with base peak *m/z* 219 are thought to be long chain compounds which form a methylsulphinyl isothiocyanate fragment, Me–SO–$(CH_2)_7$–NCS, with MW 219 [4]. The identification of β-amyrin and several terpenes was not unexpected since they have been found in several *Brassica* species (Cruciferae) [5, 6]. The main aliphatic constituents were found to be alkan-1-ols, chiefly hexacosan-1-ol (Table 1, peak 14).

Work by Ismail *et al.* [1] suggests that relatively low MW volatile compounds which contribute to the flavour and aroma of the material may be trapped in the cuticular wax of fruits and leaves. Therefore, samples of wax from watercress flowers and leaves, dissolved in ether, were analysed on a capillary column and the results are shown in Table 2. Many compounds were identified in the leaf wax sample which were lower homologues of the classes of wax compounds found using TLC and prep. TLC. The aldehydes identified have been reported in the volatiles of several other vegetables [7]; nonanal is a major component of the epicuticular wax of plums and is important in the overall aroma of this fruit [1]. The same aldehyde is also reported to be significant in the aroma of cauliflower and broccoli [8], and it may contribute to the aroma of watercress since it appears as a large peak in the leaf wax sample and it has a low threshold value of 1 mg/l. in paraffin oil [9]. Phenyl acetaldehyde [10] and benzaldehyde [11] have been identified in tomato volatiles and these compounds are thought to be formed by oxidative degradation of lipids [12]. Under more rigorous conditions these compounds may be of glucosinolate origin [13]. Acetophenone was the only ketone identified in watercress leaf wax but its occurrence has been reported in tomato [11]. In watercress, this compound, together with other aromatic compounds identified in the leaf wax (phenol, toluene, vinylbenzene and 2-methyltetrahydrofuran), may arise from the lipid material present by the oxidation of aldehydes [12].

Since long chain hydrocarbons are prevalent in most higher plant waxes, including watercress, it was not surprising to find lower members (octane and undecane) as well as C_{20} and C_{25} hydrocarbons in the leaf wax sample. Naphthalene has been reported in the volatiles of many foods [7] and was found in the leaf wax of watercress. Dimethyl trisulphide was identified in the watercress leaf wax sample but not the disulphide, which was found in the headspace collection [14]. The trisulphide has been detected in both fresh and cooked cabbage

Table 1. Constituents of whole epicuticular wax of watercress

Compound	Peak No.*	Relative amount (%)
2-Phenethyl isothiocyanate	1	5.3
Butyl phthalate + nonadecane	2	0.3
Hexadecan-1-ol + henicosane	3	2.0
Tricosane	4	0.7
Pentacosane	5	0.3
Docosan-1-ol + docosan-1-yl acetate + phthalate	6	1.5
Unidentified, base peak *m/z* 101 + hydrocarbon + phthalate	7	3.9
Tricosan-1-ol	8	0.08
Unidentified, base peak *m/z* 219 + heptacosane	9	1.8
Tetracosan-1-ol + tetracosan-1-yl acetate	10	6.8
Unidentified, base peak *m/z* 101 + nonacosane	11	4.4
Pentacosan-1-ol	12	0.05
Unidentified, base peak *m/z* 219 + hentriacontane	13	16.3
Hexacosan-1-ol + hexacosan-1-yl acetate	14	31.8
Unidentified, base peak *m/z* 101 + dotriacontane	15	2.6
Heptacosan-1-ol + heptacosan-1-yl acetate	16	0.3
Unidentified, base peak *m/z* 219 + tritriacontane	17	11.6
Octacosan-1-ol + octacosan-1-yl acetate	18	3.8
Tetratriacontane	19	0.2
Unidentified	20	2.0
Triacontan-1-ol + pentatriacontane	21	0.5
β-Amyrin	22	1.3

*Order of elution on 90 × 0.2 cm glass column packed with 1% Dexsil 300 on 100–120 mesh Supelcoport, sample 5 μl.

Table 2. Constituents of surface wax extracts from watercress leaves and flowers

Compound	Leaves		Flowers	
	Peak No.*	Relative amount (%)	Peak No.†	Relative amount (%)
Pent-3-en-2-one	—	—	1	0.9
Hepta-1,5-diene-3,4-diol	1	1.2	2	1.5
2-Methyltetrahydrofuran	2	1.2	3	0.4
Toluene	3	0.1	—	—
Octane	4	0.3	—	—
Ethylbenzene	5	0.08	—	—
Heptanal	6	0.6	4	0.2
Vinylbenzene	7	2.2	5	0.2
Dimethyl trisulphide	8	0.2	6	1.3
4-Hydroxy-4-methylpentan-2-one	—	—	7	0.03
Nonanal	13	1.2	8	0.7
Acetic acid	—	—	9	1.5
Benzaldehyde	9	0.4	—	—
Unknown, base peak *m/z* 41	—	—	10	0.4
Unknown hydrocarbon	—	—	11	0.7
Unknown, base peak *m/z* 43	10	0.07	—	—
1*H*-Indene	11	0.06	—	—
Nonan-1-ol	12	0.02	—	—
2-Methylpropanoic acid	—	—	12	0.4
Thiocyanate or sulphide M^+ 94 (32%), base peak *m/z* 41‡	—	—	13	0.4
Thiocyanate or sulphide M^+ 108 (5%), base peak *m/z* 41‡	—	—	14	1.4
2-Methylbutanoic acid	—	—	15	0.7
Unknown, base peak *m/z* 45	—	—	16	1.0
Phenylacetaldehyde	14	0.08	—	—
Acetophenone	15	0.03	—	—

Table 2. (Contd.)

Compound	Leaves		Flowers	
	Peak No.*	Relative amount (%)	Peak No.†	Relative amount (%)
Possibly hexyl isothiocyanate M^+ 143	16	0.2	—	—
Decanal	17	0.2	—	—
Unknown, base peak *m/z* 41 M^+ 141	18	0.2	—	—
Naphthalene	19	0.4	—	—
Thiocyanate or sulphide M^+ 155 (3%), base peak *m/z* 41‡	23	6.6	17	16.4
Possible aromatic aldehyde M^+ 148 (0.8%), base peak *m/z* 91	—	—	18	2.2
Hexanoic acid	—	—	19	0.09
Thiocyanate or sulphide M^+ 169 (0.5%), base peak *m/z* 41‡	28	5.2	20	10.4
Benzyl alcohol	20	0.03	—	—
Possible isothiocyanate M^+ 157 (3%), base peak *m/z* 43‡	21	0.3	—	—
2-Phenethyl alcohol	22	0.1	21	0.7
Phenol	24	0.06	22	0.3
Benzothiazole	25	0.04	—	—
Unknown, base peak *m/z* 43	—	—	23	1.0
Possible isothiocyanate M^+ 171 (0.8%), base peak *m/z* 43‡	26	0.2	—	—
3-Phenylpropionitrile	27	9.2	24	16.9
Biphenyl	29	0.02	—	—
Propane-1,2,3-triol triacetate	30	0.06	—	—
Benzyl isothiocyanate	31	0.07	25	0.2
Ethyl tetradecanoate	—	—	26	1.0
Undecane	32	0.06	—	—
Substituted phenol base peak *m/z* 150	33	0.1	27	2.8
Unknown, base peak *m/z* 59	34	0.07	—	—
2-Phenethyl isothiocyanate	35	31.6	28	25.5
Vinyl phenyl ether			29	3.3
Unknown hydrocarbon	38	0.4	30	0.3
1*H*-Indole	36	0.3	31	0.4
Diethyl-1,2-benzene dicarboxylate	37	0.7	—	—
A phenylbutyl isothiocyanate	39	0.2	—	—
Icosane	40	0.2	—	—
7-Methylthioheptyl isothiocyanate	41	8.5	32	2.4
Pentacosane	42	0.9	33	0.3
Hexadecan-1-ol or hexadecene	43	15.9	—	—
8-Methylthiooctyl isothiocyanate	44	1.6	34	0.9
Unknown hydrocarbon	45	0.4	35	1.0
Hexadecanoic acid	46	2.7	—	—

*Order of elution on 70 m × 0.7 mm glass open tubular capillary column coated with SF-96-200, sample size 0.5 μl.
†Order of elution on 56 m × 0.5 mm glass Carbowax coated SCOT column, sample size 0.5 μl.
‡Homologous series increasing by CH_2.

[15, 16] and is considered to be important in the aroma of cooked brassicas.

Several isothiocyanates were positively identified in the leaf sample. Both 2-phenethyl isothiocyanate and 3-phenylpropionitrile, which were detected in previous work on watercress volatiles [17], were found in the leaf wax. Benzyl isothiocyanate, but not its nitrile, which were reported together in garden cress [18] and watercress leaves [19], were detected as well as several other glucosinolate products found in garden cress seedlings [20]. A phenylbutyl isothiocyanate was also identified. The two methylthioalkyl isothiocyanates have also been found in watercress seeds [19] as well as the wax. The volatile compounds identified comprised *ca* 14% of the total extract.

The petals of many flowers are known to have a fine waxy bloom due to a smooth, non-crystalline wax layer [21]. The sample of the wax from watercress flowers contained many compounds found in the leaf wax in addition to several short chain fatty acids, hydrocarbons and ketones. Generally, the leaf wax of watercress contained similar compounds to that of the flowers, most being components of the epicuticular wax except for the glucosinolate degradation products, of which phenethyl isothiocyanate was the major compound. The volatiles trapped in the surface lipid layer are, therefore, thought to contribute to the overall aroma of the watercress plants, particularly as many of the compounds accumulate during deterioration of the plant [14].

EXPERIMENTAL

Plant material. Watercress was grown in natural environment watercress bed simulation tanks [22] and stems cut (18–20 cm

long) 12–18 weeks from sowing. Older, flowering watercress was also used.

Isolation of surface layer of stems and leaves. The epicuticular wax was removed from 100 g watercress, previously washed in glass distilled H_2O. Each sprig (*ca* 18 cm long) was immersed in 250 ml Et_2O, purified as described by Spence and Tucknott [14], for 30 sec with the cut stem ends held above the solvent. Na_2SO_4 (25 g, pyrolysed at 550° for 5 hr) was added to the Et_2O washings which were stored overnight at −20°. After vacuum sublimation [22] the non-volatile wax remaining in the flask was dissolved in Et_2O, transferred to a weighed ampoule and the Et_2O evaporated off in an atmosphere of N_2. This sample from stems and leaves is referred to as the leaf wax sample.

Isolation of surface layer of watercress flowers. The wax was removed from flowers by immersing 30 g flower sprigs in 100 ml Et_2O for 30 min as described above.

Analysis of wax constituents. The qualitative composition of the wax extract dissolved in $CHCl_3$ was determined using published TLC, prep. TLC and GC/MS methods [23, 5]. Samples were derivatized using acetylation and silylation for GC analysis. GC/MS analysis was also carried out on the total wax extract dissolved in Et_2O.

Gas chromatography. GC analyses were carried out using a Hewlett-Packard 5730A GC with an FID and fitted with a 90 × 0.2 cm glass column packed with 1% Dexsil 300 on 100–120 mesh Supelcoport (Supelco) and a 90 × 0.2 cm glass column packed with 3% SP2100 on 100–120 mesh Supelcoport for the wax extracts in $CHCl_3$. The temp. was programmed from 130 to 350° at 6°/min, then isothermally. N_2 carrier-gas flow was 30 ml/min and the injection port and detector temp. were 250° and 300°, respectively.

A Hewlett-Packard 5830 GC with an FID, fitted with a 70 m × 0.7 mm glass open tubular capillary column coated with SF-96-200, was used for the analysis of Et_2O extracts of leaves and stems and flowers. The temp. was programmed from 65 to 190° at 2°/min, then isothermally. N_2 carrier-gas flow was 50 ml/min and the injection port and detector temp. 250°.

Gas chromatography/mass spectrometry. MS were obtained using an LKB 9000 coupled GC/MS operating at 70 eV. A 90 × 0.32 cm stainless steel column packed with 1% Dexsil 300 on 100–120 mesh Supelcoport with temp. programming from 120 to 270° at 6°/min was used. He carrier-gas flow was 30 ml/min and the separator temp. was 250°.

A Finnigan 4000 coupled GC/MS with a 2100 data system, operated at 50 eV was also used. The same column and conditions were used as on the LKB 9000 and also three other columns: (1) 180 × 0.2 cm glass column packed with 3% SP2100 on 100–120 mesh Supelcoport, separator temp. at 280° and temp. programming from 130 to 275° at 6°/min. (2) 56 m × 0.5 mm glass Carbowax coated SCOT column, separator temp. at 225° and temp. programming from 65 to 190° at 2°/min. (3) 70 m × 0.7 mm glass open tubular capillary column coated with SF-96-200, separator temp. at 225° and temp. programming from 65 to 190° at 2°/min.

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 the lit.

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REFERENCES

1. Ismail, H. M., Brown, G. A., Tucknott, O. G., Holloway, P. J. and Williams, A. A. (1977) *Phytochemistry* **16**, 769.
2. Spence, R.-M. M. (1982) *J. Food Technol.* **17**, 633.
3. Spence, R.-M. M. and Tucknott, O. G. (1983) *Phytochemistry* (in press).
4. Spencer, G. F. and Daxenbichler, M. E. (1980) *J. Sci. Food. Agric.* **31**, 359.
5. Holloway, P. J., Jeffree, C. E. and Baker, E. A. (1976) *Phytochemistry* **15**, 1768.
6. Holloway, P. J., Brown, G. A., Baker, E. A. and Macey, M. J. K. (1977) *Chem. Phys. Lipids* **19**, 114.
7. Van Straten, S. (1977) *Volatile Compounds in Food* 4th edn. Central Institute for Nutrition and Food Research, T.N.O., Zeist, The Netherlands.
8. Buttery, R. G., Guadagni, D. G., Ling, L. C., Seifert, R. M. and Lipton, W. (1976) *J. Agric. Food Chem.* **24**, 829.
9. Stahl, W. H. (1973) *Compilation of Odour and Taste Threshold Values* (Stahl, W. H., ed.). American Society for Testing Materials, Philadelphia.
10. Kazeniac, S. J. and Hall, R. M. (1970) *J. Food Sci.* **35**, 519.
11. Buttery, R. G., Seifert, R. M., Guadagni, D. G. and Ling, L. C. (1971) *J. Agric. Food Chem.* **19**, 524.
12. Forss, D. A. (1972) *Progress in the Chemistry of Fats and Other Lipids* (Holman, R. T., ed.) Vol. XIII, Part 4, pp. 181–258. Pergamon Press, Oxford.
13. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1369.
14. Spence, R.-M. M. and Tucknott, O. G. (1983) *J. Sci. Food Agric.* (in press).
15. Bailey, S. D., Bazinet, M. L., Driscoll, J. L. and McCarthy, A. I. (1961) *J. Food Sci.* **26**, 163.
16. Maruyama, F. T. (1970) *J. Food Sci.* **35**, 540.
17. MacLeod, A. J. and Islam, R. (1975) *J. Sci. Food Agric.* **26**, 1545.
18. MacLeod, A. J. and Islam, R. (1976) *J. Sci. Food Agric.* **27**, 909.
19. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1657.
20. Gil, V. and MacLeod, A. J. (1980) *Phytochemistry* **19**, 1365.
21. Martin, M. and Juniper, B. E. (1970) *The Cuticles of Plants.* Edward Arnold, London.
22. Spence, R.-M. M. (1980) Ph. D. Thesis, University of Bath.
23. Baker, E. A. and Holloway, P. J. (1975) *Phytochemistry* **14**, 2463.