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SHORT COMMUNICATION

VOLATILE SUBSTANCES DERIVED FROM THE CUTICLE WAX OF CRANBERRY

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Abstract—Thirteen volatile substances have been identified in cranberry (*Vaccinium macrocarpon*, var. Howes) cuticle wax by gas chromatography and mass spectrometry including α-terpineol, benzoic acid and benzyl benzoate. The presence of benzoic acid and other benzenoid and terpenoid compounds in cuticle wax suggests these substances might function in a defensive mechanism.

INTRODUCTION

DURING an investigation of cranberry cuticle wax[1] it was noted that the isolated wax possessed a distinct odour and that the odour was apparently released only after the intact wax was dissolved by extraction of the berries in $CHCl_3$. This fact suggested the possibility that surface wax might function as a carrier for lower molecular weight compounds by greatly reducing their inherent volatility.

Defence against pathogenic attack is a function sometimes attributed to cuticular wax. It is quite possible that wax protects the plant by providing a chemical barrier containing substances toxic to pathogens and there are some observations in the literature to support this supposition.[2] The presence of volatile microbiocides,[3] insecticides[4] and other biologically active agents in plant extracts suggests a possible defence role for these substances in the cuticle. To exert its effect, the defensive agent must be present in sufficient concentration of an active form and must be contacted by the invading pathogen. These conditions may not be operative within the plant tissues but may be fulfilled within the cuticle where these substances may be dissolved or imbedded in cuticle wax and their volatility greatly reduced. This factor, plus the degree of concentration attainable in a relatively thin membrane, could provide an effective barrier to pathogenic attack and assumes special interest in studies of the factors underlying resistance to disease.

RESULTS AND DISCUSSION

Cranberry cuticle wax was removed from intact fruit by rapid immersion in Freon 21 (monofluorodichloromethane) in the cold. The solvent was removed and the volatiles vacuum-distilled onto a cold finger.

Thirteen compounds were identified in the volatile complex on the basis of GLC retentions and MS fragmentation patterns (Table 1). Quantitative data in Table 1 should be taken as minima as volatiles were incompletely extracted. Gas chromatograms revealed numerous components occurring at lower concentrations that were not identified. Although all the

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TABLE 1. VOLATILE SUBSTANCES DERIVED FROM CRANBERRY CUTICLE WAX

Component	% of volatile fraction	% of total wax × 100 (minimum)
Ethyl acetate	2·32	1·58
2-Pentanol	0·33	0·23
Hexanal	0·47	0·32
1-Pentanol	0·42	0 29
Octanal	0·47	0·32
Nonanal	0·58	0·39
Acetophenone	0·27	0·20
Octanol	0·66	0 45
α-Terpineol	1·47	1 00
Benzyl alcohol	0 19	0 14
2-Phenyl ethanol	0·20	0·14
Benzoic acid	84·50	57·70
Benzyl benzoate	1·05	0·71
Total unidentified	6·21	4 85

components identified in the wax were previously found in cranberry press cake and juice extracts[5,6] the quantitative proportions were quite different.

As the cranberry is noted for its high content of benzoic acid,[7,8] it is not entirely surprising to find this compound in the cuticular wax. Benzoic acid is commonly employed as a preservative in foods and pharmaceuticals and its antimicrobial properties are well documented.[9,10] It is only natural, therefore, to speculate that benzoic acid in cuticle wax contributes to a chemical defence mechanism of the fruit against disease. Although benzoic acid occurs at a level of about 2 $\mu g/cm^2$ it should be kept in mind that this material is concentrated in a wax layer approximately 3·5 μ thick. Benzyl benzoate, benzyl alcohol and α-terpineol are reported to possess antimicrobial and insect repelling properties,[10,11] but they may occur at levels too low to contribute to any defensive function. These components, as well as the others identified, probably contribute to the odour observed.

EXPERIMENTAL

Extraction of cuticle wax and wax volatiles. 1·5 kg (representing 9060 cm^2 of surface area) of whole intact cranberries, as previously described,[1] was immersed twice for 10 sec in cold 3 l. of Dupont Freon 21 (monofluorodichloromethane). The above procedure was designed to obtain a volatile sample uncontaminated with substances from internal tissue. By leaving some 40% of the wax still on the berry, this barrier contributes to preventing internal leaching. If the extracted berries were re-extracted twice with solvent, an additional amount of wax and volatiles could be removed. A fifth extraction yielded small amounts of wax but no detectable volatiles, giving further assurance that the volatile substances were derived from the wax and not from internal leaching.

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Isolation of volatile substances. The wax extract was concentrated at room temp. (b.p. Freon 21 = 8·9°) to 50 ml, ($CaSO_4$) and finally passed through a short column of $CaSO_4$. The remaining solvent was removed under slight vacuum and the wax vacuum-distilled with agitation at 100° and 1 mm Hg. Volatiles were trapped on two liquid N_2 cooled fingers in series (21·1 mg). The volatiles were taken up in Et_2O and analysed by GLC and GLC–MS. After the identification of benzoic acid, this compound was removed from the volatiles complex by extraction with 5% Na_2CO_3 and the non-acidic volatiles analysed.

Gas chromatography and mass spectroscopy. Analytical GLC was carried out using a Perkin–Elmer 900 gas chromatograph and the following column parameters: 2·4 m × 3 mm column coated with 10% UCON-550X on 70/80 mesh acid washed and silanized Chromosorb W and programmed at 50° for 3 min, then 10°/min to 210° with a flow rate of 30 ml/min. Methyl palmitate was employed as an internal standard and peak areas determined by triangulation. Mass spectra were taken on a GLC–MS system consisting of a Varian Aerograph 1200 GLC connected to a Hitachi–Perkin–Elmer RMU-6A mass spectrometer.

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