Studies on the Enzymatic Hydrolysis of Bound Aroma Constituents from Raspberry Fruit Pulp

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HRGC and HRGC–MS identifications of bound volatiles from raspberry fruit (*Rubus idaeus* cv. Héritage) pulp were achieved after isolation of an extract obtained by Amberlite XAD-2 adsorption and methanol elution followed by hydrolysis with a commercial pectinase enzyme. In total, 57 bound aglycons were identified for the first time in raspberry fruit originating from fatty acid, phenylpropanoid, and terpene metabolisms. Among them, the following C_{13} norisoprenoids were detected: 3-hydroxy-α-ionone, 3-hydroxy-β-ionone, 3-hydroxy-5,6-epoxy-β-ionone (tent.), 4-hydroxy-β-ionone, 3,4-didehydro-β-ionone, α-ionol, 3-oxo-α-ionol, 3-oxo-7,8-dihydro-α-ionol, 4-oxo-β-ionol, and 3-hydroxy-β-damascone.

INTRODUCTION

In the past, the composition of volatiles from raspberry fruit has been extensively studied, and approximately 230 aroma substances have been identified to date (Maarse, 1989). Among them, C_{13} norisoprenoids and 4-(4-hydroxyphenyl)-2-butanone ("raspberry ketone") are regarded to be key components of the raspberry aroma (Honkanen et al., 1980; Deifel, 1989). Due to the important role of glycosidically bound aroma compounds as flavor precursors (Williams et al., 1989), recently, studies of glycoconjugates of raspberry fruit volatiles have been carried out, leading to structural elucidation of the β-D-glucosides of raspberry ketone and its hydroxylated derivative, i.e., 4-(3,4-dihydroxyphenyl)-2-butanone (Pabst et al., 1990). This paper reports for the first time the occurrence of glycosidically bound volatiles in raspberry fruit.

EXPERIMENTAL PROCEDURES

Solvents. All solvents used were of high purity at purchase (Aldrich) and were redistilled before use.

Fruits. Frozen raspberry fruit pulp (*Rubus idaeus* cv. Héritage) was kindly provided by Pernod Ricard, Centre de Recherche, Créteil, France.

Isolation of the Glycosidic Extract (Gunata et al., 1985). After adjustment to pH 7 (NaOH), 300 g of fruit pulp was lyophilized and the lyophilisate extracted with 1 L of 80% aqueous methanol at room temperature. The extract was centrifuged at 10000*g* for 30 min and the centrifugate concentrated under reduced pressure. The aqueous residue was subjected to liquid chromatography on Amberlite XAD-2 adsorbent (glass column, 25 × 500 mm). After the residue was washed with 1500 mL of H_2O, elution was performed with 500 mL of methanol. The eluate was concentrated to dryness under reduced pressure (rotavapor), redissolved in 50 mL of 0.2 M citrate–phosphate buffer (pH 5.0), and extracted with diethyl ether to remove any remaining volatiles.

Enzymatic Hydrolysis. In a typical experiment, 100 μL of a nonselective pectinase (Rohapect D5L; Röhm, Darmstadt, FRG) was added to the glycosidic extract (50 mL) and the mixture incubated at 35 °C overnight. After addition of 3-octen-1-ol (45 μg) as standard, the liberated aglycons were continuously extracted (16 h) with diethyl ether and the dried (anhydrous Na_2SO_4), concentrated (Kuderna Danish, 45 °C) extract was subjected to HRGC and HRGC–MS analyses. In the same manner, a blank test without addition of enzyme was carried out.

Capillary Gas Chromatography (HRGC). A Girdel 300 gas chromatograph with FID equipped with a Chrompack fused silica CP-Wax-58-CB WCOT capillary column [30 m; 0.25 mm (i.d.); df = 0.22 μm] was used. Split/splitless (1.5 mL/min/25 mL/min) injection was employed. The temperature program was increased from 40 to 220 °C by 3 °C/min increments and held for 20 min at 220 °C. The flow rates for the carrier gas were 3 mL/min of H_2 and for the detector gases 25 mL/min of H_2 and 250 mL/min of air, respectively. The injector temperature was kept at 200 °C and the detector temperature at 250 °C.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). HRGC–MS analyses were performed with a Nermag R10-10C mass spectrometer coupled to a Girdel 31 gas chromatograph. The same column as mentioned for HRGC analysis was used. The carrier gas (He) velocity was 2.5 mL/min, and the column was directly connected to the ion source. The split/splitless (1.5 mL/min/25 mL/min) injection port was maintained at 240 °C, and the oven temperature was programmed from 40 to 220 °C at 3 °C/min. Electron impact mass spectra were recorded at 70 eV with a source temperature of 150 °C.

Results of qualitative analyses were verified by comparison of HRGC retention (R_t) and mass spectral data with those of authentic reference substances.

Reference Compounds. α-Ionol (**20**) was synthesized from α-ionone by $LiAlH_4$ reduction and subsequent LC silica gel purification of the product: mass spectral data (*m/z*, %) 95 (100), 43 (45), 138 (32), 79 (16), 91 (15), 41 (15), 93 (13), 123 (11). 3-Oxo-7,8-dihydro-α-ionol (**45**) was a donated sample. The other components listed in Table I were available in our laboratory from previous studies.

RESULTS AND DISCUSSION

A typical HRGC–MS separation of the bound aroma compounds from raspberry fruit pulp is outlined in Figure 1. Table I shows the observed elution of individual aglycons in order of increasing retention time (R_t). Quantitative evaluation was not carried out. A total of 57 compounds was identified for the first time as bound aroma constituents from raspberry fruit pulp. In a blank test performed without enzyme addition, none of the bound raspberry volatiles were detected. The identified aglycons mainly fall into three categories biogenetically derived from (i) fatty acid, (ii) shikimate, and (iii) monoterpenoid and C_{13} norisoprenoid metabolisms.

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Table I. Compounds Identified in Raspberry Fruit Pulp by HRGC and HRGC–MS after Enzymatic Hydrolysis (Rohapect D5L) of a Methanolic Eluate Obtained from XAD-Separated Fraction

peak[a]	R_t[b]	compound
1	1308	2-heptanol
2	1336	1-hexanol
3	1364	(*Z*)-3-hexen-1-ol
4	1389	(*E*)-2-hexen-1-ol
5	1431	acetic acid
6	1446	6-methyl-5-hepten-2-ol
7	1472	theaspirane A
8	1506	theaspirane B
9	1535	linalool
10	1538	1-octanol
11	1570	terpinen-4-ol
12	1606	butanoic acid
13	1645	3-methylbutanoic acid
14	1661	α-terpineol
15	1753	myrtenol
16	1773	nerol
17	1819	hexanoic acid
18	1824	geraniol
19	1841	benzyl alcohol
20	1868	α-ionol
21	1873	2-phenylethanol
22	1917	δ-octalactone
23	1957	3,4-didehydro-β-ionone
24	1977	phenol
25	2000	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone
26	2007	3-phenyl-1-propanol
27	2035	octanoic acid
28	2052	*p*-cresol
29	2131	eugenol
30	2144	δ-decalactone
31	2162	4-vinylguaiacol
32	2251	cinnamyl alcohol
33	2260	(*E*)-2,6-dimethylocta-2,7-diene-1,6-diol
34	2311	vinylphenylcarbinol
35	2316	isoeugenol
36	2369	4-vinylphenol
37	2400	benzoic acid
38	2504	3-hydroxy-β-damascone
39	2524	vanillin
40	2543	3-hydroxy-α-ionone
41	2551	4-vinylsyringol
42	2568	4-hydroxy-β-ionone
43	2591	3-oxo-α-ionol
44	2600	4-oxo-β-ionol
45	2633	3-oxo-7,8-dihydro-α-ionol
46	2641	3-hydroxy-β-ionone
47	2677	propiovanillone
48	–	3-hydroxy-5,6-epoxy-β-ionone (tent.)[c]
49	2735	(*Z*)-cinnamic acid
50	2751	4-(4-hydroxy-3-methoxyphenyl)-2-butanone (zingerone)
51	2807	2-(4-hydroxy-3-methoxyphenyl)-ethanol
52	2822	(*E*)-cinnamic acid
53	2886	hexadecanoic acid
54	2941	3-(4-hydroxy-3-methoxyphenyl)-propan-1-ol
55	2952	4-(4-hydroxyphenyl)-2-butanone (raspberry ketone)
56	2978	tyrosol
57	2985	4-hydroxyacetophenone

[a] The peak numbers correspond to the numbers in Figure 1. [b] R_t is the linear retention index based on a series of *n*-hydrocarbons. The R_t values given were coincident (±5) with that of authentic reference compounds. [c] tent. indicates the compound was tentatively assigned by means of published MS data (Hohler, 1986). For HRGC and HRGC–MS conditions, see Experimental Procedures.

Fatty Acid Derived Group. Several alkanols and alkenols as well as fatty acids were detected among the aglycons liberated by Rohapect D5L. Recently, during precursor studies of apple fruit volatiles, various glycosidically bound forms of simple aliphatic alcohols have been found for the first time in nature (Schwab and Schreier, 1990). Similarly, the occurrence of corresponding glycoconjugates can be postulated for raspberry fruit.

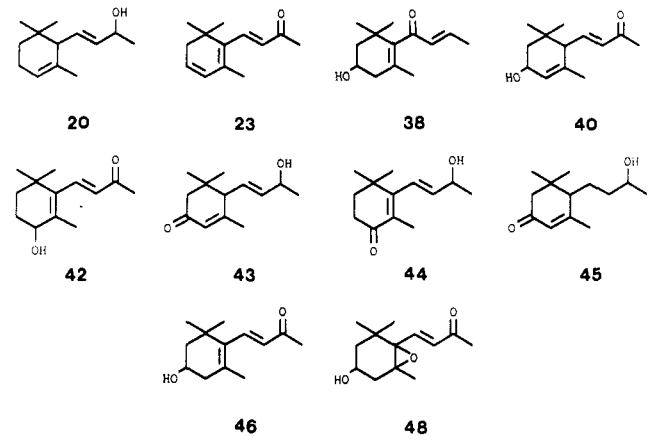

Figure 1. Structures of C_{13} norisoprenoids released by Rohapect D5L hydrolysis of a XAD-2-separated raspberry extract. The compound numbers correspond to the numbers in Table I.

Surprisingly, in this class of aglycons δ-lactones have been detected for the first time as bound volatiles in nature. They may be derived from the corresponding 5-hydroxycarboxylic acids, but any information about the structures of their native precursors is still lacking. δ-Octa- and δ-decalactone also occur as free volatiles in raspberry fruit (Maarse, 1989).

Shikimate-Derived Group. Examination of the aromatic substances listed in Table I indicates that C_6–C_1 to C_6–C_4 compounds, exhibiting a variety of hydroxy and methoxy substitution patterns, are present in the raspberry precursor fraction. These aromatic compounds are known to be derived in the plant's metabolism from phenylpropanoids via different side-chain degradation and elongation pathways (Gross, 1981). Recently, the importance of this group of secondary plant metabolites has been elucidated as the precursor fraction of grape flavor components (Williams et al., 1989).

Monoterpenes and C_{13} Norisoprenoids. The monoterpene alcohols linalool (**9**), terpinen-4-ol (**11**), α-terpineol (**14**), myrtenol (**15**), nerol (**16**), and geraniol (**17**) were identified that are all known as free aroma volatiles in raspberry fruit (Maarse, 1989). The diol **33** has not as yet been observed in raspberry; however, the aglycon has been identified in tobacco (Behr et al., 1978), papaya fruit (Winterhalter et al., 1986), and Morio-Muskat grapes and wine (Rapp et al., 1986). In addition, these authors along with Bock et al. (1986) have shown that the diol **33** is the major transformation product of linalool (**9**) by the fungus *Botrytis cinerea*. Previously, the β-D-glucoside of **33** has been found in *Betula alba* leaves and *Chaenomeles japonica* fruit (Tschesche et al., 1977), while, more recently, it has been detected in sour cherry fruit (Schwab et al., 1990).

The C_{13} norisoprenoid compounds comprise nine ionone derivatives and one damascone derivative (Figure 2). Due to a lack of authentic reference material, 3-hydroxy-5,6-epoxy-β-ionone (**48**) was tentatively identified on the basis of published MS data alone (Hohler, 1986). Compound **48** has been detected recently as β-D-glucoside in *Epimedium grandiflorum* Morr. var. thunbergianum (Miyase et al., 1987). Among the other 3-oxygenated ionone compounds, **43** and **46** have been found in earlier studies as bound volatiles from quince fruit (Winterhalter and Schreier, 1988).

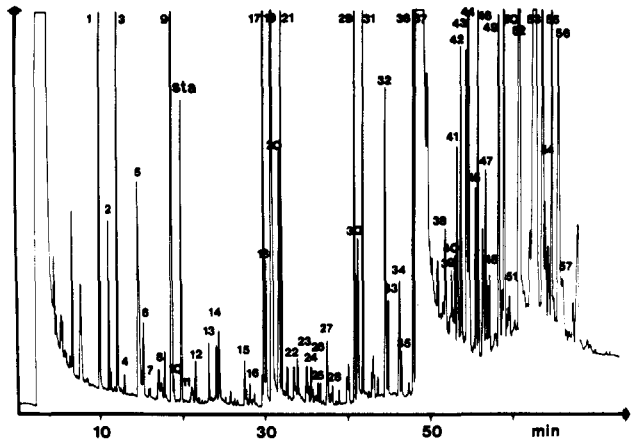

Figure 2. HRGC separation of raspberry fruit aglycons on a Chrompack fused silica CP-Wax-58-CB WCOT capillary column (30 m × 0.25 mm (i.d.); df = 0.22 μm). The numbers correspond to the numbers outlined in Table I. Sta = standard, 3-octen-1-ol.

Previous investigations on tobacco flavor have shown that 3-hydroxy-α-ionone (**40**) can be converted to **43** by oxidation of the hydroxy group and reduction of the keto moiety (Enzell, 1981). Compound **46** can be regarded as a precursor of the didehydro derivative **23** that may originate from **46** by simple dehydration. 3-Oxo-7,8-dihydro-α-ionol (**45**), also known as blumenol C (Galbraight and Horn, 1972), has recently been identified among the bound volatiles from purple passion fruit (Winterhalter, 1990). Whereas α-ionol (**20**) has been found among the volatiles of raspberry spirits (Postel and Adam, 1983), 3-hydroxy-β-damascone (**38**) has not been detected in raspberry until now. Compound **38** is also known as a glucosidically bound tobacco flavor constituent (Kodama et al., 1984).

The 4-oxygenated ionone derivatives **42** and **44** are known volatile constituents of *Osmanthus* absolute (Kaiser and Lamparsky, 1978) as well as free and bound components from quince and purple passion fruits (Winterhalter and Schreier, 1988; Winterhalter, 1990).

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Registry No. 2-Heptanol, 543-49-7; 1-hexanol, 111-27-3; (*Z*)-3-hexen-1-ol, 928-96-1; (*E*)-2-hexen-1-ol, 928-95-0; acetic acid, 64-19-7; 6-methyl-5-hepten-2-ol, 1569-60-4; theaspirane A, 66537-39-1; theaspirane B, 66537-40-4; linalool, 78-70-6; 1-octanol, 111-87-5; terpinen-4-ol, 562-74-3; butanoic acid, 107-92-6; 3-methylbutanoic acid, 503-74-2; α-terpineol, 98-55-5; myrtenol, 515-00-4; nerol, 106-25-2; hexanoic acid, 142-62-1; geraniol, 106-24-1; benzyl alcohol, 100-51-6; α-ionol, 25312-34-9; 2-phenylethanol, 60-12-8; δ-octalactone, 698-76-0; 3,4-didehydro-β-ionone, 14398-35-7; phenol, 108-95-2; 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, 3658-77-3; 3-phenyl-1-propanol, 122-97-4; octanoic acid, 124-07-2; *p*-cresol, 106-44-5; eugenol, 97-53-0; δ-decalactone, 705-86-2; 4-vinylguaiacol, 7786-61-0; cinnamyl alcohol, 104-54-1; (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol, 51724-50-6; vinylphenylcarbinol, 4393-06-0; isoeugenol, 97-54-1; 4-vinylphenol, 2628-17-3; benzoic acid, 65-85-0; 3-hydroxy-β-damascone, 35734-61-3; vanillin, 121-33-5; 3-hydroxy-α-ionone, 129966-83-2; 4-vinylsyringol, 28343-22-8; 4-hydroxy-β-ionone, 116296-75-4; 3-oxo-α-ionol, 34318-21-3; 4-oxo-β-ionol, 80945-23-9; 3-oxo-7,8-dihydro-α-ionol, 129966-84-3; 3-hydroxy-β-ionone, 14398-34-6; propiovanillone, 1835-14-9; 3-hydroxy-5,6-epoxy-β-ionone, 38274-01-0; (*Z*)-cinnamic acid, 102-94-3; zingerone, 122-48-5; 2-(4-hydroxy-3-methoxyphenyl)-ethanol), 2380-78-1; (*E*)-cinnamic acid, 140-10-3; hexadecanoic acid, 57-10-3; 3-(4-hydroxy-3-methoxyphenyl)propan-1-ol, 2305-13-7; raspberry ketone, 5471-51-2; tyrosol, 501-94-0; 4-hydroxyacetophenone, 99-93-4.