

Glycosidically Bound Aroma Compounds in the Fruits of *Prunus* Species: Apricot (*P. armeniaca*, L.), Peach (*P. persica*, L.), Yellow Plum (*P. domestica*, L. ssp. *Syriaca*)

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HRGC and HRGC-MS identifications of bound aroma compounds from fruits of *Prunus* species (apricot, *P. armeniaca*, L.; peach, *P. persica*, L.; yellow plum, *P. domestica*, L. ssp. *syriaca*) were achieved after isolation of extracts obtained by Amberlite XAD-2 adsorption and methanol elution followed by simultaneous enzyme catalysis extraction (SECE) using β -glucosidase (emulsin). In total, 47 bound aglycons were identified, from which 21 originated from the terpene metabolism. Among them, the following monoterpene diols were found: 3,7-dimethyloct-1-ene-3,7-diol; (*E*)-2,6-dimethylocta-2,7-diene-1,6-diol; 2,6-dimethyl-1,8-octanediol; and (2*E*,6*Z*)-3,7-dimethyl-2,6-octadiene-1,8-diol. The group of C_{13} norisoprenoids comprises 3- and 4-hydroxy- β -damascones, 3-hydroxy-7,8-dihydro- β -ionone, 3-oxo- α -ionol, 3-hydroxy- β -ionol, 3-hydroxy-7,8-dihydro- β -ionol, 3-hydroxy- β -ionone, 3-hydroxy-5,6-epoxy- β -ionone, dehydrovomifoliol, and vomifoliol. In addition, in peach the isomeric marmelolactones were detected.

INTRODUCTION

Whereas the composition of volatiles from fruits of *Prunus* species, such as apricot (*P. armeniaca*, L.) (Tang and Jennings, 1967, 1968; Chairote et al., 1981; Guichard and Souty, 1988; Takeoka et al., 1990), peach (*P. persica*, L.) (Jennings and Sevenants, 1964; Lim and Romani, 1964; Sevenants and Jennings, 1966; Do et al., 1969; Broderick, 1975; Spencer et al., 1978; Horvat et al., 1990), and yellow plum (Vernin et al., 1985a,b) has been extensively studied, information about their glycosidically bound aroma constituents is still lacking. Previous studies carried out on grapes (Williams et al., 1982a,b), passion fruit (Engel and Tressl, 1983; Winterhalter, 1990), apple (Schwab and Schreier, 1988), papaya (Schwab et al., 1989), quince (Winterhalter and Schreier, 1988), sour cherry (Schwab and Schreier, 1990a), and raspberry (Pabst et al., 1991) have shown that volatile secondary metabolites, including fatty acid and shikimate derived as well as terpenoid compounds, accumulate in these fruits as nonvolatile conjugates. These investigations also demonstrated the important role of glycoconjugates as flavor precursors. This paper concerns the results obtained after enzymatic hydrolysis of glycosidic extracts separated from apricot, peach, and yellow plum fruits.

EXPERIMENTAL PROCEDURES

Fruits. Fresh ripe fruits (*P. armeniaca*, L. cv. Reale; *P. persica*, L. cv. Redhaven; *P. domestica*, L. ssp. *syriaca* cv. Nancy) were obtained from the local market.

Isolation of Glycosidic Extracts. Each of 5-kg fruits was cut, and the seeds were removed. After homogenization with 1 L of 0.2 M phosphate buffer (pH 7.5) containing 0.2 M glucono- δ -lactone and centrifugation (30 min, 10000g), the supernatant was subjected to LC chromatography on Amberlite XAD-2 adsorbent using a 25 \times 900 mm glass column (Gunata et al., 1985). After being washed with 1500 mL of H₂O and 500 mL of pentane, the extract was isolated by eluting with 1000 mL of MeOH. The MeOH fraction was concentrated under reduced pressure to dryness (rotavapor) and redissolved in 80 mL of 0.2 M phosphate buffer (pH 5.5). Remaining volatiles were removed by diethyl ether extraction.

SECE. Simultaneous enzyme catalysis extraction (SECE) was

performed at 37 °C over 7 days by using 50 mg of emulsin (Boehringer) as previously described in detail (Schwab and Schreier, 1988).

Capillary Gas Chromatography (HRGC). A Carlo Erba Fractovap 4100 gas chromatograph equipped with FID was used. Two types of WCOT fused silica capillary columns were employed: (a) J&W DB-Wax (30 m, 0.25 mm i.d., film thickness 0.25 μ m); (b) J&W DB-1701-30W (30 m, 0.32 mm i.d., film thickness 0.25 μ m). Split injection (1:30) was used. The temperature programs were 3 min isothermal at 50 °C increased at 4 °C/min up to 240 °C (a) and 1 min isothermal at 60 °C increased at 4 °C/min up to 270 °C (b). The flow rate for the carrier gas was 2.0 mL/min of He, for the makeup gas 30 mL/min of N₂, and for the detector gases 30 mL/min of H₂ and 300 mL/min of air, respectively. The injector temperature was kept at 220 °C and the detector temperature at 250 °C. Volumes of 1.0 μ L were injected.

Capillary Gas Chromatography-Mass Spectrometry (HRGC-MS). A Varian Aerograph 1440 gas chromatograph equipped with a split injector was combined by direct coupling to a Finnigan MAT 44 mass spectrometer with PCDS data system. The same types of columns as mentioned above for HRGC analysis were used. The conditions were as follows: temperature program (a) from 40 to 230 °C at 4 °C/min, (b) from 50 to 270 °C at 3.5 °C/min; carrier gas flow rate, 2.5 mL/min of He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.7 mA. Volumes of 1.0 μ L were injected.

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

Reference Compounds. Ketoalcohol 37 was prepared by hydroboration of 3,4-didehydro-7,8-dihydro- β -ionone (Wolf and Zink, 1973) and showed the following mass spectral data (m/z , %): 210 (M^+ , <1), 192 (10), 177 (7), 159 (17), 149 (8), 133 (12), 121 (37), 119 (100), 107 (25), 93 (26), 79 (17), 67 (15), 55 (18), 43 (93). Compound 45 was obtained by epoxidation of keto alcohol 43 (Winterhalter and Schreier, 1988) with *m*-chloroperbenzoic acid. Mass spectral data (m/z , %): 224 (M^+ , <1), 209 (<1), 191 (2), 165 (1), 151 (2), 149 (2), 137 (2), 123 (73), 109 (6), 95 (8), 79 (5), 67 (4), 55 (8), 43 (100). The other reference compounds were available in our laboratory or commercially obtained.

RESULTS AND DISCUSSION

Using β -glucosidase (emulsin), simultaneous enzyme catalysis extraction (SECE) (Schwab and Schreier, 1988)

Table I. Compounds Identified in Apricot (I), Peach (II), and Yellow Plum (III) Fruits by HRGC and HRGC-MS after Enzymatic Hydrolysis of Methanolic Eluates Obtained from XAD-Separated Fraction

no. ^a	compound	R _i ^b	identified in		
			I	II	III
1	1-butanol	1136	+		
2	3-methyl-1-butanol	1215	+	+	+
3	3-hydroxy-2-butanone	1253	+	+	+
4	1-hexanol	1353	+	+	+
5	(E)-2-hexen-1-ol	1390	+	+	+
6	acetic acid	1415	+	+	+
7A	(Z)-linalool oxide, furanoid	1431	+		
7B	(E)-linalool oxide, furanoid	1461	+		
8	benzaldehyde	1502	+	+	+
9	linalool	1544	+	+	+
10	butanoic acid	1598	+	+	+
11	phenylacetaldehyde	1617	+		
12/13	2- and 3-methylbutanoic acid	1649	+	+	+
14	α -terpineol	1680	+	+	+
15A	(Z)-linalool oxide, pyranoid	1723	+	+	
15B	(E)-linalool oxide, pyranoid	1746	+	+	
16	methyl salicylate	1757			+
17	nerol	1790	+		
18	hexanoic acid	1840	+	+	+
19	geraniol	1848	+	+	+
20	benzyl alcohol	1864	+	+	+
21	2-phenylethanol	1899	+	+	+
22	3,7-dimethyloct-1-ene-3,7-diol	1964	+		
23	phenol	1978	+	+	+
24	octanoic acid	2050	+	+	+
25A	marmelolactone, I	2095	+		
26	eugenol	2098	+	+	+
25B	marmelolactone, II	2116	+		
26	4-vinylguaiaicol	2181	+	+	+
27	cinnamyl alcohol	2263	+		
28	(E)-2,6-dimethylocta-2,7-diene-1,6-diol	2294	+	+	+
29	4-vinylphenol	2384	+	+	+
30	benzoic acid	2420	+	+	+
31	2,6-dimethyl-1,8-octanediol	2435	+		
32	4-hydroxy- β -damascone	2451	+		
33	dodecanoic acid	2472	+	+	+
34	3-hydroxy- β -damascone	2504	+		
35	vanillin	2569	+	+	+
36	(2E,6Z)-3,7-dimethyl-2,6-octadiene-1,8-diol	2585	+	+	
37	3-hydroxy-7,8-dihydro- β -ionone	2589	+	+	
38	3-oxo- α -ionol	2656	+	+	
39	3-hydroxy- β -ionol	1881 ^c	+	+	
40	tyrosol	1904 ^c	+	+	+
41	3-hydroxy-7,8-dihydro- β -ionol	1910 ^c	+	+	+
42	4-hydroxyacetophenone	1934 ^c	+	+	+
43	3-hydroxy- β -ionone	1977 ^c	+	+	+
44	4-(4-hydroxyphenyl)-2-butanone	1989 ^c	+		
45	3-hydroxy-5,6-epoxy- β -ionone	2001 ^c	+	+	+
46	vomifoliol	2221 ^c	+	+	+
47	dehydrovomifoliol	2314 ^c	+	+	+

^a The numbers correspond to the numbers in Figures 1 and 2.

^b R_i = Linear retention index based on a series of hydrocarbons. The R_i values given were coincident with that of authentic reference compounds (DB-Wax; except for ^cR_i on DB-1701). For HRGC and HRGC-MS conditions, see Experimental Procedures.

of extracts obtained from apricot, peach, and yellow plum fruit pulp by XAD-2 adsorption and methanolic elution was performed over 7 days at 37 °C. The enzymatically released aglycons were then identified by HRGC and HRGC-MS. In total, 47 substances were detected for the first time as bound aroma constituents in the fruits of *Prunus* species mentioned above (Table I). Quantitative evaluation was not carried out. In agreement with previous studies (Williams et al., 1989; Pabst et al., 1991), they mainly fell into three categories biogenetically derived from fatty acid, phenylpropanoid, and terpene metabolisms. Whereas for the aroma substances for the two first-mentioned categories and the monoterpene alcohols the

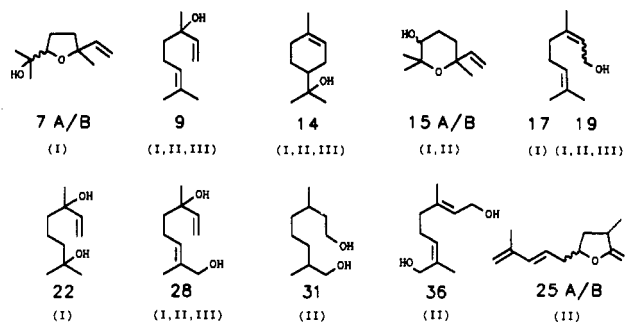


Figure 1. Structures of monoterpene aglycons identified in apricot (I), peach (II), and yellow plum (III) fruits.

principal biogenetic pathways of their formation and metabolism have been elucidated (Croteau, 1984), the situation is less clear concerning monoterpene diols and C₁₃ norisoprenoids (Enzell, 1985; Strauss et al., 1986).

Monoterpene Conjugates. The significance of glycosidically bound monoterpenes to the flavor of numerous fruits is well documented (Williams et al., 1982a,b; Engel and Tressl, 1983; Strauss et al., 1986). In addition to those monoterpenes occurring free in the fruit, further volatiles can be formed in juices by acid hydrolysis of flavorless monoterpene conjugates. Besides this acid-catalyzed generation of flavor compounds, considerable attention has also been drawn in past years to the enzymatic liberation of monoterpenes from their precursor forms (Gunata et al., 1989). The bound aroma fraction is often much larger than the free aroma fraction (for the *Prunus* species under study by factors ranging from 3 to 8) with absolute concentrations in the order of a few milligrams per liter. This together with the particularly low flavor thresholds and the aromatic properties of monoterpenes makes the glycosidic fraction of fruits an important unexploited aroma potential.

In the present study also for *Prunus* species a broad range of bound monoterpenes was found (Figure 1). The conjugates consisted predominantly of two groups of related structures: first, the common alcohols linalool (9), α -terpineol (14), nerol (17), and geraniol (19); second, several hydroxylated monoterpene derivatives 22, 28, 31, and 36, the so-called polyols. Contrary to the monoterpenes 9, 14, 17, and 19, the polyols, when enzymatically released from their bound forms, will not exhibit any flavor properties unless they are thermally degraded to more volatile products (Williams et al., 1980). With regard to the sugar moieties involved in conjugating monoterpenes, numerous examples can be found for the monoterpenes mentioned above (Ishag et al., 1985; Stahl-Biskup, 1987).

In the case of cyclic ethers 7 and 15 less information is available. Both structures, which are biogenetically most likely derived from 6,7-epoxylinalool (Winterhalter et al., 1986), have been identified as aglycons in *Vitis vinifera* grapes (Strauss et al., 1987; Winterhalter et al., 1990). Recently, two conjugated forms of the cis-furanoid linalool oxide 7A have been isolated from Riesling wine and elucidated as the 6-O- α -L-arabinofuranosyl- β -D-glucopyranoside and the β -D-glucopyranoside, respectively (Winterhalter et al., 1990).

As to the natural occurrence of the bound polyols 22, 28, 31, and 36 only conjugates of the diol 28 have been fully characterized until now. The β -D-glucopyranoside of 28, known as betulalbuside A, has been identified in leaf extracts of *Betula alba* and fruits of *Chaenomeles japonica* (Tschesche et al., 1977). In grapes, in addition to the β -D-glucopyranoside form, also the 6- α -L-arabinofuranosyl- β -D-glucopyranoside of 28 has been found

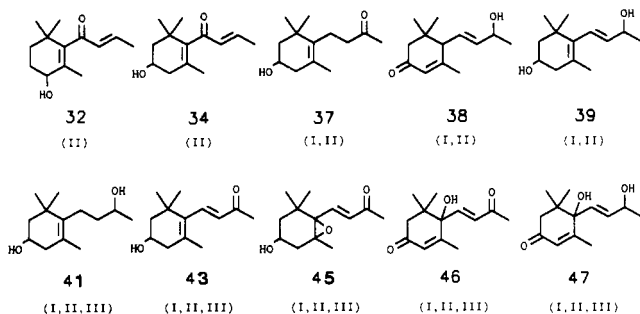


Figure 2. Structures of C₁₃ norisoprenoid aglycons identified in apricot (I), peach (II), and yellow plum (III) fruits.

(Strauss et al., 1988). In the same study, the role of 28 as potential progenitor of monoterpene flavorants has been investigated. Thermal treatment at pH 3 revealed the formation of a number of conversion products including 3,9-epoxy-*p*-menth-1-ene, the so-called dill ether (Schreier et al., 1981).

C₁₃ Norisoprenoid Conjugates. Enzymatic treatment of glycosidic extracts from each of the three *Prunus* species under investigation liberated numerous C₁₃ norisoprenoid aglycons (Figure 2), plant constituents, which are generally considered to be derived from carotenoids by oxidative degradation (Enzell, 1985). Only in the bound fraction of peach were two damascone derivatives detected. The 3-hydroxy derivative 34 has already been reported as bound constituent of several fruit tissues, such as grape (Strauss et al., 1987), apple (Schwab and Schreier, 1988), and papaya (Schwab et al., 1989). Additionally, the β-D-glucopyranoside of 34 has been previously isolated from flue-cured Virginia tobacco (Anderson et al., 1978).

The second damascone derivative bearing the hydroxy group in the 4-position was rarely found previously. The identification as aglycon in peach is the first report of the occurrence of a bound form of 32 in nature. However, 32 has been earlier seen as a free volatile in Virginia tobacco (Bolt et al., 1983). In the same study dehydration reaction with 32 led to the formation of the potent flavorant damascenone, but nothing is known about the behaviour of the glycosidically bound 32 under natural pH conditions.

A further important group of *Prunus* aglycons consisted of a series of five biogenetically presumably related 3-hydroxyionone derivatives, i.e., 37, 39, 41, 43, and 45. Among these norisoprenoids, 39, 41, and 43 have been identified as major aglycons in quince fruit (Winterhalter and Schreier, 1988). In the same study, the role of diol 39 as flavor precursor has been elucidated. In biomimetic studies with 39 the formation of numerous degradation products has been observed, which are regarded as important contributors of the overall flavor impression of quince fruit. In addition, diol 39 and the keto derivative 43 have been identified as free compounds in Burley tobacco (Fujimori et al., 1976).

The side chain saturated keto alcohol 37 is a new compound in fruit tissues. Previously, it has only been found as a conjugate in a water extract of *Epimedium grandiflorum* var. *thunbergianum* (Miyase et al., 1988). The same plant contained also the β-D-glucopyranoside of the epoxy compound 45 (Miyase et al., 1987).

The remaining 3-keto derivatives 38, 46, and 47 are common constituents in plant tissues. Several conjugated forms of 3-oxo-α-ionol (38) have earlier been reported, including the β-D-glucopyranoside (Mpondo Mpondo et al., 1989) and the α-L-arabinopyranosyl 6-β-D-glucopyranoside (Chulia et al., 1987). Vomifoliol (46) has been found several times in plants, mainly as the β-D-glucoside, the so-called roseoside, and recently also as a 1-O-β-D-xylopy-

ranosyl 6-O-β-D-glucopyranoside in apple fruit (Schwab and Schreier, 1990).

Lastly, attention has to be drawn to the identification of isomeric marmelolactones 25A/B in the aglycon fraction of peach. These lactones have previously been isolated from quince essential oil and are regarded as key flavor components in quince fruit (Tsuneya et al., 1980). Studies regarding the nature of the conjugates giving rise to the formation of 25A/B will remain the subject for continuing research.

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