

# Studies of Aroma Constituents Bound as Glycosides in Tomato

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Glycosidically bound volatiles in fresh tomato have been studied. The glycosides were isolated from an aqueous extract of tomatoes by adsorption onto a column of Amberlite XAD-2, followed by washing of the column with hexane and subsequent elution using methanol; the volatiles were later released from the methanol extract by enzyme-mediated hydrolysis using either a  $\beta$ -glucosidase or a pectinase. Major volatile compounds identified by GC/MS included 2-phenylethanol, benzyl alcohol, benzoic acid, and several shikimate-type products. Also found in small quantities were many monoterpene alcohols and C<sub>13</sub> norisoprenoids. The role of glycosides as possible flavor precursors in tomato is discussed.

## INTRODUCTION

Tomato volatiles have been the subject of many studies. Buttery et al. (1990a), for example, noted that fresh and processed tomatoes contain more than 400 volatile compounds. The characteristic aroma of tomato arises from a complex mixture of compounds, many of which have very low flavor thresholds. Most of these important flavor compounds are present, at least in the free form, only at extremely low levels. Recent studies on fruits and vegetables have shown that a significant portion of volatile flavor compounds may occur in many plants as nonvolatile precursors, most often as glycosides (Williams et al., 1989). Free volatile compounds of sensory significance may be released from these odorless precursors by enzyme-mediated hydrolysis. Such a procedure allows the isolation of trace plant constituents in higher yield and offers a potential new or enhanced source of natural flavor material.

Glycosides have been found both in fresh and in processed tomatoes (*Lycopersicon esculentum*). Fleuriot and Macheix (1980) reported the glucosides and glucosyl esters of *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. Many other known tomato constituents, e.g., benzyl alcohol and 2-phenylethanol, have also been isolated as glycosides from natural sources (Williams et al., 1983). Meanwhile, studies on flavor precursors in grape (Strauss et al., 1987; Williams et al., 1989), apple (Schwab and Schreier, 1988), papaya (Schwab et al., 1989), ginger (Wu et al., 1990), pineapple (Wu et al., 1991), quince fruit (Winterhalter and Schreier, 1988), and raspberry fruit pulp (Pabst et al., 1991) have provided evidence of the existence of glycosides of many monoterpene alcohols, C<sub>13</sub> norisoprenoids, and shikimate-type products in nature; all are important classes of flavor compound in tomatoes. Recently, Buttery et al. (1990b) isolated glycosidic fractions from fresh and processed tomatoes and identified several important volatile aglycons following acid hydrolysis. However, they found only a few volatile compounds in small quantities using enzyme-mediated hydrolysis. The present work demonstrates the presence of many glycosides in fresh tomato by enzyme-mediated hydrolysis.

## EXPERIMENTAL PROCEDURES

**Materials.** Ripe tomatoes (*L. esculentum*) were purchased from a local market. HPLC grade hexane, dichloromethane, and methanol were obtained from Fisher Scientific Co. (Springfield,

NJ). Amberlite XAD-2 nonionic polymeric adsorbent was purchased from Mallinckrodt, Inc. (Paris, KY). The methylation reagent MethElute and deuteromethylation reagent Tri-Deuter-8 were purchased from Pierce (Rockford, IL). Almond  $\beta$ -glucosidase was purchased from Sigma Chemical Co. (St. Louis, MO). Pectinol 60G, a pectinase, was obtained from Genencor, Inc. (South San Francisco, CA). Zingerone was manufactured at Givaudan Corp. (Clifton, NJ). 1-Decanol was purchased from Aldrich Chemical Co. (Milwaukee, WI).

**Isolation and Enzyme-Mediated Hydrolysis of Glycosides.** Tomatoes (1.15 kg) were sliced and blended in 100 mL of distilled water. After centrifugation and filtration to remove suspended matter, approximately 750 mL of clear juice was obtained. Compounds occurring as glycosides were isolated by XAD-2 column chromatography according to the method described by Gunata et al. (1985) with slight modification. The column was washed first with water (1.5 L) and then hexane (1 L) and was next eluted using methanol (1.5 L). The methanol eluate was dried over anhydrous sodium sulfate, concentrated to dryness under vacuum, and redissolved in 25 mL of 0.2 M citric acid/phosphate buffer (pH 5). This solution was washed exhaustively with dichloromethane (3 × 50 mL) to remove free volatiles. Almond  $\beta$ -glucosidase (30 mg) or pectinol 60G (30 mg) was then added, and the mixture was incubated for 72 h at 37 °C. The volatile compounds released by enzymatic hydrolysis were extracted with dichloromethane (3 × 20 mL). The combined dichloromethane extracts were divided into two equal fractions designated A and B. Fraction A was dried over anhydrous sodium sulfate and then concentrated under vacuum to a final volume of 5 mL. Fraction B was washed with 20 mL of 5% NaOH solution and concentrated in the same manner. A blank experiment was also conducted; 25 mL of the citric acid/phosphate buffer alone was incubated with enzyme, extracted, and concentrated as above.

**Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS).** GC was carried out on a Hewlett-Packard 5890/Series II gas chromatograph equipped with an FID and HP 3365 ChemStation software. GC/MS was carried out on a Finnigan 4500 mass spectrometer coupled to a Varian 3600 gas chromatograph. All operating conditions are the same as previously described (Wu et al., 1990).

A J&W fused silica DB-Wax capillary column (30 m × 0.25 mm i.d., film thickness 0.25  $\mu$ m) was used to analyze fraction B. Fraction A, which contained mostly acids and phenols, was methylated with MethElute prior to the GC and GC/MS analyses, using a J&W fused silica DB-1 capillary column (30 m × 0.25 mm i.d., film thickness 0.25  $\mu$ m). Under these conditions phenolic and acidic functional groups, respectively, were converted into their corresponding methoxy derivatives and methyl esters. Uncertainty may arise in cases where the original aglycon molecules contain methoxy or methyl ester functional groups. To determine whether these functional groups were part of the original molecule or were added during methylation, fraction A

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**Table I. Neutral Compounds in Tomato Bound as Glycosides**

no.	compound	$I_k^a$	concn, ppb
1	2-methyl-1-butanol	1223	77
2	1-pentanol	1270	tr <sup>b</sup>
3	4-methyl-1-pentanol	1333	9
4	3-methyl-1-pentanol	1345	12
5	1-hexanol	1372	tr
6	6-methylhept-5-en-2-ol	1478	10
7	benzaldehyde	1509	4
8	linalool	1557	7
9	$\alpha$ -terpineol	1687	11
10	citronellol	1735	tr
11	nerol	1794	tr
12	geraniol	1834	2
13	benzyl alcohol	1845	295
14	2-phenylethanol	1880	825
15	3-phenylpropanol	2037	tr
16	( <i>Z</i> )-2,6-dimethylocta-2,7-diene-1,6-diol	2245	tr
17	unidentified monoterpene alcohol	2265	70
18	( <i>E</i> )-2,6-dimethylocta-2,7-diene-1,6-diol	2296	15
19	3-hydroxy- $\beta$ -damascone	2544	11
20	3-oxo- $\alpha$ -ionol	2644	39
21	3-hydroxy-7,8-dihydro- $\beta$ -ionone	2566	tr
22	3-hydroxy- $\alpha$ -ionol	2656	tr
23	unidentified norisoprenoid	2715	tr
24	megastigma-5-en-7-yne-3,9-diol	2758	5

<sup>a</sup> Kovats retention indices (DB-Wax) based on *n*-hydrocarbons.

<sup>b</sup> Sub parts per billion level.

was also deuterated with Tri-Deuter-8 and analyzed by GC/MS in the same manner.

Zingerone and 1-decanol were added to fractions A and B, respectively, as the internal standards for quantitative analyses. The concentrations thus calculated referred to that of the isolated aglycons rather than the original concentrations in fresh tomato. Since FID response factors were not calculated for individual components in the samples, the results should be considered only semiquantitative.

## RESULTS AND DISCUSSION

The methanol fraction from the XAD column was washed with dichloromethane until no significant odor could be detected. After hydrolysis using  $\beta$ -glucosidase or pectinol 60G, an aroma resembling that of dry tomato had developed. The volatile compounds released by enzymatic hydrolysis are summarized in Tables I and II. All identifications were made by comparing mass spectra (MS) and retention indices (RI) to either the published data or a proprietary Givaudan database. For certain compounds, whose authentic MS or RI data are not available, the structures were tentatively deduced through interpreting their mass spectra. Major characteristic mass ions from a few selected compounds are summarized in Table III.

It should be pointed out that although enzymes specific to the glycoside linkage were used, there is no absolute proof that every product of such enzymatic hydrolysis must be derived from a glycoside precursor unless the particular glycoside can be isolated and identified. For example, phenolic acids occur widely in the plant kingdom in various forms of conjugate other than glycosides (Barz et al., 1985). There was, however, indirect evidence that glycosides do occur in both fresh and processed tomato (Buttery et al., 1990b). Compounds released by the glycoside-specific enzyme have been described in recent literature as "glycosidically bound".

2-Phenylethanol and benzyl alcohol were the major components found in the bound fraction of tomato.

Fraction A also contained numerous acids and phenols at a relatively high level in comparison to the levels of neutral components. Many of the latter could be detected only in fraction B after the removal of acids and phenols by caustic wash. Some of these compounds are discussed below.

**Monoterpene Alcohols.** Monoterpene alcohols are important constituents in many essential oils, although only a few of them (8–12, 16–18) were found glycosidically bound in tomato. Similar monoterpene alcohols have been found in the bound fraction of many other plants. However, studies on grape have shown that several other enzymes, e.g., pectinase Rohapect C (Williams et al., 1982) and acid phosphatase (Schwab et al., 1989), exhibit a considerably higher activity over  $\beta$ -glucosidase in releasing certain monoterpene alcohols. It is possible that grapes may contain monoterpene alcohol precursors on which  $\beta$ -glucosidase possesses only weak activity. In the present study we used both  $\beta$ -glucosidase and pectinol 60G yet observed no significant difference in the amount and/or type of monoterpene alcohols released.

**C<sub>13</sub> Norisoprenoids.** C<sub>13</sub> norisoprenoids are important aroma constituents both in fresh and in processed tomato (Buttery et al., 1990a). Because of their extremely low odor thresholds,  $\beta$ -damascenone and  $\beta$ -ionone are considered to be major contributors to tomato flavor (Buttery et al., 1988, 1990c). In a previous paper (Marlatt et al., 1991), we have described six C<sub>13</sub> norisoprenoids (19–24) released from glycosidic precursors in tomatoes by  $\beta$ -glucosidase. Only four of these C<sub>13</sub> norisoprenoids, (19, 20, 23, 24) were detected when pectinol 60G was used. Among these, 19 and 24 are of particular interest because they play a central role in the mechanism proposed by Ohloff et al. (1973) and Isoe et al. (1973) to explain the formation of  $\beta$ -damascenone through a carotenoid degradation process. According to this mechanism, megastigma-6,7-diene-3,5,9-triol, a carotenoid degradation product, dehydrates to 24, which can be converted into  $\beta$ -damascenone and 19 under strong acidic conditions. 24 has been identified in Burley tobacco (Fujimori et al., 1975) and grape (Sefton et al., 1989). Hydrolytic studies by Sefton et al. (1989) have shown that 24 is the precursor of 19 and  $\beta$ -damascenone during wine storage. Buttery et al. (1990b) reported the presence of  $\beta$ -damascenone, but not 24, in the glycosidically bound fraction of fresh tomato. However, under their drastic isolation conditions, 24 has probably gone through rearrangement or degradation to form  $\beta$ -damascenone. Under the much milder isolation conditions used in this study, we were able to detect 19 and 24, but not  $\beta$ -damascenone, in the glycosidically bound fraction of fresh tomatoes.

**Acids and Phenols.** Both aliphatic and aromatic acids were found in the glycosidically bound fraction of fresh tomato. Buttery et al. (1990b) found 3-methylbutyric acid (25) as the major aglycon in processed tomato. They subsequently reported that 3-methylbutyric acid was essential to the desirable aroma of processed tomato (Buttery et al., 1990c). Other acids, including 2-methylbutyric acid (26), hexanoic acid (28), and benzoic acid (30), have also been found to occur in nature as glycosides (Schwab and Schreier, 1988; Pabst et al., 1991).

Phenolic acids, such as *p*-coumaric acid (42), ferulic acid (49), caffeic acid (50), and sinapic acid (52), are widely distributed in the plant kingdom. They most often occur in the form of conjugates such as esters, amides, or glycosides (Barz et al., 1985). These phenylpropanoic acids are derived from carbohydrates through the complex shikimate pathway. Further metabolism, involving mostly side-chain reactions, leads to numerous aromatic natural

Table II. Acids and Phenols in Tomato Bound as Glycosides

no.	compound	$I_k^b$	concn, ppb
25	3-methylbutyric acid	766 <sup>c</sup>	44
26	2-methylbutyric acid	768 <sup>c</sup>	12
27	3-methylpentanoic acid	866 <sup>c</sup>	13
28	hexanoic acid	906 <sup>c</sup>	18
29	3-methyl-2-pentenoic acid	926 <sup>c</sup>	6
30	benzoic acid	1093 <sup>c</sup>	1040
31	4-hydroxybenzaldehyde	1213 <sup>c</sup>	12
32	4-hydroxyacetophenone	1310 <sup>c</sup>	10
33	4-hydroxybenzoic acid	1337 <sup>c</sup>	49
34	2-methoxy-4-allylphenol (eugenol)	1369 <sup>c</sup>	74
35	3-methoxy-4-hydroxybenzaldehyde (vanillin)	1427 <sup>c</sup>	25
36	3-(4-hydroxyphenyl)-1-propanol	1449 <sup>c</sup>	215
37	3-(4-hydroxyphenyl)propionic acid <sup>e</sup>	1489 <sup>c</sup>	106
38	3-methoxy-4-hydroxyacetophenone	1515 <sup>c</sup>	44
39	2-(3-methoxy-4-hydroxyphenyl)ethanol	1531 <sup>c</sup>	tr <sup>d</sup>
40	3-methoxy-4-hydroxybenzoic acid	1543 <sup>c</sup>	32
41	4-hydroxy-( <i>Z</i> )-cinnamic acid	1544 <sup>c</sup>	23
42	4-hydroxy-( <i>E</i> )-cinnamic acid ( <i>p</i> -coumaric acid)	1631 <sup>c</sup>	101
43	3,5-dimethoxy-4-hydroxyacetophenone	1632 <sup>c</sup>	tr
44	3-(3-methoxy-4-hydroxyphenyl)-1-propanol	1661 <sup>c</sup>	242
45	3-(3-methoxy-4-hydroxyphenyl)propionic acid <sup>e</sup>	1684 <sup>c</sup>	828
46	3-methoxy-4-hydroxy-( <i>Z</i> )-cinnamic acid	1730 <sup>c</sup>	99
47	3-(3,5-dimethoxy-4-hydroxyphenyl)-1-propanol <sup>e</sup>	1804 <sup>c</sup>	tr
48	3-(3,5-dimethoxy-4-hydroxyphenyl)propionic acid <sup>e</sup>	1815 <sup>c</sup>	tr
49	3-methoxy-4-hydroxy-( <i>E</i> )-cinnamic acid (ferulic acid)	1835 <sup>c</sup>	348
50	3,4-dihydroxy-( <i>E</i> )-cinnamic acid (caffeic acid)	1835 <sup>c</sup>	28
51	3,5-dimethoxy-4-hydroxy-( <i>Z</i> )-cinnamic acid	1851 <sup>c</sup>	tr
52	3,5-dimethoxy-4-hydroxy-( <i>E</i> )-cinnamic acid (sinapic acid)	1966 <sup>c</sup>	tr

<sup>a</sup> Identified by GC/MS as methoxy derivatives or methyl esters. <sup>b</sup> Kovats retention indices (DB-1) based on *n*-hydrocarbons. <sup>c</sup>  $I_k$  of derivatives where acidic and phenolic groups were methylated. <sup>d</sup> Sub parts per billion level. <sup>e</sup> Tentative assignment.

Table III. Mass Ion Data of Selected Compounds in Tomato Bound Fractions

no.	compound	mass ion (intensity)
17	unidentified monoterpene alcohol	116 (1), 110 (1), 98 (20), 97 (12), 95 (6), 81 (11), 70 (17), 69 (24), 68 (20) 56 (25), 55 (100), 43 (25)
18	( <i>E</i> )-2,6-dimethylocta-2,7-diene-1,6-diol	137 (3), 119 (6), 110 (6), 96 (8), 93 (11), 82 (17), 79 (15), 71 (56), 68 (25), 67 (51), 55 (36), 43 (100)
20	3-oxo- $\alpha$ -ionol	208 (1), 192 (2), 152 (16), 134 (23), 119 (9), 119 (100), 107 (12), 93 (14), 69 (9), 55 (10), 43 (87)
21	3-hydroxy-7,8-dihydro- $\beta$ -ionone	192 (M - H <sub>2</sub> O, 6), 159 (9), 149 (6), 121 (25), 119 (100), 107 (12), 93 (14), 69 (9), 55 (10), 43 (87)
22	3-hydroxy- $\alpha$ -ionol	192 (M - H <sub>2</sub> O, 4), 174 (23), 159 (100), 144 (25), 133 (32), 129 (18), 117 (26), 105 (20), 91 (37), 77 (18), 41 (38)
23	unidentified C <sub>13</sub> norisoprenoid	210 (8), 177 (12), 150 (28), 135 (71), 109 (44), 108 (58), 95 (53), 69 (60), 55 (45), 43 (93), 41 (100)
24	megastigm-5-en-7-yne-3,9-diol	208 (12), 193 (20), 175 (9), 149 (11), 131 (11), 105 (14), 91 (19), 55 (13), 43 (100)
36	3-(4-hydroxyphenyl)-1-propanol <sup>a</sup>	166 (24), 148 (7), 122 (11), 121 (100), 105 (4), 91 (11), 77 (13)
37	3-(4-hydroxyphenyl)propionic acid <sup>a</sup>	194 (19), 163 (3), 134 (17), 122 (10), 121 (100), 105 (5), 91 (12), 77 (11)
44	3-(3-methoxy-4-hydroxyphenyl)-1-propanol <sup>a</sup>	196 (42), 152 (44), 151 (100), 137 (11), 121 (14), 107 (14), 91 (15), 79 (10), 77 (15)
45	3-(3-methoxy-4-hydroxyphenyl)propionic acid <sup>a</sup>	224 (34), 193 (4), 164 (15), 152 (11), 151 (100), 149 (12), 121 (8), 107 (10), 91 (12), 77 (13)
48	3-(3,5-dimethoxy-4-hydroxyphenyl)propionic acid <sup>a</sup>	254 (75), 239 (8), 181 (100), 151 (38), 121 (12), 91 (16), 77 (15)

<sup>a</sup> Mass spectra of derivatives where acidic and phenolic groups were methylated.

products. It is therefore not surprising that the enzymatic hydrolysis released a large number of these shikimate-type compounds. In the present study we found that they exceeded 40% of the total weight of aglycons detected in fresh tomato. Fleuriet and Macheix (1980) described the occurrence of glycosides of the four common phenolic acids, i.e., 42, 49, 50, and 52, in tomatoes. Many other shikimate-type compounds listed in Table II, including 31, 32, 34, 35, 39, and 44, were also present in the bound fraction of Riesling wine (Strauss et al., 1987), grape juice (Winterhalter et al., 1990), and raspberry fruit pulp (Pabst et al., 1991). However, they are reported here for the first time to be bound as glycosides in fresh tomato. On the other hand, a series of shikimate-type ketones, including propovanillone, zingerone, and raspberry ketone, which occur in bound form as glycosides in several natural sources [for

example, see Winterhalter et al. (1990)], were not found in fresh tomato.

Because of their sensory significance, many of the aglycons found in the present study have long been recognized as important contributors to tomato flavor (Buttery et al., 1990c; Kazeniak and Hall, 1970; Stevens, 1970). The effect of glycosides on tomato flavor, however, is still not completely understood. Further work is needed to isolate these glycosides, to elucidate their structures, and to understand their role as flavor precursors.

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