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GLYCOSIDICALLY BOUND AROMA COMPONENTS FROM SOUR CHERRY

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Key Word Index—*Prunus cerasus*; sour cherry; Rosaceae; aroma metabolites; benzyl β-D-glucoside; bound aroma components; 6-hydroxy-2,6-dimethyl-octa-2(*E*),7-dienyl β-D-glucoside; 2-methoxy-4-(2-propenyl)phenyl β-D-glucoside; 2-phenylethyl β-D-glucoside.

Abstract—Benzyl β-D-glucoside, 2-phenylethyl β-D-glucoside, 6-hydroxy-2,6-dimethyl-octa-2(*E*),7-dienyl β-D-glucoside and 2-methoxy-4-(2-propenyl)phenyl β-D-glucoside were isolated from sour cherry fruit pulp by liquid chromatography. Identifications were performed, after per-*O*-methylation, by comparison of HRGC, HRGC-MS and HRGC-FTIR data with those of authentic β-D-glucosides synthesized under modified Koenigs–Knorr conditions.

INTRODUCTION

Sour cherry (*Prunus cerasus* L.) is one of the most important fruit species in northern and central Europe. Although a number of studies have been carried out on its qualitative and quantitative aroma composition [1–5], information about precursors and metabolites of the aroma constituents is scarce. Among these flavourless sour cherry fruit constituents only cyanogenic glycosides have been investigated [6, 7]. Glycosidically bound aroma components have not been studied as yet. This paper concerns the isolation and identification of a number of these non-volatile aroma metabolites from sour cherry fruit pulp.

RESULTS AND DISCUSSION

After simultaneous distillation extraction (SDE) [8] at pH 1 of a glycosidic fraction obtained from sour cherry fruit pulp by LC, HRGC, HRGC-MS and HRGC-FTIR analyses revealed the occurrence of benzyl alcohol (**1**), 2-phenylethanol (**2**) and 2-methoxy-4-(2-propenyl)phenol (eugenol) (**3**) in the hydrolysate. Enzymic hydrolysis of the glycosidic isolate using commerical β-glucosidase (emulsin) also gave these aglycones and, additionally, 6-hydroxy-2,6-dimethyl-octa-2(*E*),7-dienol (**5**). The results suggested that these components, of which **1**–**3** are well-known as sour cherry aroma constituents, were also present as glycosides in the fruit pulp.

In order to study the constituents of the glycosidic isolate, HRGC, HRGC-MS and HRGC-FTIR analyses of the per-*O*-methylated and, in part, per-*O*-trimethylsilylated material were carried out. The HRGC separation of the per-*O*-methylated glycosidic extract from sour cherry fruit pulp is shown in Fig. 1. By means of these techniques, we first of all identified benzyl β-D-glucoside and 2-phenylethyl β-D-glucosides [9] synthesized under modified Koenigs–Knorr condition [10] (Table 1).

The mass spectra of derivatives of components G3 and G4 (Fig. 1) showed fragment ions characteristic for per-*O*-methylated carbohydrates (*m/z* 88 and 101) as well as *m/z* 91 and 151 suggesting the occurrence of per-*O*-methylated benzyl glycosides [9]. Due to the high linear retention indices of 2710 and 2740 determined for G3 and G4, respectively, benzyl disaccharide glycosides were postulated. As the structures of glycosidic conjugates of eugenol (**3**) and 6-hydroxy-2,6-dimethyl-octa-2(*E*),7-dienol (**5**) seemed to be more interesting, G3 and G4 were not further studied.

As the mass spectral data of per-*O*-trimethylsilylated G1 was coincident with that previously published for 6-(trimethylsiloxy-)-2,6-dimethyl-octa-2(E),7-dienyl-tetra-*O*-(trimethylsilyl-) β-D-glucoside (**4b**) [11], 6-hydroxy-2,6-dimethyl-octa-2(*E*),7-dienyl β-D-glucoside (**4d**) was synthesized as reference compound. For this synthesis, 6-acetoxy-2,6-dimethyl-octa-2(*E*),7-dienol (**4**) was prepared by allylic oxidation of 6-acetoxy-2,6-dimethyl-octa-2,7-diene using selenium dioxide [12]. The acetyl function was introduced to protect the tertiary OH group, and thus prevent rearrangement reactions. After liquid chromatographic purification 6-acetoxy-2,6-dimethyl-octa-2(*E*),7-dienol (**4**) was glucosidized with α-D-acetobromoglucose and deacetylated with sodium methanolate. The NMR data of the glucoside derivatives are shown in Table 2; their linear retention data are outlined in Table 3. The mass spectral and vapour phase FTIR data are summarized in Tables 3 and 4, respectively. The chromatographic and spectroscopic data of synthesized **4d** and the compound isolated from the glycosidic extract were coincident. Thus, the occurrence of 6-hydroxy-2,6-dimethyl-octa-2(*E*),7-dienyl β-D-glucoside (**4d**) in sour cherry fruit was confirmed.

The mass spectrum obtained for compound G2 suggested the occurrence of a per-*O*-methylated glycoside of eugenol (**3**) (Fig. 1). Therefore 2-methoxy-4-(2-propenyl)phenyl β-D-glucoside (**3d**) was synthesized by the modified Koenigs–Knorr method previously published by Paulsen *et al.* [10]. The NMR data of the synthesized glucoside **3d** and its derivatives are shown in Table 5. The mass spectral and vapour phase FTIR data

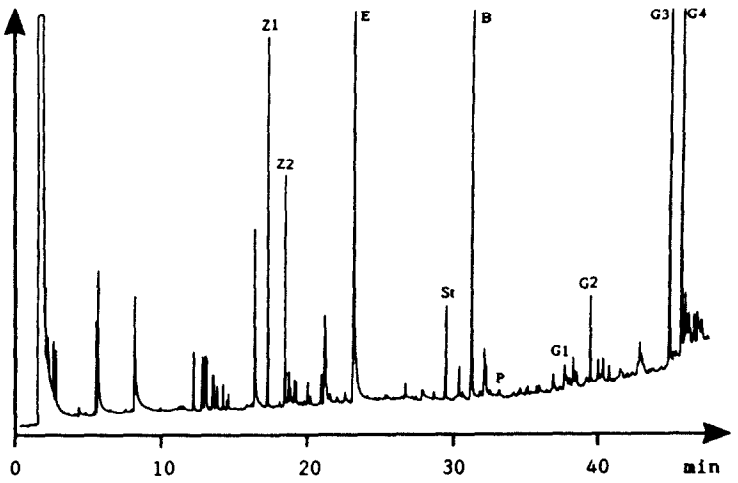
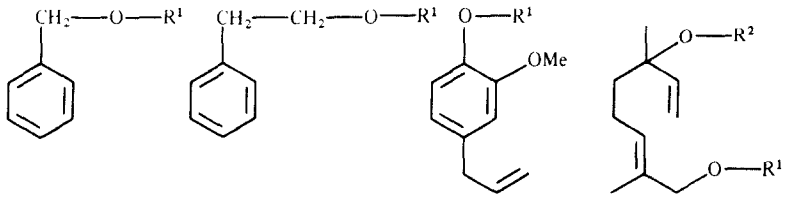


Fig. 1. HRGC separation (J&W 30 m × 0.25 mm i.d. fused silica WCOT DB-5 capillary column, d.f. = 0.25 μm) of a per-*O*-methylated glycosidic extract isolated from sour cherries. Z1,Z2 = per-*O*-methylated carbohydrates; E = methyl 4-methoxycinnamate; St = standard: per-*O*-methylated phenyl β-D-glucoside; B = 1c; P = 2c; Gl = 3c; G2 = 4c (cf. Table 1); G3,G4 = per-*O*-methylated benzyl disaccharide glycosides.

Table 1. Structures of β-D-glucosides from sour cherry fruit and their derivatives



R^1 = H	**1**	**2**	**3**	**4**	R^2 = acetyl
R^1 = 2,3,4,6-tetraacetyl-β-D-glucose	**1a**	**2a**	**3a**	**5**	R^2 = H
				4a	R^2 = acetyl
R^1 = 2,3,4,6-tetratrimethylsilyl-β-D-glucose	**1b**	**2b**	**3b**	**4b**	R^2 = trimethylsilyl
R^1 = 2,3,4,6-tetramethyl-β-D-glucose	**1c**	**2c**	**3c**	**4c**	R^2 = methyl
R^1 = -β-D-glucose	**1d**	**2d**	**3d**	**4d**	R^2 = H

are outlined in Table 6 and 7, respectively. From these data the presence of 2-methoxy-4-(2-propenyl)phenyl β-D-glucoside in sour cherry fruit pulp was confirmed.

While a lot of information is available about the occurrence of benzyl and 2-phenylethyl β-D-glucoside (**1d, 2d**) in plant tissues [9, 13–16], **3d** has been found only in **Melissa officinalis** leaves [17]. Other natural glycosidic conjugates of **3** have been described, i.e. sasanquin (eugenol primeveroside) [18] and gein (eugenol vicianoside) [19]. Compound **4d** was detected first in *Betula alba* leaves and fruits of *Chaenomeles japonica* [11]. Later, it was found also in grapes [20].

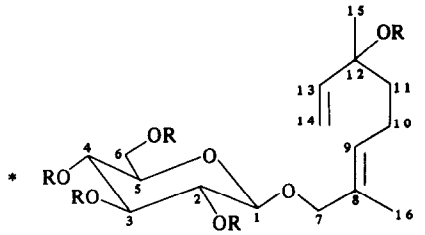
EXPERIMENTAL

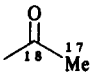
EIMS was determined at 70 eV by HRGC-MS, scanning from *m/z* 41 to 499 with total ion current monitoring. HRGC, HRGC-MS and HRGC-FTIR were carried out using a fused silica WCOT column (30 m × 0.259 mm, d*f* = 0.25 μm) coated with DB 5. Split injection (1:50) was used (1 μl). The column was programmed at 5°/min from 60° to 300°. FID temp. 300°; carrier gas He 2 ml/min. Light pipe and transfer lines were held at 280°; vapour phase spectra were recorded from 700–4000 cm^{-1} with 1 cm/sec. Linear R_t, MS and FTIR data were compared with those of synthesized reference compounds. NMR spectra were measured at 200 MHz in $CDCl_3$ for acetylated glucosides and CD_3OD for glucosides, respectively.

Isolation and fractionation of glycosides. To 1 kg sour cherries (*Prunus cerasus* L. var. Schattenmorelle; seeds removed) 1 l of 0.2 M K-Pi buffer, (pH 7.4) containing 0.2 M glucono-δ-lactone and 3.5 mg phenyl β-D-glucoside (internal standard) was added. After centrifugation (30 min, 20000 g) the supernatant was subjected to LC on XAD adsorbent (glass column, 25 × 900 mm). After washing with 1.5 l distilled water and 500 ml pentane, isolation of glycosides was performed by eluting with EtOAc (750 ml). The EtOAc fraction was concd under red. pres. to

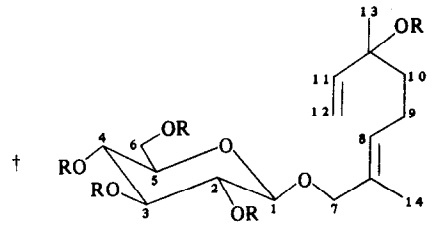
Table 2. NMR data of chemically synthesized compounds **4a** and **d** (TMS, 200 mHz, coupling constants in Hz; $CDCl_3$ as solvent for **4a** and CD_3OD for **4d**)

C	4a	4a [11]	4a [20]	4d	H†	4a	4a [11]	4d
1	98.4	98.85	98.8	102.6	1	4.44 *d* (8)	4.47 *d* (7.5)	4.25 *d* (8)
2	71.5	71.8	71.4	74.7	2	4.8–5.3 *m*	4.9–5.3 *m*	3.2–4.1 *m*
3	72.6	73.1	73.0	78.0	3	4.8–5.3 *m*	4.9–5.3 *m*	3.2–4.1 *m*
4	68.3	71.5	68.6	71.6	4	4.8–5.3 *m*	4.9–5.3 *m*	3.2–4.1 *m*
5	71.1	71.8	71.8	77.0	5	3.6–3.8 *m*		3.2–4.1 *m*
6	61.8	62.15	62.1	62.7	6a	4.10 *m*	4–4, 21 *m*	3,2–4.1 *m*
7	71.0	68.7	72.8	74.7	6*b*	4.25 *m*	4–4, 21 *m*	3.2–4.1 *m*
8	131.0	130.95	130.9	129.4	7	4.4–4.6 *m*	4–4, 21 *m*	4.2–4.4 *m*
9	128.0	129.9	129.7	127.4	8	5.40 *t* (9)	5.3–5.5 *t*	5.37 *m*
10	22.5	22.5	22.5	23.0	9	1.7–2.3 *m*	1.7–2.3 *m*	1.7–2.3 *m*
11	39.7	41.75	41.7	41.7	10	1.7–2.3 *m*	1.7–2.3 *m*	1.7–2.3 *m*
12	74.9	75.15	75.2	75.7	11	5.52 *m*	5.92 *dd*	5.62 *m*
13	141.1	145.1	144.9	142.5	12	4.8–5.3 *m*	4.9–5.3 *m*	5.0–5.3 *m*
14	113.9	112.0	112.0	114.2	13	1.23 *s*	1.28 *s*	1.28 *s*
15	29.5	27.95	28.0	29.8	14	1.60 *s*	1.6 *s*	1.63 *s*
16	13.8	13.65	13.5	13.9	15	1.96 *s*		
17	20.4	20.47	20.6			1.98 *s*		
18	169.1	170.19	169.3			2.01 *s*		
	169.2	170.5	170.3			2.06 *s*		
	170.1	171.08						
	170.5	171.49						



4a R = 

4d R = −H



4a R = 

4d R = −H

dryness and redissolved in 5 ml dist. H_2O. Fatty acids were separated by Et_2O and $CHCl_3$ extraction and the aq. phase concd *in vacuo* to dryness.

Hydrolysis of glycosides (a). Simultaneous Distillation Extraction (SDE) of the EtOAc isolate was performed with pentane–Et_2O (1:1, 60 ml) over 2 hr using the SDE apparatus described by Schultz *et al.* [8]. The organic layer was dried over Na_2SO_4 sicc. and carefully concentrated. (b). Enzymic hydrolysis of the EtOAc isolate was carried out at 30° for 24 hr in 5 ml 0.2 M K-Pi buffer (pH 5.0) using 4 mg almond β-glucosidase (emulsin). Aglycones were extraced with Et_2O and dried over Na_2SO_4 sicc. The liberated compounds from (a) and (b) were analysed by HRGC, HRGC-MS and HRGC-FTIR.

Synthesis of 6-acetoxy-2,6-dimethyl-octa-2(E),7-dienol (**4**). 6-Acetoxy-2,6-dimethyl-octa-2-7-diene (linalyl acetate; 19.3 g, 0.1 mol) and SeO_2 (5.5 g; 0.05 mol) were dissolved in 150 ml 95% EtOH and the soln was heated at 80° for 2 hr. After filtration through Celite, the solvent was removed under red. pres. The residue was redissolved in 1 l Et_2OH, extracted with 500 ml NaCl soln. The organic layer was sepd and dried over Na_2SO_4. After removing the solvent, the residue was subjected to silica gel chromatography (200 g silica gel 60, Merck) using pentane–Et_2O mixtures with increasing polarity (200 ml Et_2O–pentane, 1:4; 200 ml Et_2O–pentane, 1:1). 6-Acetoxy-2,6-dimethyl-octa-2(*E*),7-dienol (**4**) was obtained in 72% yield. R_t (DB-5) 1525. EIMS (70 eV) *m/z* (rel. int.): 41 (41), 43 (100), 45 (19), 53 (16), 55 (19), 60 (15), 67 (22), 68 (17), 71 (14), 79 (40), 80 (19), 84 (14), 91 (22), 93 (42), 94 (43), 119 (18), 121 (16), 134 (5), 152 (2). FTIR (vapour phase) ν (cm^{-1}): 3669, 3646, 3097, 3019, 2985, 2938, 2876, 1756, 1462, 1415, 1373, 1242, 1087, 926, 833.

Table 3. Mass spectral (70 eV) and linear retention data of compounds **4a, b** and **c**

4a 2689*		**4a** [11]†		**4a** [20]		**4b** 2610*		**4b** [11]†		**4c** 2279*	
m/z	%	*m/z*	%	*m/z*	%	*m/z*	%	*m/z*	%	*m/z*	%
41	9	71	30	41	38	45	14	73	77.8	41	29
42	22	109	100	43	100	55	9	103	45.1	43	38
43	100	115	22	55	44	68	10	135	75.3	45	100
55	6	119	21	67	41	73	92	147	38	55	39
67	7	134	24	68	30	75	17	225	74.7	57	6
68	7	135	14	69	34	79	11	191	64.9	59	13
69	6	152	31	71	38	93	18	204	58.5	67	21
79	8	169	86	81	35	103	13	217	100	71	23
80	6	200	2.8	82	31	107	10	271	11.8	75	21
81	7	211	2.6	93	30	135	18	361	86.5	79	13
91	7	229	3	95	15	147	25	377	14	85	42
92	7	271	3	96	27	169	9	451	56	88	43
93	25	331	5	97	24	191	10	467	4	89	13
97	8			103	11	203	14	497	0.42	93	29
103	5			107	16	204	100	512	0.21	101	46
109	34			109	43	205	25	587	0.49	107	21
110	2			110	24	217	23	602	0.49	111	17
115	3			115	17	218	9	677	0.48	115	33
119	3			127	18	233	5	692	0.03	119	4
127	9			134	8	243	8			134	4
134	8			135	10	271	6			135	17
135	7			139	15	361	11			147	17
139	9			152	18					157	3
169	31			169	34					167	8
211	1			331	3					187	9
229	1										
245	1										
271	1										
331	1										

*Linear retention data (J&W DB-5, 30 m × 0.25 mm i.d., df = 0.25 μm).
†Mass range *m/z* 70–700.

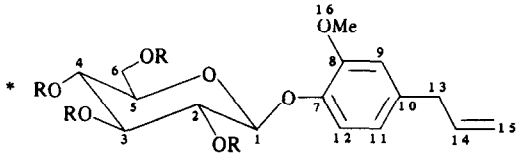
Table 4. Vapour phase FTIR data of **4a** and **4c**

Wavenumber (cm^{-1})		
4a	**4c**	Kind of vibration
3043	3083	C–H ν of double bond
3017		C–H ν of double bond
2956		C–H ν of single bond
2925	2929	C–H ν of single bond
2846	2843	C–H ν of C–O–Me group
1766		C=O ν of acetyl function
1648	1647	C=C ν
1434	1455	C–H δ of single bond
1366	1374	–Mc symmetric δ
1219		–C–O–C ν of acetyl function
	1108	C–O ν of Ac group
1046		=C–O–C ν
953	949	C–H δ
920	937	C–H δ of double bond
813	818	C–H δ of double bond

Synthesis of β-D-glucosides. (a) 2,3,4,6-Tetra-*O*-acetyl-β-D-glucosides. Glucosides (**3a, 4a**) were synthesized under the following modified Koenigs–Knorr conditions [10]. To 71.4 mM of the corresponding alcohol (3 or 4) in 50 ml CH_2Cl_2, 7 g Drierite® and 21.5 mM Ag_2O were added and the mixture stirred in the dark at room temp. for 30 min. Twenty mM α-D-acetobromoglucose in 50 ml CH_2Cl_2 were added within 20 min. After stirring the mixture in the dark for 3 days, it was filtered through Celite, evapd *in vacuo*, redissolved in 90 ml pentane–Et_2O (2:1) and extracted with 90 ml MeOH (50%). The crude product was purified by LC on silica gel using pentane–EtOAc (3:1) as solvent. (b) Deacetylation of compounds **3a** and **4a**. To a soln of 300 mg **3a** (**4a**) in 20 ml MeOH, 20 ml 0.02 M sodium methanolate soln was added. After 12 hr the mixture was neutralized by adding Dowex 50 WX8 (20–50 mesh, H^+ form) and filtered. The crude products were purified by LC on silica gel using EtOAc–MeOH (9:1) as solvent. (c) Derivatization. Per-*O*-methylation was performed by the method of Finne *et al.* [21]. Per-*O*-trimethylsilylated products were obtained after dissolving 5 mg sample in 0.8 ml pyridine adding 0.3 ml silyl 21 (HMDS: TMCS 2:1; Macherey & Nagel) and heating for 20 min at 80°.

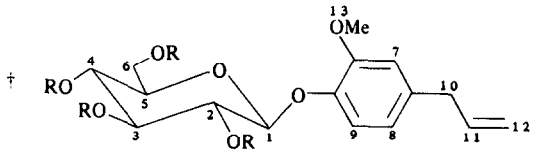
Table 5. NMR data of chemically synthesized compounds **3a** and **d** (TMS, 200 MHz, coupling constants in Hz; $CDCl_3$ as solvent for **3a** and CD_3OD for **3d**)

C*	3a	3d	H†	3a	3d
1	100.7	102.9	1	4.48 *d* (8)	4.18 *d* (8)
2	71.0	74.8	2	4.90 *dd* (8) (9)	3.30 *dd* (8) (9)
3	72.6	78.0	3	5.09 *t* (9)	3.67 *t* (9)
4	68.2	71.2	4	5.03 *t* (9)	3.46 *t* (9)
5	71.6	77.8	5	3.70 *m*	3.39 *m*
6	61.7	62.7	6a	4.06 *m*	3.73 *m*
7	144.2	146.2	6b	4.20 *m*	3.90 *m*
8	150.3	150.6	7	6.64 *s*	6.81 *s*
9	112.9	114.1	8	6.94 *dd* (8) (1)	7.07 *d* (8)
10	136.5	136.4	9	6.58 *dd* (8) (1)	6.71 *dd* (8) (1)
11	120.4	122.0	10a	3.41 *d* (1)	3.52 *s*
12	120.0	118.2	10b	3.24 *d* (8)	3.35 *d* (8)
13	39.6	40.7	11	5.84 *m*	5.95 *m*
14	137.0	138.9	12	5.13 *m*	5.05 *m*
15	115.7	115.9	13	3.71 *s*	3.83 *s*
16	55.7	56.7	14	1.90 *s*	
17	20.4			1.95 *s*	
18	168.9			1.98 *s*	
	169.1			2.02 *s*	
	169.9				
	170.2				



3a R = 

3d R = −H



3a R = 

3d R = −H

Table 6. Mass spectral (70 eV) and linear retention data of **3a**, **b** and **c**

	3a 2737*		3b 2638*		3c 2515*		3a 2737*		3b 2638*		3c 2515*	
m/z	%	*m/z*	%	*m/z*	%	*m/z*	%	*m/z*	%	*m/z*	%
43	100	45	12	41	30	332	3	217	17	91	9
44	9	55	7	43	12			221	12	95	10
81	5	69	8	45	100			236	35	99	11
97	6	73	100	55	13			240	6	101	54
103	6	74	18	59	21			259	5	103	11
109	42	75	26	65	3			273	7	111	35
127	11	81	7	69	4			289	9	116	15
139	6	97	8	70	4			306	3	127	9
164	12	103	22	71	33			378	3	155	9
169	59	117	8	72	9					164	7
170	7	129	12	73	17					187	14
211	4	147	11	75	26					218	31
229	2	164	58	77	6					219	4
271	5	169	10	85	8						
330	2	199	8	88	13						
331	12	206	13	89	20						

Table 6. *Continued*

*Linear retention data (J&W DB-5, 30 m × 0.25 mm i.d., df = 0.25 μm).

Table 7. Vapour phase FTIR data of **3a** and **c**

Wavenumber (cm^{-1})		
3a	**3c**	Kind of vibration
	3090	C–H ν of double bond
3069	3039	C–H ν of double bond
2933	2947	C–H ν of single bond
2838	2847	C–H ν of Ac group
	1772	C=O ν of acetyl function
1669	1643	C=C ν
1574	1587	C=C ν
1505	1505	–C–O–C ν
1454	1414	C–H δ of single bond
1387	1373	–Me symmetric δ
	1205	–C–O–C ν of acetyl function
1108		C–O ν of Ac group
	1036	=C–O–C ν
925	916	C–H δ of aromatic function
723	802	C–H δ of aromatic function

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