

ARYL β -D-GLUCOSIDES FROM *CARICA PAPAYA* FRUIT

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Abstract—Benzyl β -D-glucoside, 2-phenylethyl β -D-glucoside, 4-hydroxyphenyl-2-ethyl β -D-glucoside and four isomeric malonated benzyl β -D-glucosides were isolated from *Carica papaya* fruit pulp by liquid chromatography on XAD adsorbent. Identifications were performed after permethylation by comparison of HRGC, HRGC-MS and HRGC-FTIR data with those of synthesized reference compounds.

INTRODUCTION

Early studies carried out on *Carica papaya* have demonstrated the occurrence of benzyl glucosinolate in the fruit [1]. This compound is a well-known precursor of aroma substances originating after cell disruption [2]. Since in the past increasing interest was devoted to various glycosidic derivatives as potential precursors of aroma compounds [3, 4], in the course of our studies on aroma precursors [5, 6] we investigated also the non-volatile carbohydrate conjugated substances from papaya fruit. In this paper we report on the occurrence of aryl β -D-glucosides.

RESULTS AND DISCUSSION

After hydrolysis at pH 1.0 of a glycosidic fraction from *C. papaya* fruit pulp obtained by liquid chromatography on XAD adsorbent, HRGC, HRGC-MS and HRGC-FTIR analyses revealed the occurrence of benzyl alcohol, 2-phenylethanol and 4-hydroxyphenyl-2-ethanol in the hydrolysate. Enzymic hydrolysis of the glycosidic isolate with a partially purified β -glucosidase from papaya fruit pulp [7] also gave these three alcohols. These results suggested that the alcohols were present in the original fruit pulp as glycosides.

To investigate the constituents of the glycosidic isolate, HRGC, HRGC-MS and HRGC-FTIR analyses of the per-*O*-methylated material were performed. By means of these techniques benzyl β -D-glucoside (1) 2-phenylethyl β -D-glucoside (2) and 4-hydroxyphenyl-2-ethyl β -D-glucoside (3) were identified (Fig. 1). Structural elucidation was performed by comparison of chromatographic and spectroscopic data with those of authentic β -D-glucosides synthesized under modified Koenigs–Knorr conditions [8].

Additionally, four substances among the HRGC separated per-*O*-methylated sugar conjugates showed carbonyl absorption at 1749 cm^{-1} indicating the occurrence of an ester structure. Preparative HPLC fractionation of the glycosidic isolate into 15 fractions led to two fractions, in which, after permethylation, 1b (in fraction 4) and 1b together with four isomeric acylated sugar con-

jugates 4a–4d (in fraction 6) were detected by HRGC, HRGC-MS and HRGC-FTIR. Since 1 was clearly separated by HPLC in fraction 4, the additional presence of 1b in fraction 6 was thought to be caused by hydrolysis during the permethylation step. This suggestion was confirmed by the occurrence of dimethyl dimethylmalonate, which was also identified in fraction 6 after permethylation. Chemical synthesis using 1 and dimethylmalonic acid monochloride as well as subsequent chromatographic and spectroscopic analysis proved the structures of per-*O*-methylated unknown compounds as dimethylmalonated benzyl tetra-*O*-methyl- β -D-glucosides (4a–4d). Since permethylation of malonic acid yielded dimethyl dimethylmalonate, the natural occurrence of malonic acid was suggested. This could be proved by chemical hydrolysis at pH 1.0 of a glycosidic fraction obtained from XAD separated material by ethyl acetate elution. After preparative TLC and methylation with diazomethane, HRGC, HRGC-MS and HRGC-FTIR analyses revealed the occurrence of dimethyl malonate. Thus, final structural elucidation of 4 as isomeric malonated benzyl β -D-glucosides was achieved (Fig. 1).

Whereas the glucosides 1–3 are known natural constituents of various sources, as found, e.g. in apricot kernels [9], *Cucurbita pepo* [10] and rose flowers [11, 12], *Vitis vinifera* fruits [13], *Rhododendron* leaves [14], bark of *Ligustrum japonicum* [15] as well as leaves of Salicaceae species [16], the finding of the malonated benzyl β -D-glucosides 4 in *C. papaya* fruit appears to be the first observation of malonyl conjugates of simple aryl β -D-glucosides. The presently known malonyl derivatives only comprise conjugates with betalains, anthocyanins, isoflavones, flavonols and flavones as well as ginsenosides [17].

EXPERIMENTAL

General. EI- and CIMS were 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 \times 0.259 mm, df = 0.25 μm) coated with DB 5. Split injection (1:50) was used

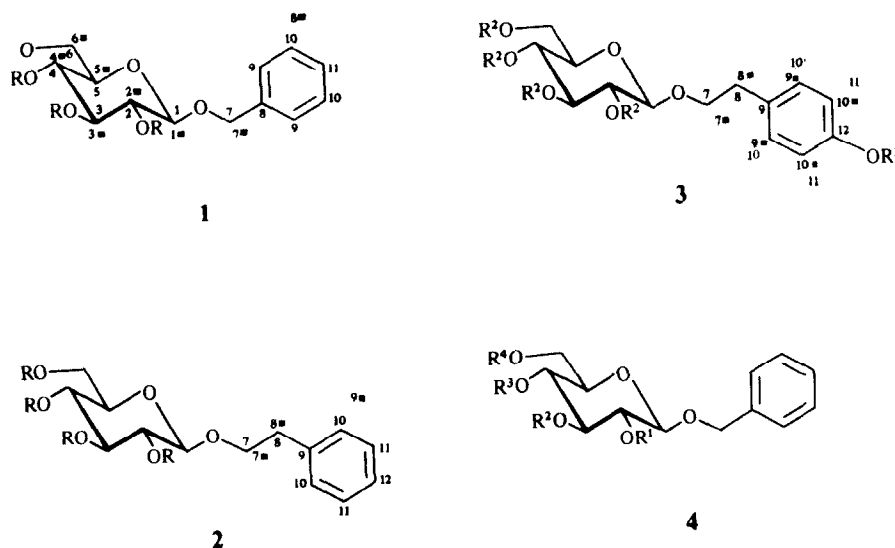


Fig. 1. Chemical structures of aryl- β -D-glucosides synthesized and identified in papaya fruit pulp. **1** ($R = H$); **1a** ($R = Ac$); **1c** ($R = Me$). **2** ($R = H$); **2a** ($R = Ac$); **2b** ($R = Me$). **3** ($R_1 = R_2 = H$); **3a** ($R_1 = H$; $R_2 = Ac$); **3b** ($R_1 = R_2 = Me$). **4** (4 isomers) **I** ($R_1 = COCH_2COOH$; $R_2 = R_3 = R_4 = H$); **II** ($R_2 = COCH_2COOH$; $R_1 = R_3 = R_4 = H$); **III** ($R_3 = COCH_2COOH$; $R_1 = R_2 = R_4 = H$); **IV** ($R_4 = COCH_2COOH$; $R_1 = R_2 = R_3 = H$). **4a** ($R_1 = COC(Me)_2COOMe$; $R_2 = R_3 = R_4 = Me$); **4b** ($R_2 = COC(Me)_2COOMe$; $R_1 = R_3 = R_4 = Me$). **4c** ($R_3 = COC(Me)_2COOMe$; $R_1 = R_2 = R_4 = Me$); **4d** ($R_4 = COC(Me)_2COOMe$; $R_1 = R_2 = R_3 = Me$). ■ H atoms.

(1 μ l). The column was prog. at 5°/min from 60 to 300°. FID temp. 300°; carrier gas He 2 ml/min. Light pipe and transfer lines were held at 200°; vapour phase spectra were recorded from 700–4000 cm^{-1} with 1 cm/sec. Linear R_f , MS and FTIR data were compared with those of synthesized reference compounds, NMR spectra were measured at 200 and 400 MHz in $CDCl_3$ for acetylated glucosides and CD_3CO-CD_3 or CD_3OD for glucosides, respectively. 1H and ^{13}C NMR data were assigned according to information available from refs [8, 18, 19]. For HRGC, HRGC-MS and HRGC-FTIR the samples were per-*O*-methylated as described in ref. [20].

Isolation and fractionation of glycosides. Fresh *C. papaya* fruits (Solo variety from Brazil; sample wt 1 kg) were peeled and the seeds removed. After homogenization with 1 l of 0.2 M Pi buffer pH 6.5 containing 0.2 M glucono- δ -lactone and 3.5 mg phenyl β -D-glucoside (int. st) and centrifugation (30 min, 20 000 *g*) the supernatant was subjected to LC chromatography on XAD adsorbent (glass column, 25 \times 900 mm). After washing with 1500 ml H_2O and 500 ml pentane, isolation of glycosides was performed by eluting with EtOAc (750 ml). The EtOAc fraction was concd under red press to dryness and redissolved in 5 ml H_2O . Fatty acids were sepd by Et_2O and $CHCl_3$ extn and the aq phase concd *in vacuo* to dryness.

The residue obtained was redissolved in 5 ml MeOH and the soln sepd into 15 fractions (each 5 ml) by prep. HPLC on Spherisorb 5 ODS (250 \times 5 mm) using H_2O -MeOH-THF (90:9:1) and RI detection. Fractions 4 and 6 were concd *in vacuo* to dryness, per-*O*-methylated and analysed by HRGC, HRGC-MS and HRGC-FTIR.

Hydrolysis of glycosides. (i) Chemical hydrolysis of the EtOAc isolate was performed by heating at pH 1.0 for 1 hr with subsequent Et_2O extn. (ii) Enzymic hydrolysis of the EtOAc isolate was carried out at 35° for 24 hr using a partially purified β -glucosidase from *C. papaya* fruit pulp [7] and subsequent extn with pentane- CH_2Cl_2 (2:1). The liberated compounds from (i) and (ii) were analysed by HRGC, HRGC-MS and HRGC-FTIR.

Identification of bound malonic acid. The EtOAc isolate was fractionated by prep. TLC ($CHCl_3$ -MeOH- H_2O , 16:9:2, double development). The material collected between R_f 0.26 and 0.30 was extd with MeOH, concd *in vacuo* to dryness, redissolved in 20 ml H_2O , acidified to pH 1.5 and refluxed for 2 hr. After Et_2O extn, methylation with CH_3N_2 , concn and drying, HRGC, HRGC-MS and HRGC-FTIR were carried out.

Synthesis of β -D-glucosides. (i) 2,3,4,6-Tetra-*O*-acetyl- β -D-glucosides. Glucosides (**1a**–**3a**) were synthesized under the following modified Koenigs-Knorr conditions [8]. To 71.4 mM of the corresponding alcohol in 50 ml CH_2Cl_2 , 7 g Drierite and 21.5 mM Ag_2O were added and the mixt stirred in the dark at room temp for 30 min. The 19.9 mM α -D-acetobromoglucose in 50 ml CH_2Cl_2 were added within 20 min. After stirring the mixt in the dark at room temp for 3 days, it was filtered through Celite, evapd *in vacuo*, redissolved in 90 ml pentane- Et_2O (2:1) and extd with 90 ml aq MeOH (50%). The crude product was purified by LC on silica gel using pentane-EtOAc (3:1) as solvent. Yields of purified compounds ranged from 12 to 22% and 15% for **1a**, **2a** and **3a**, respectively. Benzyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucoside (**1a**). R_f 2571. EIMS m/z (%) 331 (1), 271 (1), 259 (1), 245 (3), 217 (1), 216 (1), 169 (2), 157 (6), 152 (8), 139 (18), 115 (3), 110 (4), 109 (2), 97 (14), 91 (50), 65 (4), 43 (100), [13]. 1H NMR, see Table 1; ^{13}C NMR, see Table 2. 2-Phenylethyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucoside (**2a**). R_f 2635. EIMS m/z (%) 347 (1), 331 (1), 287 (1), 259 (1), 243 (1), 200 (1), 169 (3), 157 (1), 145 (1), 115 (2), 112 (2), 109 (4), 105 (23), 104 (17), 103 (4), 98 (3), 91 (1), 81 (6), 79 (3), 76 (2), 70 (2), 69 (2), 65 (1), 43 (100), [13]. 1H NMR, see Table 1; ^{13}C NMR, see Table 2. 4-Hydroxyphenyl-2-ethyl 2,3,4,6-tetra-*O*-acetyl- β -D-glucoside (**3a**). R_f 2782. EIMS m/z (%) 169 (6), 122 (5), 121 (27), 120 (23), 109 (9), 107 (7), 103 (7), 91 (6), 79 (6), 77 (7), 68 (6), 55 (5), 43 (100).

(ii) **Deacetylation of compounds 1a, 2a and 3a.** To a soln of 600 mg **1a** (**2a**, **3a**) in 20 ml MeOH, 20 ml 0.02 M NaOMe soln was added. After 12 hr the reaction mixt was neutralized by adding Dowex 50 WX8 (20–50 mesh, H^+ form) and filtered. The

Table 1. ^1H NMR spectral data of compounds **1**, **1a**, **2**, **2a** and **3** (coupling constants in Hz)

	1	2	Glucose-		5	6	Acetyl-	7	Aglycon-moiety		10
			3	4					8	9	
1 *†	4.42 <i>d</i> (7.7)	3.42 <i>dd</i> (7.7) (9)	3.79 <i>t</i> (9)	3.46 <i>t</i> (9)	3.32 <i>ddd</i> (2.5) (5) (9)	3.88 <i>dd</i> (2.5) (12)	—	4.64 <i>d</i> (12.5)	7.31 <i>m</i>	—	—
						3.71 <i>dd</i> (5) (12)		4.91 <i>d</i> (12.5)	—	—	—
1a *‡	4.56 <i>d</i> (7.7)	5.07 <i>dd</i> (7.7) (9)	5.18 <i>t</i> (9)	5.11 <i>t</i> (9)	3.68 <i>ddd</i> (2.5) (5) (9)	4.28 <i>dd</i> (5) (12)	2.09 <i>s</i>	4.62 <i>d</i> (12.5)	7.32 <i>m</i>	—	—
						4.16 <i>dd</i> (2.5) (12)	2.01 <i>s</i>	4.91 <i>d</i> (12.5)	—	—	—
							1.99 <i>s</i>	—	—	—	—
							1.98 <i>s</i>	—	—	—	—
2 *†	4.35 <i>d</i> (8)	3.42 <i>dd</i> (8) (9)	3.70 <i>t</i> (9)	3.32 <i>t</i> (9)	3.22 <i>ddd</i> (2.5) (5) (9)	3.84 <i>dd</i> (2.5) (12)	—	4.05 <i>m</i>	2.91 <i>dt</i> (7) (2)	7.23 <i>m</i>	—
						3.70 <i>dd</i> (5) (12)					
2a *‡	4.48 <i>d</i> (8)	4.99 <i>dd</i> (8) (9)	5.18 <i>t</i> (9)	5.08 <i>t</i> (9)	3.68 <i>ddd</i> (2.5) (5) (9)	4.27 <i>dd</i> (5) (12)	2.09 <i>s</i>	4.12 <i>m</i>	2.89 <i>dt</i> (7) (3)	7.27 <i>m</i>	—
						4.13 <i>dd</i> (2.5) (12)	2.04 <i>s</i>				
							2.01 <i>s</i>				
							1.99 <i>s</i>				
3 §	4.29 <i>d</i> (8)	3.43 <i>dd</i> (8) (9)	3.67 <i>t</i> (9)	3.35 <i>t</i> (9)	3.23 <i>ddd</i> (2.5) (5) (9)	3.74 <i>dd</i> (5) (12)	—	3.86 <i>d</i> (7)	2.84 <i>t</i> (7)	7.04 <i>s</i>	6.66 <i>s</i>
						3.69 <i>dd</i> (2.5) (12)				7.08 <i>s</i>	6.70 <i>s</i>

* 400 MHz; † $\text{CD}_3\text{--CO--CD}_3$.‡ CDCl_3 ; § 200 MHz; || CD_3OD .

crude products were purified by LC on silica gel using EtOAc–MeOH (9:1) as solvent. Yields of purified compounds ranged from 94 to 87% and 80% for **1**, **2** and **3**, respectively. Benzyl β -D-glucoside (**1**). ^1H NMR, see Table 1; ^{13}C NMR, see Table 2. 2-Phenylethyl β -D-glucoside (**2**). ^1H NMR, see Table 1; ^{13}C NMR, see Table 2. 4-Hydroxyphenyl-2-ethyl β -D-glucoside (**3**). ^1H NMR, see Table 1; ^{13}C NMR, see Table 2.

(iii) *Derivatization*. Per-*O*-methylation of compounds **1–4** was performed by the method of ref. [20]. Benzyl 2,3,4,6-tetra-*O*-methyl- β -D-glucoside (**1b**). *R*_f 2054. EIMS *m/z* (%) 225 (6), 165 (6), 151 (35), 147 (17), 115 (50), 102 (12), 101 (91), 91 (97), 89 (14), 88 (100), 75 (18), 73 (17), 71 (18), 65 (13), 59 (9), 45 (53), 41 (9). FTIR (vapour phase) ν cm^{-1} 3072, 3038, 2989, 2939, 2840,

1459, 1366, 1102, 992, 937. 2-Phenylethyl 2,3,4,6-tetra-*O*-methyl- β -D-glucoside (**2b**). *R*_f 2153. EIMS *m/z* (%) 165 (30), 105 (58), 101 (36), 89 (8), 88 (100), 79 (7), 77 (7), 75 (14), 73 (13), 71 (16), 59 (6), 45 (40), 41 (9), FTIR (vapour phase) ν cm^{-1} 3070, 3035, 2989, 2939, 2840, 1498, 1456, 1369, 1102, 990, 4-Methoxyphenyl-2-ethyl 2,3,4,6-tetra-*O*-methyl β -D-glucoside (**3b**). *R*_f 2417. EIMS *m/z* (%) 196 (1), 195 (2), 136 (6), 135 (47), 134 (26), 121 (9), 101 (33), 91 (7), 89 (9), 88 (100), 79 (6), 77 (8), 75 (14), 73 (12), 71 (14), 65 (5), 59 (6), 53 (5), 45 (51). FTIR (vapour phase) ν cm^{-1} 3035, 2989, 2941, 2839, 1623, 1512, 1466, 1370, 1249, 1108, 990, 942, 818.

Synthesis of acylated benzyl β -D-glucosides. The synthesis of dimethylmalonic acid monochloride was performed as described

Table 2. ^{13}C NMR spectral data of compounds **1**, **1a**, **2**, **2a** and **3**

	Glucose-						Acetyl-		Aglycon-moiety					
	1	2	3	4	5	6			7	8	9	10	11	12
1 *†	103.3d	75.0d	78.1d	71.9d	77.5d	63.1t	—	—	71.0t	139.3s	128.6d	128.9d	128.2d	—
1a *‡	99.2d	71.3d	72.8d	68.5d	71.7d	61.9t	20.4q	169.0s 169.1s 169.9s 170.3s	70.6t	136.6s	128.3d	127.8d	127.6d	—
2 *†	103.6d	74.3d	77.5d	71.3d	76.9d	62.5t	—	—	70.4t	36.4t	139.4s	129.3d	128.5d	126.4d
2a *‡	100.6d	71.2d	72.8d	68.5d	71.8d	61.9t	20.4q	169.0s 169.2s 170.0s 170.4s	70.4t	35.9t	138.4s	128.8d	128.2d	126.2d
3 §	104.3d	75.0d	78.0d	72.0d	77.8d	62.7t	—	—	71.6t	36.3t	130.9s	130.7d	117.8d	156.7s

* 400 MHz; † $\text{CD}_3\text{-CO-CD}_3$.‡ CDCl_3 ; § 200 MHz; || CD_3OD .

in ref [21]. A soln of 70 mg dimethylmalonic acid monochloride in 200 μl pyridine was carefully added under cooling to a soln of 100 mg **1** in 300 μl dry pyridine. After 12 hr at room temp, 5 ml dist H_2O was added. The reaction mixture was then extracted three times with 5 ml EtOAc. After evaporating the solvent *in vacuo* and subsequent per-*O*-methylation, HRGC, HRGC-MS and HRGC-FTIR analyses revealed the presence of four isomers (I–IV) exhibiting R_f , FTIR and MS data corresponding to those of papaya fruit constituents. Per-*O*-methylated malonated benzyl β -D-glucoside (Isomer I). R_f 2535. EIMS m/z (%) 279 (2), 261 (1), 215 (4), 151 (4), 143 (3), 129 (3), 127 (2), 115 (33), 111 (10), 101 (83), 91 (100), 88 (97), 87 (31), 75 (17), 73 (31), 71 (17), 69 (14), 65 (14), 59 (17), 45 (33), 41 (21), CIMS (2-methylpropane) m/z (%) 302 (100), 270 (45), 188 (9), 156 (67), 91 (18). FTIR (vapour phase) $\nu\text{ cm}^{-1}$ 3070, 3040, 2991, 2957, 2846, 1749, 1469, 1394, 1365, 1257, 1114. Per-*O*-methylated malonated benzyl β -D-glucoside (Isomer II). R_f 2573. EIMS m/z (%) 215 (8), 175 (8), 156 (5), 147 (10), 143 (10), 129 (39), 115 (13), 111 (9), 102 (18), 101 (100), 91 (95), 88 (14), 87 (8), 75 (8), 73 (37), 71 (24), 69 (9), 65 (11), 59 (12), 45 (25), 41 (13). FTIR (vapour phase), cf. isomer I. Per-*O*-methylated malonated benzyl β -D-glucoside (Isomer III). R_f 2584. EIMS m/z (%) 215 (23), 157 (19), 151 (5), 143 (23), 129 (40), 115 (22), 111 (21), 101 (56), 91 (100), 88 (65), 87 (5), 75 (14), 73 (42), 71 (19), 69 (14), 65 (9), 59 (14), 45 (56), 41 (19). CIMS (2-methylpropane) m/z (%) 334 (7), 216 (7), 188 (43), 156 (100), 91 (14). FTIR (vapour phase), cf. isomer I. Per-*O*-methylated malonated benzyl β -D-glucoside (Isomer IV). R_f 2607. EIMS m/z (%) 289 (5), 215 (5), 202 (9), 187 (7), 147 (19), 143 (12), 129 (63), 115 (35), 111 (11), 101 (100), 91 (95), 88 (7), 87 (5), 75 (21), 73 (28), 71 (32), 69 (11), 65 (12), 59 (11), 45 (63), 41 (14). CIMS (2-methylpropane) m/z (%) 334 (7), 188 (100), 156 (7), 130 (10), 91 (9). FTIR (vapour phase), cf. isomer I.

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