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ALIPHATIC AND AROMATIC GLYCOSIDES FROM THE CELL CULTURES OF *LYCOPERSICON ESCULENTUM*

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; cell cultures; benzyl glycosides; androsin; isopentenylgentiobioside.

Abstract—The β-D-glucoside, gentiobioside and 6-*O*-α-L-arabinosyl-β-D-glucoside of benzyl alcohol, androsin and a new simple aliphatic glycoside, isopentylgentiobioside, have been found in the cell cultures of *Lycopersicon esculentum*. Their structures were elucidated from chemical and spectroscopic evidence.

INTRODUCTION

Volatile alcohols such as monoterpene alcohols, benzyl alcohol and 2-phenylethanol play an important role in the flavour of many plants. Generally, these compounds are stored in the cells as glycosides [1]. In the course of our studies on metabolites from plant cell cultures, we have studied the cell suspension cultures of *Lycopersicon esculentum* L.

RESULTS AND DISCUSSION

From the ethanolic aqueous extract of cell suspension cultures of *L. esculentum* followed by reversed-phase column chromatography and HPLC, five compounds were isolated: isopentylgentiobioside (**1**), androsin (**2**) and three benzyl glycosides: the glucoside (**3**), 6-*O*-α-L-arabinosyl-β-D-glucoside (**4**) and gentiobioside (**5**) of benzyl alcohol.

Isopentylgentiobioside (**1**) had the molecular formula $C_{17}H_{32}O_{11}$ (FAB mass spectral and NMR data). The ^1H and ^{13}C NMR spectra indicated the presence of two sugar moieties and an aliphatic aglycone. The high-field ^1H NMR spectrum shows two methyls as a doublet at δ 0.76 (6H, $J = 6.6$ Hz) a methylene as multiplets at δ 1.38 and a methine at δ 1.45. The COSY spectrum indicated a coupling between the two methyls and the methine at δ 1.45 that was coupled with the methylene at δ 1.38 that was in turn coupled with the methylene signal at δ 3.55–3.62, partially overlapped by a proton of an ABX signal. These data suggest that the aglycone is isopentyl alcohol. The ^{13}C NMR spectrum with signals at δ 22.9 (*q*), 25.6 (*d*), 38.9 (*t*) and 70.0 (*t*) confirms the presence of isopentyl alcohol as a glycoside. Acid hydrolysis of compound **1** yielded D-glucose, identified by high-performance anion exchange (HPAE). The β-configuration of both glycosidic linkages was evident from the presence of two doublets at δ 4.38 and δ 4.32 (1H, each, $J = 7.8$ Hz) in the ^1H NMR spectrum of **1**. The 1–6 interglycoside linkage was established on the basis of the glycosylation shift [2–4] observed on C-6 of the first glucose unit. The identification of ^{13}C signals (Table 1) belonging to each single monosaccharide residue was possible by two-dimensional NMR experiments (COSY, TOCSY, HETCOR and HMBC). Consequently, compound **1** is characterized as isopentyl-β-D-glucopyranosyl(1–6)β-D-glucopyranoside.

Compound **2** had a molecular formula $C_{17}H_{32}O_{11}$ (FAB mass spectral and NMR data). The IR bands at 1685 and 1640 cm^{-1} were characteristic of an α,β-unsaturated ketone, while the UV absorption at 270 nm strongly suggested an aromatic ketone. The ^1H NMR spectrum showed a methyl ketone at δ 2.64, a methoxyl group at δ 3.95, three aromatic protons at δ 7.26 (*d*, $J = 8.5$ Hz), 7.62 (*d*, $J = 1.5$ Hz) and 7.73 (*dd*, $J = 8.5$ and 1.5 Hz) characteristic of a 1,2,4-trisubstituted aromatic ring, a proton doublet at δ 5.18 ($J = 7.2$ Hz) and signals between δ 3.92–3.40 indicated the presence of a sugar moiety. The relative position of substituents on the aromatic ring was established as indicated in formula **2**, by NOESY from the presence of NOEs between methyl ketone (δ 2.64) and H-2 (δ 7.62) and H-6 (δ 7.73), the methoxyl group and H-2 and the anomeric proton (δ 5.18) and H-5 (δ 7.26). These data suggest that the compound **2** is androsin and the ^{13}C chemical shifts of **2** were in excellent agreement with those previously reported [5]. Androsin was previously isolated from *Penstemon pinifolius* (Scrophulariaceae) [5], *Apocynum androsaemifolium* (Apocynaceae) [6] and *Neolloydia texensis* (Cactaceae) [7]. The HMBC

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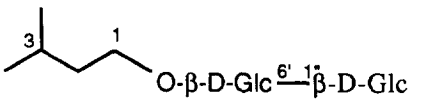
FORMULAE



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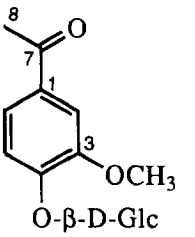

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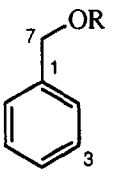

3 R = β-D-Glc

4 R = β-D-Glc 6'-1" β-D-Glc

5 R = β-D-Glc 6'-1" α-L-Ara (p)

experiment showed correlations between the methoxyl protons and carbon atom at δ 149.6, and the anomeric proton with carbon atom at δ 151.6 which allowed for the identification of the C-3 and C-4 chemical shifts, that are exchanged in comparison with those reported [5].

The spectral data of compound **3** were in excellent agreement with those of benzyl-β-D-glucopyranoside, previously isolated from various sources [8–10] and recently isolated from tomato fruit [11].

Compounds **4–5** showed similar related NMR spectra (Experimental and Table 1) indicating the presence, in both compounds, of a benzyl disaccharide glycoside.

Compound **4** had a molecular formula $C_{18}H_{26}O_{10}$ and acid hydrolysis yielded L-arabinose and D-glucose. In the ^{1}H NMR spectrum, two anomeric protons at

Table 1. ^{13}C NMR spectral data of compounds **1–5** (125 MHz, $CD_3OD + D_2O$)*

	1	2	3	4	5
Aglycone moiety					
1	70.0 *t*	132.6 *s*	138.0 *s*	138.1 *s*	137.7 *s*
2	38.9 *t*	112.4 *d*	129.6 *d*†	129.4 *d*†	129.6 *d*†
3	25.6 *d*	149.6 *s*	129.5 *d*†	129.3 *d*†	129.5 *d*†
4	22.9 *q*	151.6 *s*	129.2 *d*	129.0 *d*	129.3 *d*
5	22.9 *q*	115.7 *d*	129.5 *d*†	129.3 *d*†	129.5 *d*†
6		124.9 *d*	129.6 *d*†	129.4 *d*†	129.6 *d*†
7		202.2 *s*	72.2 *t*	72.2 *t*	72.5 *t*
8		26.7 *q*			
OMe		56.8 *q*			
Sugar moiety					
1′	103.7 *d*	101.0 *d*	102.5 *d*	102.7 *d*	102.7 *d*
2′	74.4 *d*	73.9 *d*	74.4 *d*	74.4 *d*	74.3 *d*
3′	77.2 *d*†	77.4 *d*†	77.2 *d*	77.1 *d*	77.0 *d*
4′	70.7 *d*	70.8 *d*	70.9 *d*	70.7 *d*	70.5 *d*
5′	76.2 *d*†	77.2 *d*†	77.2 *d*	76.2 *d*	76.2 *d*
6′	69.5 *t*	61.9 *t*	62.0 *t*	69.3 *t*	69.5 *t*
1″	104.0 *d*			104.8 *d*	104.1 *d*
2″	74.4 *d*			71.9 *d*	74.3 *d*
3″	77.1 *d*†			73.6 *d*	76.8 *d*†
4″	70.9 *d*			69.4 *d*	70.7 *d*
5″	77.1 *d*†			67.0 *t*	76.7 *d*†
6″	62.0 *t*				61.7 *t*

*Signal assignments were based on COSY, TOCSY, HETCOR and HMBC spectra. Chemical shifts were referenced on the solvent peak $\delta_{MeOH} = 49.0$.

†Interchangeable signals.

δ 4.26 (H″-1, *d*, $J = 7.4$ Hz) and 4.41 (H′-1, *d*, $J = 8.0$ Hz) were observed. Along with the benzyl moiety the ^{13}C NMR spectrum of **4** revealed the presence of six signals derived from a glucopyranosyl, and five signals from an arabinopyranosyl moiety, that are in agreement with the corresponding signals of linalyl-α-L-arabinopyranosyl(1-6)-β-D-glucopyranoside [12]. Thus, **4** is benzyl - α - L - arabinopyranosyl(1 - 6) - β - D - glucopyranoside. Williams and his co-worker [8] isolated from *Vitis vinifera* benzyl - α - L - arabinofuranosyl(1 - 6) - β - D - glucopyranoside, establishing the structure by EI-mass spectrometry of the acetylated derivative.

Compound **5** had a molecular formula $C_{19}H_{28}O_{11}$ and acid hydrolysis yielded D-glucose. The carbon signals due to the sugar moiety of **5**, as well as of two anomeric proton signals at δ 4.45 (H″-1, *d*, $J = 8.0$ Hz) and 4.47 (H′-1, *d*, $J = 8.0$ Hz), indicated the presence of a gentiobioside moiety. Thus, compound **5** is benzyl-β-D-glucopyranosyl(1–6)-β-D-glucopyranoside.

EXPERIMENTAL

General. IR cards 3M (KC-0061) were purchased from Carlo Erba Reagenti Italy. FAB-mass spectra were recorded at 25 kEv (2 μA) using glycerol (G) as matrix. ^1H and ^{13}C NMR were recorded at 500 and 125 MHz, respectively. The 2D NMR spectra were obtained using Bruker's microprograms. Chromatographies were performed using Lichroprep RP-18 (40–63 μm). Semiprep. HPLC purifications were carried out with Spherisorb S5ODS2 column (25 cm × 4.6 mm i.d.) and with a RI detector. Sugar analyses were performed on HPAE-PAD (Dionex) equipped with a Carbopac PA1 column eluted with 15 mM NaOH (1 ml min^{-1}) and with a Pulsed Amperometric detector. Commercial seeds of *Lycopersicon esculentum* L. var. S. Marzano were purchased from Bulsem Salerno (Italy).

Cell cultures. For callus induction, plant sections from sterile grown *L. esculentum* var. S. Marzano were cultured on MS basal medium supplemented with (mg l^{-1}): myoinositol (100), nicotinic acid (0.5), pyridoxine hydrochloride (0.5), thiamine hydrochloride (0.1), glycine (2), sucrose (30 000) and agar (9000). This medium was supplemented with (M): *p*-chlorophenoxyacetic acid (10^{-5}), 2-4-dichlorophenoxyacetic acid (2×10^{-6}) and 6-benzylaminopurine (10^{-6}). The initial callus was transferred to fresh medium after 4 weeks and the resulting culture was maintained as above. Suspension cultures were initiated from 4th generation callus by transfer of *ca* 3 g callus into 100 ml liquid medium. Suspension cultures were maintained in 250 ml flasks by transfer of *ca* 1 g fresh weight tissue (*ca* 10 ml) into 100 ml fresh medium every 21 days. Cultures were maintained at 24°, 150 rpm, in continuous light.

Extraction and isolation of compounds. Tissue (25 g dry weight) was extracted with 70% aqueous EtOH (3 × 500 ml). The combined extracts were concentrated *in vacuo* and the aqueous residue extracted with $CHCl_3$. The aqueous phase was introduced onto a RP-18 (30 g) column, and eluted with H_2O, H_2O–MeOH (1:1) and MeOH (300 ml for each solvent). The H_2O–MeOH fr. was chromatographed on Lobar RP-18 column eluted with a solvent gradient from H_2O to MeOH. Frs eluted with H_2O–MeOH (2:3) were further purified on reversed-phase HPLC (H_2O/MeOH; 4:1, flow 3 ml min^{-1}) recovering compounds **1–5**.

Isopentyl - β - D - glucopyranosyl(1–6) - β - D - *glucopyranoside* (**1**). Yield 15 mg; $[\alpha]_D$ −41.5° (H_2O; *c* 0.01); FAB-MS *m/z* (rel. int.): 413 $[M + H]^+$ (100), 251 (65), 163 (20); ^1H NMR: δ 0.76 (6H, *d*, $J =$ 6.6 Hz, H-4 and H-5), 1.38 (2H, *m*, H-2), 1.45 (1H, *m*, H-3), 3.15 (2H, *m*, H′-2 and H″-2), 3.26–3.36 (5H, *m*, H′-3, H′-4, H′-5, H″-3 and H″-5), 3.47 (1H, *m*, H″-4), 3.55–3.62 (3H, *m*, H-1 and H′-6), 3.72 (1H, *dd*, $J =$ 11.7 and 5.6 Hz, H″-6), 3.78 (1H, *dd*, $J = 12.0$ and 1.8 Hz, H′-6), 4.05 (1H, *dd*, $J = 11.7$ and 1.8 Hz, H″-6), 4.32 (1H, *d*, $J = 7.8$ Hz, H″-1), 4.38 (1H, *d*, $J =$ 7.8 Hz, H′-1); ^{13}C NMR: see Table 1.

Androsin (**2**). Yield 10 mg; $[\alpha]_D$ −47.1° (H_2O; *c* 0.01); UV λ_{max} (H_2O) nm (log ε): 305 (3.87), 270 (3.99), 222 (4.15); IR ν_{max} (dry film) cm^{-1}: 3600–3100, 2960–2850, 1685, 1640, 1575, 1510; FAB-MS *m/z* (rel. int.): 329 $[M + H]^+$ (100); ^1H NMR: δ 2.64 (3H, *s*, H-8), 3.40–3.75 (2H, *m*), 3.72 (1H, *dd*, $J =$ 11.8 and 5.5 Hz, H′-6), 3.92 (1H, *dd*, $J = 11.8$ and 1.8 Hz, H′-6), 3.95 (3H, *s*, OMe), 5.18 (1H, *d*, $J =$ 7.2 Hz, H′-1), 7.26 (1H, *d*, $J = 8.5$ Hz, H-5), 7.62 (1H, *d*, $J = 1.5$ Hz, H-2), 7.73 (1H, *dd*, $J = 8.5$ and 1.5 Hz, H-6); ^{13}C NMR: see Table 1.

Benzyl-β-D-glucopyranoside (**3**). Yield 10 mg; $[\alpha]_D$ −46° (H_2O; *c* 0.01); IR ν_{max} (dry film) cm^{-1}: 3600–3100, 2960–2850, 1645, 1570, 1500; FAB-MS *m/z* (rel. int.): 271 $[M + H]^+$ (100); ^1H NMR: δ 3.10 (1H, *m*, H′2), 3.20–3.30 (3H, *m*, H′-3, H′-4 and H′-5), 3.50 (1H, *dd*, $J = 12.4$ and 5.8 Hz, H′-6), 3.70 (1H, *dd*, $J = 12.4$ and 1.4 Hz, H′-6), 4.30 (1H, *d*, $J = 7.9$ Hz, H′-1), 4.50 (1H, *d*, $J = 11.8$ Hz, H-7), 4.75 (1H, *d*, $J = 11.8$ Hz, H-7), 7.18–7.25 (5H, *m*, H-2-6); ^{13}C NMR: see Table 1.

Benzyl - α - L - arabinopyranosyl(1–6)β - D - *glucopyranoside* (**4**). Yield 21 mg; $[\alpha]_D$ −39° (H_2O; *c* 0.02); IR ν_{max} (dry film) cm^{-1}: 3600–3100, 2960–2850, 1640, 1575, 1510; FAB-MS *m/z* (rel. int.): 403 $[M + H]^+$ (100), 307 (45), 295 (30); ^1H NMR: δ 3.18 (1H, *m*, H′2), 3.30–3.35 (2H, *m*), 3.45–3.50 (4H, *m*), 3.71 (1H, *dd*, $J = 11.7$ and 5.5 Hz, H′-6), 3.80 (2H), 4.02 (1H, *dd*, $J = 11.7$ and 1.6 Hz, H′-6), 4.26 (1H, *d*, $J = 7.4$ Hz, H″-1), 4.41 (1H, *d*, $J = 8.0$ Hz, H′-1), 4.60 (1H, *d*, $J = 11.7$ Hz, H-7), 4.80 (1H, *d*, $J = 11.7$ Hz, H-7), 7.25–7.35 (5H, *m*, H-2-6); ^{13}C NMR: see Table 1.

Benzyl - β - D - glucopyranosyl(1–6) - β - D - *gluco - pyranoside* (**5**). Yield 18 mg; $[\alpha]_D$ −76.2° (H_2O; *c* 0.015); IR ν_{max} (dry film) cm^{-1}: 3600–3100, 2960–2850, 1640, 1575, 1510; FAB-MS *m/z* (rel. int.): 433 $[M + H]^+$ (100), 271 (55); ^1H NMR: δ 3.27 (2H, *m*, H′-2 and H″-2), 3.35–3.45 (5H, *m*, H′-3, H′-5, H″-3, H″-4 and H″-5), 3.54 (1H, *m*, H′-4), 3.66 (1H, *dd*, $J = 12.2$ and 5.3 Hz, H″-6), 3.80 (1H, *dd*, $J = 11.7$ and

5.4 Hz, H′-6), 3.86 (1H, *dd*, J = 12.2 and 1.8 Hz, H″-6), 4.14 (1H, *dd*, J = 11.7 and 1.6 Hz, H′-6), 4.45 (1H, *d*, J = 8.0 Hz, H″-1), 4.47 (1H, *d*, J = 8.0 Hz, H′-1), 4.67 (1H, *d*, J = 11.7 Hz, H-7), 4.87 (1H, *d*, J = 11.7 Hz, H-7), 7.30–7.40 (5H, *m*, H-2-6); ^{13}C NMR: see Table 1.

Acid hydrolysis of compounds **1–5**. Compounds **1–5** (5 mg each) were heated in 2 N HCl (0.5 ml) at reflux for 30 min. The reaction mixts were extracted with EtOAc and the solvents evapd to dryness under N_2. The H_2O-soluble residues of the hydrolysate were analysed by HPAE-PAD giving D-glucose from **1–3** and **5**, and D-glucose and L-arabinose from **4**.

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