Pergamon

0031-9422(95)00679-6

Phytochemistry, Vol. 41, No. 3, pp. 787–789, 1996

0031-9422/96 $15.00 + 0.00

SUCROSE ESTERS FROM THE SURFACE LIPIDS OF *PETUNIA HYBRIDA*

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(*Received in revised form* 28 *July* 1995)

Key Word Index—*Petunia hybrida*; Solanaceae; wild tobacco; leaf surface lipids; sucrose ester.

Abstract—Three sucrose ester types were isolated and identified from the leaf surface lipids of *Petunia hybrida*. They contained both unbranched and branched fatty acids (from C2 to C8). Further, one of these sucrose esters contained malonic acids. The structures of the sucrose esters were 2,3,4-*O*-tri-acyl-α-D-glucopyranosyl-1-*O*-malonyl-4-*O*-acyl-6-*O*-acetyl-β-D-fructofuranoside, 2,3,4-*O*-tri-acyl-α-D-glucopyranosyl-4-*O*-acyl-6-*O*-acetyl-β-D-fructofuranoside and 2,3,4,6-*O*-tetra-acyl-α-D-glucopyranosyl-β-D-fructofuranoside.

INTRODUCTION

Many glucose and sucrose esters bearing a range of short to medium-chain fatty acyl substitution patterns are known in the surface exudates from leaves of the Solanaceae genera (e.g. *Datura* [1], *Lycopersicon* [2], *Nicotiana* [3–6] and *Solanum* [7]). It was demonstrated that sucrose esters were formed in the glandular trichomes in tobacco leaves [8]. These leaf surface lipids have biological activity against plants and microorganisms [9, 10]. It was reported that sucrose esters in leaf surface lipids of *Petunia hybrida* contain straight-chain fatty acids from C4 to C8 in addition to methylbutyryl groups [11]. However, the precise structures of the sucrose esters in leaf surface lipids of *P. hybrida* have not been determined. This report addresses the isolation and characterization of sucrose esters in the leaf surface lipids of *P. hybrida*.

Table 1. Compositions (mol %) of fatty acids of sucrose esters isolated from the leaf surface lipids of *P. hybrida*

	Compounds		
Fatty acids	1	2	3
Acetic acid	20.0	20.2	0.7
Butanoic acid	0.3	0.4	1.2
3-Methyl butanoic acid	41.3	40.5	51.2
Pentanoic acid	3.7	3.6	3.2
4-Methylpentanoic acid	8.5	8.7	7.3
Hexanoic acid	10.8	12.0	12.7
5-Methyl hexanoic acid	3.0	2.6	5.3
Heptanoic acid	7.5	7.8	11.1
6-Methyl heptanoic acid	2.2	1.9	3.6
Octanoic acid	2.7	2.3	3.7
Total	100.0	100.0	100.0

RESULTS AND DISCUSSION

Three glycolipids (**1–3**) were purified from the chloroform extracts of the leaf surface lipids of *P. hybrida*. The R_f values on HPTLC (Merck Art 5642) for **1, 2** and **3** were 0.68, 0.68 and 0.48, respectively, when developed with chloroform–acetone (5:5). Mild alkaline hydrolysis of **1–3** gave sucrose, short-chain fatty acids (C-4 to C-8), and branched chain fatty acids (C-5 to C-8) (Table 1). Methanolysis of **1** gave dimethyl malonate.

The IR spectra of the three glycolipids had absorption peaks at around 3400 cm^{-1} (OH proton) and 1740 cm^{-1} (ester carbonyl). The ^1H NMR assignments by spin–spin decoupling and ^{13}C–^1H COSY experiments on the three glycolipids indicated the presence of sucrose (Table 2), and **2** and **3** had an acetyl signal around δ2.1. By downfield chemical shift observations in the ^1H NMR spectra, various positions were found to be esterified, i.e. in **1** (the f3 and g6), in **2** (the f1, f3 and g6), and in **3** (the f1, f3, f6 and g6). The ^{13}C NMR spectra from ^{13}C–^1H COSY experiments on the three glycopids indicated the presence of sucrose-ring carbons (Table 3). Carbonyl carbons of esters were observed from δ170 to 180. Compound **1** had seven signals at δ165.8 (f1, carboxyacid of malonylester), 169.3 (f1, carbonyl of malonylester), 171.4 (f6), 173.1 (g3), 173.8 (f4), 175.2 (g4) and 175.8 (g2), **2** had five signals at δ171.3 (f6), 173.1 (g3), 173.6 (f4), 175.1 (g4) and 175.6 (g2), and **3** had four signals at δ173.3 (g3), 173.9 (g6), 175.2 (g4) and 176.7 (g2). The malonyl ester position, acetyl and other fatty acyl positions in the glycolipids were determined by ^1H-^{13}C NMR spectroscopy using the HMBC technique [12]. Compounds **1** and **2** had acetyl signals at f6, respectively.

Thus the structures were determined to be as follows: **1** was 2,3,4-*O*-tri-acyl-α-D-glucopyranosyl-1-*O*-malonyl-

Table 2. ^{1}H NMR spectral data (δ ppm) for the glucopyranosyl (g) and fructofuranosyl (f) protons of sucrose esters isolated from the leaf surface lipids of *P. hybrida*

Compound	g1	g2	g3	g4	g5	g6	f1	f3	f4	f5	f6
1	5.66	4.90	5.53	5.12	4.37	4.15	4.20	4.20	4.09	3.90	4.30
2	5.65	4.95	5.65	5.12	4.35	4.25	3.63	4.23	4.09	3.92	4.32
3	5.68	4.87	5.53	5.15	4.35	4.23	3.56	4.29	4.29	3.82	3.77

Table 3. ^{13}C NMR spectral data (δ ppm) for the glucopyranosyl (g) and fructofuranosyl (f) carbons of sucrose esters isolated from the leaf surface lipids of *P. hybrida*

Compound	g1	g2	g3	g4	g5	g6	f1	f2	f3	f4	f5	f6
1	89.1	70.1	69.5	67.9	68.7	61.9	64.9	103.0	77.3	74.3	78.7	63.3
2	89.2	70.2	69.5	68.0	68.6	61.9	64.3	104.6	78.0	75.0	80.7	63.8
3	89.4	70.7	69.5	67.6	68.7	61.4	64.2	104.8	78.0	73.8	82.1	61.0

Table 4. FAB-mass spectral data for the molecular ions of sucrose esters isolated from the leaf surface liplids of *P. hybrida*

		Composition of fatty acids and malonyl ester				
Compound	M_r + Na	C-2	C-5	C-6	C-7	Malonyl ester
1	913.39	1	—	2	2	1
	899.39	1	—	3	1	1
	885.37	1	—	4	—	1
	871.35	1	1	3	—	1
	857.34	1	2	2	—	1
	843.32	1	3	1	—	1
2	813.42	1	—	3	1	—
	799.41	1	—	4	—	—
	785.38	1	1	3	—	—
	771.37	1	2	2	—	—
	757.34	1	3	1	—	—
	743.32	1	4	—	—	—
3	785.40	—	—	2	2	—
	771.38	—	—	3	1	—
	757.36	—	—	4	—	—
	743.35	—	1	3	—	—
	729.34	—	2	2	—	—
	715.33	—	3	1	—	—
	701.32	—	4	—	—	—

4-*O*-acyl-6-*O*-acetyl-β-D-fructofuranoside, **2** was 2,3,4-*O*-tri-acyl-α-D-glucopyranosyl-4-*O*-acyl-6-*O*-acetyl-β-D-fructofuranoside and **3** was 2,3,4,6-*O*-tetra-acyl-α-D-glucopyranosyl-β-D-fructofuranoside.

The structures of sucrose esters were confirmed by FAB-mass spectra analyses (Table 4). The results indicated that **1** contained acetyl, malonyl and fatty acyl molecules (C5, C6 and C7) and **2** contained acetyl and fatty acyl molecules (C5, C6 and C7). It was also shown that **3** contained fatty acyl molecules (C5, C6 and C7).

The present experiments showed that *P. hybrida* had different types of sucrose esters from other Solanaceae species in terms of their ester positions and fatty acid compositions. The sucrose esters with both short-chain fatty acid and malonyl esters appear to be unique to this species. We could not study the location of the sucrose esters on the leaf of *P. hybrida* here, though chloroform might have extracted both lipids of cuticle layer and the wall just below. Because, it was demonstrated that sucrose esters were formed in the glandular trichomes supplied carbon sources such as sucrose and glucose from epidermal cells in tobacco leaves [8]. Therefore, we concluded that the three sucrose esters in the leaf surface lipids of *P. hybrida* were also delivered from the glandular trichomes.

EXPERIMENTAL

Plant materials. Petunia plants (*P. hybrida*) were grown in soil in 120 cm^2 pots in a greenhouse at 28°. The leaf surface lipids were extracted by dipping the leaves of three-month-old plants in $CHCl_3$ for 10 sec. The $CHCl_3$ extracts were concentrated and kept at $-20°$ until used.

Isolation of sucrose esters. The stored lipids (0.2 wt% of the fresh leaves of *P. hybrida*) were subjected to chromatography on a silica gel (Wakogel C300) column and sepd into 4 frs by successive elution with $CHCl_3$ (1), $CHCl_3$–Me_2CO (1:1), Me_2CO, and MeOH. The relative wt% of each fr. was 28.6, 38.0, 4.5 and 28.9%, respectively. The $CHCl_3$–Me_2CO (1:1) eluate was further purified on a silica gel (Wakogel C300) column and sepd by successive elution with hexane–Me_2CO (8:2), hexane–Me_2CO (7:3), hexane–Me_2CO (5:5) and Me_2CO. The hexane–Me_2CO (8:2) eluate was further purified by HPLC (YMC-Pack A-014 Sil). Compounds **1** and **2** were eluted at 14.10 and 15.62 ml with hexane–Me_2CO (6:4, 2.0 ml min^{-1}). The hexane–Me_2CO (7:3) eluate was further purified by HPLC (YMC-Pack A-014 Sil).

Compound **3** was eluted at 21.25 ml with hexane–Me_2CO (6:4, 2.0 ml min^{-1}).

Alkaline hydrolysis of compounds **1–3**. To identify the sugar moieties, about 2.0 mg of each lipid was saponified with 2 N KOH at 110° for 30 min. The resulting saponified solubles were subjected (×2) to HPTLC with a solvent system of *n*-BuOH–iPrOH–H_2O (3:12:4).

Fatty acid analysis. The alkaline hydrolyzates of the sucrose esters were acidified with 6 N HCl. After precipitating the salt by adding a few drops of ethyl ether, each solution was subjected directly to fused silica capillary gas chromatography using FID (Unisole 400 (FS), Chromato Research Ltd, 70–160°, 4° min^{-1}). The peaks on the chart were identified by comparing their retention times with those of standard fatty acids.

Methanolysis of **1**. **1** was methanolized with 5% methanoic H_2SO_4 in MeOH. After methanolysis, the hexane extract was analysed by GC-MS, and, dimethyl malonate (m/z 132, 101, 74, 59) was identified.

Assignment of the ester positions in the glycolipids. The ester positions in the individual glycolipids were determined by the HMBC experiment, using a delay time of 80 msec.

Spectroscopy. All spectral data were obtained with the following instruments. ^{1}H NMR spectra on a Bruker AC-300 (300 MHz, $CDCl_3$); Two-dimensional NMR spectra on a Bruker AM-500 (500 MHz, $CDCl_3$); GC-MS spectra on a Hewlett-Packerd HP-5970; IR spectra on a Jasco FT/IR 5000; FAB-MS spectra on a Shimazu Kuretos series II.

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