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3,4-DI-*O*- AND 2,3,4-TRI-*O*-ACYLATED GLUCOSE ESTERS FROM THE GLANDULAR TRICHOMES OF NONTUBEROUS *SOLANUM* SPECIES

RUSSELL R. KING, LARRY A. CALHOUN* and RUDRA P. SINGH

Research Station, Research Branch, Agriculture Canada, Fredericton, New Brunswick, Canada, E3B 4Z7; *Department of Chemistry, University of New Brunswick, Fredericton, New Brunswick, Canada, E3B 6E2

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Abstract—Complexes of 3,4-di-O- and 2,3,4-tri-*O*-acylated glucose esters were identified as the major nonvolatile constituents in the exudate from type B glandular trichomes of certain nontuberous *Solanum* species. The principal glucose ester was resolved by reversed phase TLC and characterized as 2-*O*-acetyl-3-*O*-isobutyryl-4-*O*-isocaprylglucopyranose.

INTRODUCTION

The aerial surfaces of many Solanaceous species are covered with glandular trichomes that utilize mucilaginous secretions to entrap or otherwise deter potential predators. In the genus *Solanum*, two distinct types of glandular trichomes predominate [1, 2]. Normally, the multicellular or type A glandular trichomes consist of short stalks with tetralobulate glandular tips. When ruptured, the glands release a combination of phenolic substances and oxidative enzymes that combine to produce a quick-setting fluid [3]. The other or type B trichomes are long slender hairs with unicellular glandular tips. These freely exude a durable sticky material.

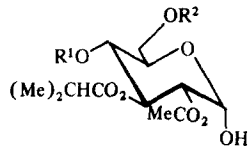
Our investigations of the type B trichome exudate from accessions of the wild potato species *Solanum berthaultii* [4, 5] and *Solanum neocardenasii* [6] revealed that complex mixtures of sucrose esters constituted the major portion of the nonvolatiles. Moreover, the sucrose substitution pattern in these compounds varied substantially (i.e. from 3,4,6-tri-*O*- and 3,3′,4,6-tetra-*O*-acylated in *S. berthaultii* accessions to 2,3,3′,4-tetra-*O*-acylated in *S. neocardenasii*). (Previously, 2,3,4,6-tetra-*O*-acylated sucrose esters had been detected in isolates of the foliage from wild *Nicotiana tabacum* species [7]). In all instances there was considerable variation in the identity of the acyl substituents. Consideration of the diversity displayed by such a limited number of sucrose esters prompted us to undertake an investigation of other appropriate Solanaceae species. This paper describes the results obtained on analysis of the exudate from the type B glandular trichomes of certain nontuberous *Solanum* species.

RESULTS AND DISCUSSION

Preliminary analysis (TLC and base hydrolysis) of the glandular exudates from nine of ten appropriate nontuberous *Solanum* species examined (specifically: *S. aethiopsicum*, *S. carolinense*, *S. lactum mill*, *S. maritinum*, *S. nigrum var chloracaepum*, *S. pyracantum*, *S. sturtianum*, *S. vignoi* and *S. villorian*) indicated that glucose esters constituted the major portion of the nonvolatiles in each. Moreover, the make up of the glucose esters (as determined by capillary GC of the acetyl derivatives) appeared identical for all nine species.

After the initial survey a large sample of the trichome exudate from the foliage of *Solanum aethiopsicum* was selected for a more detailed chemical analysis. Sequential fractionation by preparative TLC on silica gel plates isolated two complexes of glucose esters from other nonvolatiles in the exudate. ^{1}H NMR [8] of these isolates indicated the presence of glucose molecules acylated at the 3-*O*- and 4-*O*-positions in the minor complex and at the 2-*O*-, 3-*O*-, and 4-*O*- positions in the major. To determine acid composition, both complexes were transesterified with sodium methoxide. Comparative capillary GLC-MS (EI) of the methyl esters identified isobutyryl, 2-methylbutyryl, isopelargonyl and isocapryl groups in both complexes. Subsequent capillary GLC-MS (EI) of the 2,3,4-tri-*O*-acylated complex as their acetyl derivatives indicated the presence of six components in significant amounts. The parent components were assigned numbers based on the order of elution of their acetyl derivatives by GLC on a DB-5 capillary column. High mass ions of the acetyl derivatives (i.e. M − 60) and their relative proportion of the complex were as follows: for **1(a)**, *m/z* 456 (6.1%); **1(b)**, *m/z* 456 (4.5%); **2 (a)**, *m/z* 470 (34.7%); **2 (b)**, *m/z* 470 (22.8%); **3 (a)**, *m/z* 484 (7.6%); and **3 (b)**, *m/z* 484 (5.8%). The similarity in mass spectral fragmentation patterns of **1 (a)** with **1 (b)**, **2 (a)** with **2 (b)** and **3 (a)** with **3 (b)** was consistent with the expected presence of 1-*O*-acetyl anomers. Other mass spectral data of note was the prominence of: (i) butyryl mass ions, i.e. *m/z* 71 in compounds **1** and **2**; (ii) capryl mass ions, i.e. *m/z* 155 in compounds **2** and **3**, (iii) pelargonyl ions, i.e. *m/z* 141 in compound **1** and (iv) methylbutyryl ions, i.e. *m/z* 85 in compound **3**.

Similar GLC-MS (EI) determinations revealed that the acetylated derivatives of the 3,4-di-*O*-acyl complex were identical to those of the acetylated 2,3,4-tri-*O*-acyl complex. Such findings are only consistent with an acetyl group occupying the 2-*O*- position in all of the 2,3,4-tri-*O*-



2 $R^1 = (Me)_2CH(CH_2)_6C(=O)-$, $R^2 = H$

4 $R^1 = H$, $R^2 = (Me)_2CH(CH_2)_6C(=O)-$

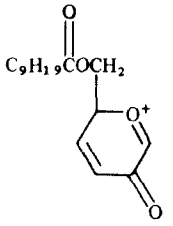


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Table 1. ^{1}H NMR data (δ) for the glucose esters

H	2 (α)	2 (β)	4 (α)
H-1	5.49	4.77	5.42
($J_{1,2}$)	(3.5)	(7.9)	(3.6)
H-2	4.94	4.91	4.93
($J_{2,3}$)	(10.3)	(9.5)	(10.3)
H-3	5.63	5.33	5.34
($J_{3,4}$)	(9.6)	(9.5)	(9.2)
H-4	5.04	5.08	3.56
($J_{4,5}$)	(9.6)	(9.5)	(9.2)
H-5	4.08	3.58	4.11
H-6	3.68	3.70	4.40
$>CHCO_2-$	2.52	2.52	2.60
$-CH_2CO_2-$	2.27	2.27	2.38
$MeCO_2$	2.07	2.07	2.09
Me_3CCO_2	1.11, 1.12	1.11, 1.12	1.17, 1.18
	(7.0) (7.0)	(7.0) (7.0)	(7.0) (6.9)
>CH–	1.56	1.56	1.56
$-CH_2-$	1.26	1.26	1.28, 1.63
Me–	0.86	0.86	0.86
	(6.5)	(6.5)	(6.5)

Table 2. ^{13}C NMR data (δ) for the carbon atoms of the glucose esters

C	2 (α)*	2 (β)	4 (α)†
1	90.26	95.50	90.43
2	69.52	73.45	70.67
3	71.28	74.46	72.38
4	68.64	68.35	69.46
5	68.95	71.46	70.17
6	61.11	61.11	62.60

†From one-bond COLOC experiment.

*By analogy with compound **4**.

acylated compounds. In addition, the substituents occupying the 3-*O*- and 4-*O*- positions must be equivalent in both complexes. Hence, it was rationalized that identification of either all of the 3-*O*- or all of the 4-*O*- substituents would suffice to assign structures for the components of both complexes. The discovery that mild hydrolysis with methanolic ammonium hydroxide catalysed ready migration of the 4-*O*-acyl substituents to the 6-*O*-positions in all these compounds, was utilized as an initial step in the assignment process. Next, prominent mass ions representative of pyroxonium structures in their individual mass spectral fragmentation patterns were used to establish the M_r of the acyl substituents at the 6-*O*-positions [9]. By these procedures the components of the glucose esters in the 2,3,4-tri-*O*-acylated complex were assigned the structures 2-*O*-acetyl-3-*O*-isobutyryl-4-*O*-isopelargonylglucopyranose (**1**), 2-*O*-acetyl-3-*O*-isobutyryl-4-*O*-isocaprylglucopyranose (**2**) and 2-*O*-acetyl-3-*O*-(2-methylbutyryl)-4-*O*-isocaprylglucopyranose (**3**). By comparison the 3,4-di-*O*-acylated complex must consist of the 2-*O*-deacetyl analogues.

Structural designations for the glucose ester isolates were further substantiated by reversed phase TLC separation and spectroscopic analysis of the compound assigned the 2-*O*-acetyl-3-*O*-isobutyryl-4-*O*-isocaprylglucopyranose structure (**2**). Thus, (i) a FAB positive ion spectrum of the compound exhibited a high mass peak at *m/z* 469 $[M+Na]^+$ corresponding to the molecular formula $C_{22}H_{38}O_9$; (ii) a two-dimensional ^{1}H homonuclear shift correlated NMR spectrum [10] showed discrete downfield signals for H-2, H-3 and H-4 (Table 1); (iii) a two-dimensional ^{13}C–^{1}H heteronuclear shift correlated NMR experiment (COLOC) [11] optimized for the detection of one band correlations [12] allowed assignment of the ^{13}C NMR signals for the glucose carbons (Table 2) and also confirmed the presence of one acetyl, one isobutyryl and one isocapryl substituents and (iv) a selective long range INEPT experiment [13] for establishing long-range ^{13}C–^{1}H shift correlations was utilized to confirm that the acetyl group occupied the C-2 position of the glucose molecule. To complete the structural assignment compound **2** was treated with methanolic ammonium hydroxide. This catalysed migration of the 4-*O*-substituent to the 6-*O*- position and yielded a transformation product (**4**) which was crystalline and anomerically homogeneous (Tables 1 and 2). As expected, its mass spectral fragmentation pattern (EI) displayed a prominent mass ion at *m/z* 281. This mass ion was deemed representative of the pyroxonium structure **5** and established [9] the presence of the isocapryl moiety at the C-6 position (also prolonged hydrolysis of **4** yielded 6-*O*-caprylglucopyranose). Thus, by the process of elimination, the isobutyryl group must occupy the 3-*O* position in the original compound.

Interestingly, the major acylation patterns displayed in the glucose ester isolates from the nontuberous *Solanum* species are identical to those found in the glucose portions of sucrose esters from *S. neocardenasii* [6]. In contrast, isocapryl groups noticeably prominent only in 3,4,6-tri-*O*-acylated sucrose esters from certain *S. be-*

rthaultii introductions [4] constitute a major substituent in the nontuberous *Solanum* glucose esters.

Since completion of the foregoing investigations [14] 2,3,4-tri-*O*-acylated glucose esters have also been identified in the trichome exudate of *Lycopersicon pennellii* (Corr.) D'Arcy (formerly *Solanum pennellii*) [15]. These findings contrast with our analysis of more bonafide wild *Lycopersicon* species in which the glycolipid portion of the trichome exudate contained mainly 2,3,4-tri-*O*-acylated sucrose esters [14] and the difference may be phylogenetically significant.

EXPERIMENTAL

Plant material. Plants were grown in a greenhouse from seeds obtained from W. J. Cody, Phanerogamic Herbarium Plant Research Institute, Ottawa, Canada. A total of 10 seedlings were examined with a binocular microscope for each species.

Analytical methods. Mp: uncorr. IR spectra were determined in $CHCl_3$. All NMR spectra were recorded in $CDCl_3$ operating at 200.057 MHz for ^1H and 50.309 MHz for ^{13}C. Chemical shifts were measured downfield from internal TMS and further details of the general procedures are outlined in a previous paper [5]. Capillary GLC studies were performed on a Varian 3500 GLC utilizing on-column injection and a 30 m × 0.25 nm i.d. fused silica capillary column with a 0.25 μm film of DB-5.

Ester isolation and identification. Composite samples (20 g) of freshly collected foliage from mature plants (preliminary studies indicated that plant or leaf age did not alter the ester type present) were extracted with $CHCl_3$ (100 ml) by soaking for 5 min. The $CHCl_3$ was removed *in vacuo* and the residue was taken up in Me_2CO (4 ml), cooled to 0° and vacuum filtered through Whatman no. 1 filter paper to remove co-extracted plant waxes. After removal of the Me_2CO *in vacuo*, a sample of the residue was subjected to thin layer chromatography (0.2 mm silica gel, developed in $CHCl_3$–MeOH (9:1) with detection by charring after a 5% H_2SO_4 in EtOH spray). For preliminary detection charred areas in the R_f range 0.3–0.6 were usually indicative of the presence of carbohydrate esters. Positive and indeterminate samples were treated with Ac_2O (2 ml) and pyridine (1 ml) with stirring at room temp. overnight. The reaction mixture could be sampled directly (or quenched in Na_2CO_3 soln, prior to extraction of the acetylated esters with an organic solvent) for comparative capillary gas liquid chromatographic analysis. Foliage (426 g) from mature plants of *Solanum aethiopsicum* were extracted by dipping (*ca* 5 sec) in a 2 l beaker containing $CHCl_3$ (1000 ml) and then into a second 2 l beaker containing a similar amount of $CHCl_3$. The combined extracts were filtered through a plug of cotton wool and the $CHCl_3$ removed *in vacuo* at room temp. The residue (1.48 g) was taken up in Me_2CO (50 ml) cooled to 0° and vacuum filtered through Whatman no. 1 filter paper to remove co-extracted plant waxes. Removal of the Me_2CO *in vacuo* yielded a yellowish viscous residue (1.22 g) which was then fractionated by TLC (10 plates of 0.5 mm silica gel 60 developed in $CHCl_3$–MeOH 9:1). The glucose ester complexes were detected (using a H_2O spray) at R_f 0.37 and 0.56, then eluted from the silica gel with Me_2CO (300 ml). Removal of the Me_2CO *in vacuo* yielded the di-*O*-acyl (93 mg) and tri-*O*-acyl (384 mg) esters respectively.

Transesterification of the glucose ester complexes. Portions of the glucose ester complex (20 mg) were dissolved in dry MeOH (5 ml) and treated for 10 min at room temp. with 0.1 M NaOMe (1.0 ml). The reaction mixture was deionized with amberlite IR-120 (H^+) resin and analysed on a 30 M SP2330 capillary column at 10 psi He and splitless injection at 30°, held for 2 min, then raised to 120° at 25°/min. The methyl esters were identified by comparative GLC retention data of purchased or prepared standards and by GC-MS (EI). Removal of the methyl esters and acetylation of the carbohydrate residue with Ac_2O–NaOAc yielded a compound with GC-MS (EI) identical to β-D-glucopyranose penta-acetate.

Acetylation and GC-MS analysis of the glucose ester complexes. A portion of each glucose ester complex (5 mg) was treated with Ac_2O (5 ml) and pyridine (1 ml) with stirring at room temp. overnight. The reaction mixture was quenched in an excess of satd Na_2CO_3 solution which was subsequently extracted with two 30 ml portions of $CHCl_3$. The $CHCl_3$ was removed *in vacuo* and the glassy residue dissolved in toluene and subjected to GC-MS (EI) analysis on a 30 × 0.32 mm i.d. fused silica capillary column containing a 0.25 μm film thickness of DB-5. A 2 μl sample was injected via on-column injection at 90° and raised to 320° at 100°/min. The column oven was held at 90°C for 2 min, raised to 250° at 25°/min. then programmed at 8°/min to 300°. Mass spectrometer: 70 eU (EI) 45 to 650 amn at 1.5 sec/scan. Data acquisition was started 13 min after injection.

4-O-acyl group migration. A portion (10 mg) of the 2,3,4-tri-*O*-acylated ester complex was dissolved in 5% methanolic NH_4OH (2 ml) and stirred with cooling in an ice H_2O bath until TLC studies ($CHCl_3$–MeOH, 9:1) indicated that the majority of starting materials had been transformed (*ca* 15 min). The solvents were removed *in vacuo* at room temp. and the residue acetylated prior to GC-MS as described previously.

2-O-*Acetyl*-3-O-*isobutyryl*-4-O-*isocaprylglucopyranose* (**2**). The purified 2,3-4-*O*-triacyl glucose ester complex was fractionated on 0.2 mm RP-C_{18} thin layer plates (Whatman) developed in Me_2CO–H_2O (7:3). The zone at R_f 0.47 after rechromatography yielded 2-*O*-acetyl-3-*O*-isobutyryl-4-*O*-isocaprylglucopyranose (**2**) as a viscous semi-solid with ν_{max} 3510 and 1735 cm^{-1}; ^1H NMR, and mass spectral data are given in the text. ^{13}C NMR signals at δ 20.68 (*q*) and 170.09 (*s*) corresponded to C-2 and C-1 respectively of the acetyl group; signals at δ 18.78 (*q*), 18.92 (*q*), 34.01 (*d*) and 176.05 (*s*) corresponded to C-3, C-3′, C-2 and C-1 respectively of the isobutyryl group; signals at δ 22.61 (2 *q*), 27.91 (*d*), 38.89 (*t*), 27.13 (*t*), 29.14 (*t*), 29.43 (*t*), 24.79 (*t*), 33.98 (*t*) and 173.50 (*s*) corresponded to C-10 through C-1 of the isocapryl group (the glucose signals are given in the text).

2-O-*Acetyl*-3-O-*isobutyryl*-6-O-*isocaprylglucopyranose* (**4**). Purified 2-*O*-acetyl-3-*O*-isobutyryl-4-*O*-isocaprylglucopyranose (**2**) was treated with 5% methanolic NH_4OH as described for the 2,3,4-tri-*O*-acylated ester complex. The reaction mixture was then fractionated on 0.25 mm silica gel thin layer plates developed in $CHCl_3$–MeOH (9:1). The zone at R_f 0.66 yielded 2-*O*-acetyl-3-*O*-isobutyryl-6-*O*-isocaprylglucopyranose (**4**) as crystals (from petrol) with mp 92–93°C; ν_{max} 3515 and 1735 cm^{-1}; ^1H NMR and mass spectral data are given in the text. ^{13}C NMR signals at δ 20.71 (*q*) and 170.05 (*s*) corresponded to C-2 and C-1 respectively of the acetyl group; signals at δ 18.65 (*q*), 19.04 (*q*), 34.12 (*d*) and 178.03 (*s*) corresponded to C-3, C-3′, C-2 and C-1 respectively of the isobutyryl group; signals at δ 22.62 (2 *q*), 27.92 (*d*), 38.91 (*t*), 27.20 (*t*), 29.13 (*t*), 29.49 (*t*), 24.89 (*t*), 34.08 (*t*) and 174.39 (*s*) corresponded to C-10 through C-1 of the isocapryl group (the glucose signals are given in the text). Prolonged hydrolysis of **4** yielded a monoacylated C_{10} derivative with ^1H NMR δ 4.41 (2H, *m*).

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