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2,3-DI-*O*- AND 1,2,3-TRI-*O*-ACYLATED GLUCOSE ESTERS FROM THE GLANDULAR TRICHOMES OF *DATURA METEL*

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Abstract—Complexes of 2,3-di-*O*- and 1,2,3-tri-*O*-acylated glucose esters were identified as the major non-volatile constituents in the exudate from type B glandular trichomes of *Datura metel*. The principal glucose esters were resolved by reversed phase TLC and characterized as 2,3-di-*O*-hexanoyl-α-glucopyranose and 1,2,3-tri-*O*-hexanoyl-α-glucopyranose.

INTRODUCTION

The aerial surfaces of many Solanaceous species are covered with glandular trichomes that utilize mucilaginous secretions to trap or otherwise deter potential predators. In the genus *Solanum*, two distinct types of glandular trichomes predominate and they are atypical of other Solanaceae genera [1, 2]. Normally, the multicellular (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. The other or type B trichomes are slender hairs with unicellular glandular tips. These glands freely exude a durable sticky material. They are most common throughout the *Solanum* and *Nicotiana* genera but also appear in other Solanaceous species such as *Datura*, *Lycopersicon* and *Petunia*.

Our investigations of the type B glandular trichome exudate from accessions of certain wild potato species have revealed that complex mixtures of sucrose esters constitute the major portion of the non-volatiles [3–5]. The sucrose substitution patterns in these compounds varied from 3,4,6-tri-*O*- and 3,3',4,6-tetra-*O*- to 2,3,3'4-tetra-*O*-acylated. For each substitution pattern there were noticeable differences in the acyl substituents present.

Investigations of the type B trichome exudate from other Solanaceae genera revealed the presence of 3,4-di-*O* and 2,3,4-tri-*O*-acylated glucose esters in numerous non-tuberous *Solanum* species [6, 7] 2,3,4-tri-*O*-acylated sucrose esters in *Lycopersicon hirsutum* f. *glabratum* and 2,3,4,6-tetra-*O*-acylated sucrose esters in *Petunia multiflora nana* [6]. Previously, 2,3,4,6-tetra-*O*-acylated glucose and sucrose esters had been reported in isolates of the foliage from wild *Nicotiana tabacum* species [8]. More recently, 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*) [9].

This paper describes the results obtained on analysis of the exudate from the type B glandular trichomes of *Datura metel* L.

RESULTS AND DISCUSSION

Preliminary analysis of the chloroform soluble extracts of foliage from available *D. metel* accessions indicated that glucose esters constituted a major portion of the nonvolatiles and the amounts varied with the density of the type B glandular trichomes present. These results were in accordance with previous findings in other genera—namely, that the type B glandular trichomes are the source of carbohydrate esters.

After the initial survey a large sample of the trichome exudate from the foliage of *D. metel* accessions was accumulated for a 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 [10] of these isolates indicated the presence of 2,3-di-*O*- and 1,2,3-tri-*O*-acylated glucose esters respectively. To identify the acyl groups present, both complexes were transesterified with sodium methoxide. Comparative capillary GC-MS (EI) of the resultant methyl esters confirmed the presence of substantial quantities of pentanoyl, hexanoyl, heptanoyl, octanoyl and undecanoyl residues in each complex.

Subsequent capillary GC-MS (EI) of the 1,2,3-tri-*O*-acylated esters as their acetyl derivatives indicated the presence of one major and several minor constituents. Fractionation of the 1,2,3-tri-*O*-acylated complex on reversed phase TLC plates isolated the major component in 54.2% yield. A FAB positive ion spectrum of the compound exhibited a high mass peak at *m/z* 497 $[M + Na]^+$ corresponding to the molecular formula $C_{24}H_{42}O_9$. A ^{1}H NMR homonuclear decoupling experiment indicated the presence of discrete downfield signals for H-1, H-2 and H-3 (Table 1). A ^{13}C NMR spectrum confirmed the presence of three hexanoyl substituents and allowed comparative assignments for the glucose carbons (Table 2). On the basis of this data the compound was assigned the structure 1,2,3-tri-*O*-hexanoyl-α-glucopyranose (**1**).

Two other distinct components comprising 18.4 and 7.9%, respectively, of the 1,2,3-tri-*O*-acylated glucose ester complex were also resolved by reversed phase TLC.

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

H	1	2
1	6.32 (*d*)	5.41 (*d*)
($J_{1,2}$)	(3.7)	(3.3)
2	5.04 (*d*, *d*)	4.83 (*d*, *d*)
($J_{2,3}$)	(3.7, 10.2)	(3.3, 10.4)
3	5.30 (*d*, *d*)	5.37 (*t*)
($J_{3,4}$)	(9.0, 10.0)	(9.6)
4	3.85 (*m*)	3.49 (*m*)
5	3.85 (*m*)	3.69 (*m*)
6	3.85 (*m*)	3.88 (*m*)
$-CH_2CO_2-$	2.41 (*t*)	2.33 (*t*)
	(7.5)	
	2.36 (*t*)	2.33 (*t*)
	(7.6)	
	2.24 (*t*)	
	(7.6)	
$-CH_2CH_2CO_2-$	1.61 (*m*)	1.60 (*m*)
$MeCH_2CH_2-$	1.30 (*m*)	1.29 (*m*)
Me	0.90 (*m*)	0.89 (*m*)

The greater of the two was determined to contain hexanoyl and pentanoyl substituents in a 2:1 ratio while the other contained hexanoyl and heptanoyl groups in a 2:1 ratio. MS (EI) analysis [7] indicated that these components were undoubtedly isomeric mixtures but they could not be further resolved by HPLC or TLC techniques.

Capillary GC-MS (EI) of the 2,3-di-*O*-acylated glucose esters as their acetyl derivatives indicated the presence of four major components in roughly equal proportions (i.e. 24.9, 20.1, 16.9 and 23.3%). These components were readily separated by reversed phase TLC but MS (EI) analysis indicated that only one of the four was not an isomeric mixture. The nonisomeric compound was readily crystalline and a FAB positive ion spectrum of it exhibited a high mass peak at *m/z* 399 $[M+Na]^+$ corresponding to the molecular formula $C_{18}H_{32}O_8$. A ^{1}H NMR homonuclear decoupling experiment indicated the presence of discrete downfield signals for H-2 and H-3 (Table 1). A ^{13}C NMR spectra subsequently confirmed the presence of two hexanoyl substituents and allowed comparative assignments for the glucose carbons (Table 2). On the basis of the preceding data, the compound was assigned the structure 2,3-di-*O*-hexanoylglucopyranose (**2**).

Table 2. ^{13}C NMR data (δ) for the sugar portions of the glucose esters*

C	1	2
1	88.99	90.31
2	73.01	71.47
3	73.85	73.03
4	68.90	70.19
5	69.37	70.67
6	61.76	62.24

*By analogy with similar compounds where assignments were made on the basis of one-bond COLOC experiments [7].

In contrast to glucose esters previously identified in the trichome exudate from other members of the Solanaceae, those associated with the genus *Datura* are rather unique. For instance, they are the only C-1 acylated glucose esters yet reported and the substituent acyl groups are predominately straight chain ones.

EXPERIMENTAL

Plant material. Plants were grown in a greenhouse from seeds obtained from W. J. Cody (Phanerogamic Herbarium Plant Research Institute, Ottawa, Canada). Seedlings were examined with a binocular microscope for the presence of glandular trichomes.

Analytical methods. Mps: uncorr. IR.: $CHCl_3$. All NMR spectra were recorded in $CDCl_3$ at 200.057 MHz for ^{1}H 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 [4]. The MS (EI) were determined on a Finnigan 4021 GC-MS coupled to an INCOS data acquisition system. Capillary GLC studies were performed on a Varian 3500 GLC utilizing on-column injection and a 30 m × 0.25 mm 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 TLC (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 $NaHCO_3$ soln, prior to extraction of the acetylated esters with an organic solvent) for comparative capillary GL analysis. Foliage (514 g) from mature plants of *D. metel* 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 (0.82 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 (0.67 g) which was then fractioned 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 water spray) at R_f 0.31 and 0.64, then eluted from the silica gel with Me_2CO (300 ml). Removal of the Me_2CO *in vacuo* yielded the di-*O*-acyl (210 mg) and tri-*O*-acyl (204 mg) esters respectively.

Transesterification of the glucose ester complexes. Portions of the glucose ester complex (10 mg) were dissolved in dry MeOH (2 ml) and treated for 10 min at room temp. with 0.1 M NaOMe (0.5 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 GC 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 sat. $NaHCO_3$ soln 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-1. 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° for 2 min, raised to 250° at 25°/min, then programmed at 8°/min to 300°. Mass spectrometer: 70 eV (EI) 45 to 650 amu at 1.5 sec/scan. Data acquisition was started 13 min after injection.

Separation and characterization of the 1,2,3-*tri*-O-*acylated glucose esters.* The purified 1,2,3-tri-*O*-acylated glucose ester complex was fractionated on 0.2 mm RP-C_{18} thin layer plates (Whatman) developed in Me_2CO–H_2O (7:3). One major and several minor zones could be distinguished by charring after spraying with 5% H_2SO_4 in EtOH. The glucose esters were eluted with Me_2CO–MeOH (4:1). The zone at R_f 0.44 (54.2% of the mixture) yielded 1,2,3-*Tri*-O-*hexanoyl-α-glucopyranose* (**1**) as a homogeneous (by capillary GC of the acetylated derivatives), viscous, semisolid, ν_{max} 3520 and 1735 cm^{-1}. Pertinent ^1H NMR and mass spectral data are given in the text. ^{13}C NMR signals for the glucose component are given in the text and ^{13}C NMR signals for the three hexanoyl groups were assigned as follows: C-1 (171.88), 172.54 and 174.92), C-2 (33.84, 34.13 and 34.23), C-3 (24.40, 24.50 and 24.61), C-4 (31.15), C-5 (22.25) and C-6 (13.84). The zone at R_f 0.47 (18.4% of the mixture) could not be further resolved but GC-MS (EI) of the acetyl derivatives showed high mass ions at 429 and 443 *m/z* which would indicate one compound with a pentanoyl group at the C-1 position and at least one other compound with a hexanoyl group at the C-1 position. Similarly, GC-MS following acetylation of the isolates from zone R_f 0.40 (7.9% of the mixture) indicated high mass ions at 443 and 457 *m/z*. These results would indicate one compound with a heptanoyl group at the C-1 position and at least one other compound with a hexanoyl group at the C-1 position.

Separation and characterization of the 2,3-*di*-O-*acylated glucose esters.* The purified 2,3-di-*O*-acylated glucose ester complex was fractionated on 0.2 mm RP-C_{18} thin layer plates (Whatman) developed in Me_2CO–H_2O (13:7) and four major zones could be distinguished by charring after spraying with 5% H_2SO_4 in EtOH. The glucose esters were eluted with Me_2CO–MeOH (4:1). The zone at R_f 0.48 (24.9% of the mixture) crystallized from Et_2O–hexane to give 2,3-di-*O*-hexanoyl-α-glucopyranose (**2**) as colourless plates with mp 112–114°, ν_{max} 3540 and 1738 cm^{-1}. Pertinent ^1H NMR and mass spectral data are given in the text. ^{13}C NMR signals for the glucose component are given in the text and ^{13}C NMR signals for the two hexanoyl groupss were assigned as follows: C-1 (173.05 and 175.08), C-2 (34.04 and 34.27), C-3 (24.54 and 24.61), C-4 (31.19), C-5 (22.27) and C-6 (13.87). The zone at R_f 0.43 (20.1% of the mixture) could not be further resolved but ^{13}C NMR analysis and GC-MS (EI) of the acetyl derivatives showed fragmentation patterns [7] that indicated a mixture of 2-*O*-hexanoyl-3-*O*-heptanoyl-α-glucopyranose and 2-*O*-heptanoyl-3-*O*-hexanoyl-α-glucopyranose. In a like manner, analysis of the zone at R_f 0.39 (16.9% of the total) indicated a mixture of 2-*O*-heptanoyl-3-*O*-octanoyl-α-glycopyranose and 2-*O*-octanoyl-3-*O*-heptanoyl-α-glucopyranose. Similarly, an analysis of the zone of R_f 0.36 (23.3% of the total) indicated a mixture of 2-*O*-hexanoyl-3-*O*-nonanoyl-α-glucopyranose and 2-*O*-nonanoyl-3-*O*-hexanoyl-α-glucopyranose.

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