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ELUCIDATION OF STRUCTURES FOR A UNIQUE CLASS OF 2,3,4,3′-TETRA-*O*-ACYLATED SUCROSE ESTERS FROM THE TYPE B GLANDULAR TRICHOMES OF *Solanum neocardenasii* HAWKES & HJERTING (PI 498129)

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ABSTRACT

A class of unique sucrose esters that comprise the greater portion of nonvolatile constituents in the exudate from type B glandular trichomes of *S. neocardenasii* Hawkes & Hjerting (PI 498129) were resolved by reversed phase t.l.c. The major components were characterized by a combination of hydrolysis studies and spectroscopic techniques as 2-*O*-acetyl-3′-*O*-hexanoyl-3,4-di-*O*-isobutyrylsucrose, 2-*O*-acetyl-3′,4-di-*O*-hexanoyl-3-*O*-isobutyrylsucrose, and 2-*O*-acetyl-3′-*O*-decanoyl-3,4-di-*O*-isobutyrylsucrose.

INTRODUCTION

The foliage of many wild potato species is covered with glandular trichomes (type A and type B) that utilize mucilaginous secretions to entrap arthropod pests[1]. The type A trichomes consists of short stalks with tetralobulate glandular tips. When ruptured, the glands release phenolic substances and oxidases[2]. These combine to produce a quick-setting fluid which rapidly darkens and hardens. In comparison, the type B trichomes are composed of long slender hairs with unicellular glandular tips. These glands freely exude a durable sticky material.

Our previous investigations involving the exudate from type B glandular trichomes of *Solanum berthaultii* Hawkes accessions demonstrated that in certain cases (*i.e.*, PI 473340) the major nonvolatile components were 3,4,6-tri-*O*-acylated sucrose esters[3]. In others (*i.e.*, PI 218215, 265857, 265858, 310925, 473330, and WRF 1727), 3,4,6,3′-tetra-*O*-acylated sucrose esters constituted the major portion of the nonvolatiles[4]. The latter compounds were the first-reported, naturally occurring sucrose esters to contain acyl substituents at both the D-glucose and D-

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fructose residues. We now report the isolation of a new but kindred class of compounds, namely 2,3,4,3′-tetra-*O*-acylated sucrose esters. These unique sucrose derivatives were isolated from the glandular trichomes of *Solanum neocardenasii* Hawkes & Hjerting (PI 498129). *S. neocardenasii* is a recently discovered wild potato species from Bolivia[5], and current investigations[6,7] indicate that it may prove of substantial interest to breeding programs attempting to expand the genetic base of host-plant resistance to insects.

RESULTS AND DISCUSSION

Leaf surface chemicals from accessions of *S. neocardenasii* (PI 498129) were obtained by means of a chloroform wash. Co-extracted plant waxes were removed by dissolving the chloroform residues in acetone. Fractionation of the acetone residues by preparative t.l.c. (silica gel) yielded a clear viscous isolate that con-

TABLE I

^{1}H-N.M.R. DATA (δ) FOR THE SUCROSE ESTERS 1–4[a]

Hydrogen atom	*Compound*			
	1	**2**	**3**	**4**
H-1 ($J_{1,2}$)	5.64d (3.7)	5.63d (3.7)	5.64d (3.6)	5.49d (3.6)
H-2 ($J_{2,3}$)	4.90dd (10.2)	4.91dd (10.0)	4.89dd (9.9)	4.86dd (10.0)
H-3 ($J_{3,4}$)	5.47t (9.8)	5.41t (10.0)	5.47t (9.9)	5.39t (9.8)
H-4 ($J_{4,5}$)	4.96t (9.8)	4.95t (10.0)	4.96t (10.0)	
H-5	4.12m	4.13m	4.10m	
H-3′ ($J_{3',4'}$)	5.24d (7.7)	5.20d (7.8)	5.23d (7.6)	
H-4′	4,.47m	4.57m	4.46m	
H-5′	3.89m	3.91m	3.90m	
CH_3CO_2–	2.03s	2.03s	2.03s	2.03s
–CH_2CO_2–	2.52t	2.27, 2.50t	2.51t	
=$CHCO_2$–	2.47, 2.53h	2.47h	2.48, 2.54h	2.60h

[a] *J* values in Hz.

stituted the bulk of materials recovered. The quantity of the isolate was directly related to the number of type B glandular trichomes present in the *S. neocardenasii* progeny sampled and, on past evidence, was assumed to be representative of the trichome exudate.

Subsequent reversed phase t.l.c. and l.c. analysis revealed the presence of three major components in the silica gel-purified isolate. After fractionation of the isolate on reversed phase t.l.c. plates, two-dimensional ^{1}H-n.m.r. spectra[8,9] revealed that all three components consisted of sucrose molecules esterified at O-2, O-3, O-4, and O-3′ (see Table I). Pertinent to these assignments was the knowledge that acylation of a secondary alcohol results in a downfield shift of the signal of the corresponding methine proton, normally to a position in the range δ 5–6. Additionally, the splitting patterns of the five methine protons in sucrose can be anticipated on the basis of published data for acylated derivatives as follows[10]: H-2 (dd, J ~10 and 2 Hz), H-3 (t, J ~10 Hz), H-4 (t, J ~10 Hz), H-3′ (d, J 5–8 Hz), and H-4′ (dd, both J 5–8 Hz).

The most preponderant (34.6%) and also most polar of the three compounds proved remarkably crystalline (the first reported natural mixed sucrose ester to be so). A two-dimensional, ^{13}C–^{1}H heteronuclear shift-correlated n.m.r. study[11,12] of this compound allowed assignment of the ^{13}C-n.m.r. signals for the sucrose carbon atoms (see Table II), and also established the presence of one acetyl, one hexanoyl, and two isobutyryl substituents. A f.a.b.-positive ion spectrum[13] confirmed the molecular formula as $C_{28}H_{46}O_{15}$ (*i.e.*, *m/z* 645.3 = M + Na^+) and demonstrated that the hexanoyl substituent was associated with the monosubstituted D-fructose unit (see Table III). To complete the structural assignment, a COLOC experi-

TABLE II

^{13}C-N.M.R. DATA (δ) FOR THE CARBOHYDRATE COMPONENT OF THE SUCROSE ESTERS 1–3

Carbon atom	*Compound*		
	1	2[a]	3[a]
1	89.47	89.48	89.48
2	70.47	70.37	70.45
3	68.83	68.91	68.81
4	68.25	68.32	68.24
5	71.80	71.34	71.94
6	60.05[b]	59.70[b]	59.82[b]
1′	64.39	64.51	64.51
2′	104.02	103.99	104.01
3′	79.33	79.73	79.59
4′	71.54	71.34	71.44
5′	82.58	82.45	82.51
6′	61.51[b]	61.62[b]	61.58[b]

[a]Comparative assignments based on the two-dimensional, ^{13}C–^{1}H heteronuclear shift-correlated n.m.r. study of compound 1. [b]Tentative assignments which may be reversed.

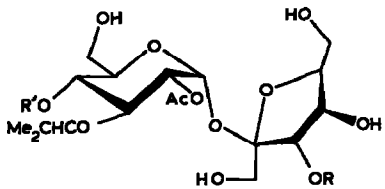
TABLE III

PROMINENT IONS (*m/z*) IN THE F.A.B.-MASS SPECTRA OF COMPOUNDS 1–3

Identity of ions	*Compound*[a]		
	1	2	3
$M^+ + 23$	645.3 (84)	673.2 (24)	701.3 (60)
$M^+ + 1$	623.2 (15)	651.2 (5)	679.3 (4)
[2,3,4-Tri-*O*-acyl-D-glucopyranosyl]$^+$	345.1 (12)	373.2 (8)	345.1 (12)
[3′-*O*-Acyl-D-fructofuranosyl]$^+$	261.1 (100)	261.1 (100)	317.2 (96)

[a]Relative proportion (%) in parentheses.

ment[14,15] established the long-range ^{13}C–^1H shift correlations, and demonstrated that the acetyl group was at O-2 of the D-glucose unit. Consequently, the structure 2-*O*-acetyl-3′-*O*-hexanoyl-3,4-*O*-isobutyrylsucrose (**1**) was assigned to the most polar and major component of the trichome exudate.



1 R = $CH_3(CH_2)_4CO$, R′ = Me_2CHCO
2 R = R′ = $CH_3(CH_2)_4CO$
3 R = $CH_3(CH_2)_8CO$, R′ = Me_2CHCO
4 R = R′ = H

^{13}C-N.m.r. and f.a.b.-positive-ion spectra of the least preponderant and second most polar sucrose ester indicated a molecular formula of $C_{30}H_{50}O_{15}$, and an acetyl-, isobutyryl-, and hexanoyl-substituted D-glucose and a hexanoyl-substituted D-fructose unit (see Table III). Similar spectral determinations of the remnant sucrose ester indicated a molecular formula of $C_{32}H_{54}O_{15}$ and an acetyl- and di(isobutyryl)-substituted D-glucose and a decanoyl-substituted D-fructose unit (see Table III). To ascertain the exact substitution pattern in the D-glucose unit of these two esters, they were subjected to partial hydrolysis with methanolic ammonium hydroxide. Characterization of a common 2-*O*-acetyl-3-*O*-isobutyrylsucrose (**4**) product by comparison with an identical compound obtained by hydrolysis of **1** allowed their structural assignments as 2-*O*-acetyl-3′,4-di-*O*-hexanoyl-3-*O*-isobutyrylsucrose (**2**) and 2-*O*-acetyl-3′-*O*-decanoyl-3,4-di-*O*-isobutyrylsucrose (**3**), respectively.

Interestingly, the *S. neocardenasii* sucrose esters are the first group studied to (a) contain hexanoyl residues, (b) remain unsubstituted at O-6, (c) contain long

chain, *i.e.*, hexanoyl and decanoyl groups at the D-fructose unit, and (d) have acetyl substituents at O-2. This uniqueness, compared to the make-up of sucrose esters previously characterized[3,4,16], suggests a potentially large diversity of such compounds still to be explored.

EXPERIMENTAL

General methods. — Melting points were determined on a Kofler hot-stage microscope and are uncorrected. I.r. spectra were determined with a Beckman IR-20A spectrophotometer for solutions in chloroform. All n.m.r. spectra were recorded for solutions in $CDCl_3$ with a Varian XL-200 spectrometer operating at 200.057 MHz for ^1H and 50.309 MHz for ^{13}C; chemical shifts were measured downfield from the signal of internal tetramethylsilane and further details of the general procedures are outlined in a previous paper[4]. F.a.b.–m.s. were recorded with a Finnigan MAT 312 mass spectrometer; Xe (99.995%, Matheson) was used as the bombardment gas at 8 kV and the resulting ions were extracted into the mass analyzer at an accelerating potential of 3 kV. L.c. determinations under elevated pressure were performed in a Perkin–Elmer RP-18 octadecyl column (10-μm particle size, 4.6 mm × 25 cm) in conjunction with a Waters model 410 refractive-index detector. Capillary g.l.c. studies were performed with a Varian 3500 g.l.c. instrument utilizing on-column injection and a fused silica capillary column (30 m × 0.25 mm i.d.) with a 0.25-μm film of DB-5.

Isolation of sucrose esters. — Plants were grown from seeds of *S. neocardenasii* (PI 498129), obtained from the Inter-Regional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin. Leaves (614 g) from mature plants were extracted by dipping them (~5 s) in a 2-L beaker containing chloroform (1000 mL) and then into a second 2-L beaker containing a similar amount of chloroform. The combined extracts were filtered through a plug of cotton wool and the chloroform removed *in vacuo* at room temperature. The residue (1.12 g) was taken up in acetone (60 mL), cooled to 0°, and vacuum-filtered through Whatman No. 1 filter paper to remove co-extracted plant waxes. Removal of the acetone *in vacuo* yielded a yellow-colored viscous residue (0.91 g) which was then fractionated by t.l.c. on five plates of 1.0-mm Silica Gel 60 developed in 6:1 chloroform–methanol. The sucrose esters were detected (by use of a water spray) at R_F 0.45 and eluted from the silica gel with 3:1 chloroform–methanol (300 mL). Removal of the chloroform and methanol *in vacuo* at room temperature yielded the mixture of sucrose esters (563 mg) as a clear viscous liquid.

Purification and characterization of the individual sucrose esters. — The purified sucrose ester mixture was fractionated on 0.2-mm, RP-C_{18} thin-layer plates (Whatman) developed in 3:1 acetone–water. Three distinct major zones could be distinguished by charring after spraying with 5% H_2SO_4 in ethanol. The sucrose esters were eluted with 4:1 chloroform–methanol. The zone at R_F 0.59 (34.6% of the mixture) readily crystallized from diethyl ether–hexane to furnish 2-*O*-acetyl-3′-

O-hexanoyl-3,4-di-*O*-isobutyrylsucrose (**1**) as colorless rosettes, m.p. 115–116°; $\nu_{max}^{CHCl_3}$ 3530 and 1735 cm^{-1}; pertinent ^1H- and ^{13}C-n.m.r., and f.a.b.-mass spectral data for the sugar component are given in the text; ^{13}C-n.m.r. signals at δ 13.94, 22.25, 31.21, 24.48, 33.96, and 174.77 were assigned to C-6–C-1, respectively, of the hexanoyl group; signals at δ 18.64, 18.79, 18.67, 18.97, 33.89 (2), 175.81, and 176.16 to C-3′, C-3, C-2, and C-1, respectively, of the isobutyryl groups; and signals at δ 20.60 and 170.20 to C-2 and C-1, respectively, of the acetyl group.

The zone at R_F 0.51 (16.8% of the mixture) after rechromatography yielded 2-*O*-acetyl-3′,4-di-*O*-hexanoyl-3-*O*-isobutyrylsucrose (**2**) as a homogeneous (by capillary g.l.c. of the acetylated derivative), viscous semi-solid; $\nu_{max}^{CHCl_3}$ 3525 and 1735 cm^{-1}; pertinent ^1H- and ^{13}C-n.m.r., and f.a.b.-mass spectral data for the sugar component are given in the text; ^{13}C-n.m.r. signals at δ 13.85, 13.96, 22.25 (2), 31.23 (2), 24.50 (2), 33.92 (2), 172.91, and 174.97 were assigned to C-6–C-1, respectively, of the hexanoyl groups; signals at δ 18.74, 18.92, 33.99, and 175.87 to C-3, C-3′, C-2, and C-1, respectively, of the isobutyryl group; and signals at δ 20.59 and 170.08 to C-2 and C-1, respectively, of the acetyl group.

After rechromatography, the zone at R_F 0.45 (27.1% of the mixture) yielded 2-*O*-acetyl-3′-*O*-decanoyl-3,4-di-*O*-isobutyrylsucrose (**3**) as a homogeneous (by capillary g.l.c. of the acetylated derivative), viscous semi-solid; $\nu_{max}^{CHCl_3}$ 3530 and 1735 cm^{-1}; pertinent ^1H- and ^{13}C-n.m.r., and f.a.b.-mass spectral data for the sugar component are given in the text; ^{13}C-n.m.r. signals at δ 14.11, 22.68, 31.89, 29.53, 29.31, 29.27, 29.20, 24.83, 34.02, and 174.86 were assigned to C-10–C-1, respectively, of the decanoyl group; signals at δ 18.67, 18.81, 18.87, 18.97, 33.89 (2), 175.79, and 176.16 to C-3, C-3′, C-2, and C-1, respectively, of the isobutyryl groups; and signals at δ 20.59 and 170.13 to C-2 and C-1, respectively, of the acetyl group.

Partial hydrolysis of the sucrose esters. — A sample (10 mg) of each sucrose ester (**1**, **2**, and **3**) was dissolved in 5% methanolic ammonium hydroxide (2 mL) and stirred at room temperature until t.l.c. monitoring (17:3 chloroform–methanol) indicated that the majority of the starting material had been degraded (~20–25 min). Analytical t.l.c. (4:1 chloroform–methanol) and capillary g.l.c. of acylated portions of the hydrolyzate were used for the matching of the hydrolysis products. A quantity of one product that was found in good yield in the hydrolyzate from all three sucrose esters showed ^1H-n.m.r. signals that were indicative of a 2,3-di-*O*-acylated sucrose (see Table I) containing an acetyl and an isobutyryl group; subsequent ^1H-n.m.r. decoupling studies confirmed its identity as 2-*O*-acetyl-3-*O*-isobutyrylsucrose (**4**).

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