*Carbohydrate Research*, 166 (1987) 113–121
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands



# ISOLATION AND CHARACTERIZATION OF 3,3′,4,6-TETRA-*O*-ACYLATED SUCROSE ESTERS FROM THE TYPE B GLANDULAR TRICHOMES OF *Solanum berthaultii* HAWKES (PI 265857)

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(Received May 27th, 1986; accepted for publication in revised form, September 8th, 1986)

## ABSTRACT

A series of polar, high-molecular-weight compounds that comprise the major portion of nonvolatile constituents in the exudate from type B glandular trichomes of *S. berthaultii* Hawkes (PI 265857) were isolated by silica gel t.l.c. and identified by spectroscopic and chemical means as a complex of 3,3′,4,6-tetra-*O*-acylated sucrose esters. Two of the principal sucrose esters were resolved by reversed-phase t.l.c. and characterized as 6-*O*-capryl-3,3′,4-tri-*O*-isobutyrylsucrose and 6-*O*-capryl-3′-*O*-isobutyryl-3,4-di-*O*-(2-methylbutyryl)sucrose.

## INTRODUCTION

The foliage of many wild potato species is covered with glandular trichomes (type A and type B) that exude chemical secretions which entrap arthropod pests[1]. Type A trichomes release a quick setting fluid when ruptured, but type B constantly exude droplets of a clear sticky substance. Our previous investigations involving the exudate from type B trichomes of *Solanum berthaultii* Hawkes (PI 473340) determined that the major portion of its nonvolatile constituents consisted of a complex of triacylated sucrose esters[2]. Chemical and spectroscopic means established that the sucrose molecules were esterified at O-3, O-4, and O-6 of the D-glucose units. The major component of the complex was isolated and characterized as 6-*O*-capryl-3,4-di-*O*-isobutyrylsucrose. We now report the isolation and characterization of 3,3′,4,6-tetra-*O*-acylated sucrose esters from the type B trichomes of another *S. berthaultii* introduction. To our knowledge, this is the first reported isolation of naturally occurring sucrose esters that contain substituents on both the D-glucose and D-fructose units. A 2,3,4,6-tetra-*O*-acylated sucrose ester complex from green tobacco leaves has been reported previously[3].

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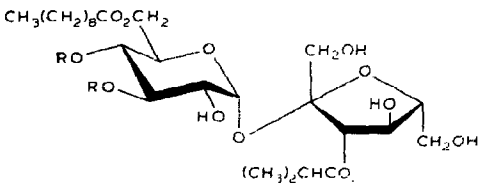
## RESULTS AND DISCUSSION

Freshly collected leaves from clone 26 of *S. berthaultii* (PI 265857) (ref. 4) (which contains an abundance of type B hairs) were extracted with chloroform. Sequential fractionation of the nonvolatile residues by preparative t.l.c. on silica gel plates yielded a clear viscous isolate that constituted the bulk of recovered materials. In crosses derived from clone 26, progeny lacking type B hairs did not yield this viscous material.

$^{13}$C-N.m.r. and two-dimensional $^{1}$H-n.m.r.[5,6] of the isolate indicated the presence of sucrose molecules esterified at O-3, O-4, and O-6 of the D-glucose and at O-3′ of the D-fructose unit. In contrast to the triacylated sucrose esters, the tetraacylated derivatives were not amenable to hydrolysis into their component sugars with invertase because of the substituted β-D-fructofuranosyl residues. To determine the acid composition, the isolate was transesterified with sodium methoxide. Capillary g.l.c.–m.s. of the methyl derivatives identified isobutyric, 2-methylbutyric, and capric acids as the major substituents.

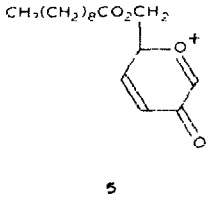
Molecular ions for the sucrose ester complex were not obtained by direct probe (e.i.) mass spectra. Only weak high-mass ions of *m/z* 457, 471, and 485 were observed. These ions could most reasonably be ascribed to the trisubstituted D-glucose units (*i.e.*, ionization is thought to occur at the ring oxygen atom and degradation of a pyranosyl ring is usually initiated by cleavage of a bond occupying the position β to it). Subsequent capillary g.l.c.–m.s. (e.i.) of the sucrose esters as their acetyl derivatives indicated the presence of four components in significant proportions. The parent components were assigned numbers **1** through **4** based on the order of elution of their acetyl derivatives by g.l.c. on a DB-5 capillary column. High-mass ions of the acetyl derivatives and their relative proportion of the complex were as follows: for **1**, *m/z* 499 (18.8%); **2**, *m/z* 513 (21.2%); **3**, *m/z* 513 (21.3%); and **4**, *m/z* 527 (29.7%). Mass ions for butyryl and capryl groups (*i.e.*, *m/z* 71 and 155, respectively) were preponderant in all four compounds. A mass ion for methylbutyryl groups (*m/z* 85) was prominent only in compounds **2**, **3**, and **4**.




**1** R = $(CH_3)_2CHCO$

**2,3** R = $(CH_3)_2CHCO$, $CH_3CH_2CH(CH_3)CO$

**4** R = $CH_3CH_2CH(CH_3)CO$




**5**

In their respective mass profiles, three of the four common prominent ions (*i.e.*, *m/z* 169, 239, and 359) can be ascribed to degraded elements of a tetraacylated D-fructofuranosyl residue containing three acetyl and one isobutyryl groups[7]. The remnant common ion (*m/z* 281) is representative of the pyroxonium structure **5** and establishes the presence of a capryl group at O-6 in all four compounds[28].

On the basis of the evidence presented, compounds **1** and **4** were assigned the structures 6-*O*-capryl-3,3′,4-tri-*O*-isobutyrylsucrose and 6-*O*-capryl-3′-*O*-isobutyryl-3,4-di-*O*-(2-methylbutyryl)sucrose, respectively. Likewise, compounds **2** and **3** consist of 6-*O*-capryl-3′-isobutyrylsucroses differing only in an interchange of isobutyryl and 2-methylbutyryl groups at O-3 and O-4.

Structural designations for compounds **1** and **4** were further elucidated by their separation and purification utilizing reversed-phase t.l.c. Similar efforts to separate compounds **2** and **3** were unsuccessful. An f.a.b.-positive ion spectrum[9] of **1** exhibited a high-mass peak at *m/z* 729.4 ($M + Na^+$) corresponding to the molecular formula $C_{34}H_{58}O_{15}$. Other significant peaks in the mass spectrum were observed at *m/z* 457 (12.8%), 369 (7.2%), 281 (22.4%), 233 (12.1%), 155 (100%), and 127 (68.6%). A two-dimensional $^1$H-n.m.r. spectrum of **1** showed discrete downfield signals for H-1, H-3, H-3′, H-4, and H-6 (see Table I). A two-dimensional, $^{13}$C–$^1$H heteronuclear, shift-correlated n.m.r. study[10,11] of compound **4** was utilized to make comparative assignments of the $^{13}$C-n.m.r. signals for the carbon atoms of the sugar skeleton of compound **1** (see Table II). For compound **1**, $^{13}$C-n.m.r. signals at $\delta$ 14.11 (q), 22.68 (t), 31.88 (t), 29.14 (t), 29.29 (2t), 29.46 (t), 24.73 (t), 33.91 (t), and 173.47 (s) corresponded to C-10 through C-1 of the capryl group; at 18.58 (q), 18.79 (2q), 18.87 (q), 18.91 (q), and 19.15 (q) to C-3 and C-3′; at 33.93 (d), 34.04 (d), and 34.19 (d) to C-2, and at 175.14 (s), 176.94 (s), and 179.11 (s) to C-1 of the isobutyryl groups. Acetylation of **1** with acetic anhydride–pyridine furnished a crystalline tetraacetate.

An f.a.b.-positive ion spectrum of **4** exhibited a high-mass peak at *m/z* 757.4 ($M + Na^+$) corresponding to the molecular formula $C_{36}H_{62}O_{15}$. Other significant peaks in the mass spectrum were observed at *m/z* 485 (12.8%), 383 (8.2%), 281 (20.8%), 233 (11.9%), 155 (100%), and 127 (66.2%). A two-dimensional, $^1$H-n.m.r. spectrum of compound **4** also showed discrete signals for H-1, H-3, H-3′, H-4, and H-6 (see Table I and Fig. 1). A two-dimensional, $^{13}$C–$^1$H heteronuclear shift-correlated n.m.r. study of **4** allowed the assignment of signals corresponding to the carbon atoms of the sugar skeleton (see Table II and Fig. 2). $^{13}$C-N.m.r. signals at $\delta$ 14.12 (q), 22.68 (t), 31.88 (t), 29.45 (t), 29.29 (2t), 29.15 (t), 24.73 (t), 33.91 (t), and 173.46 (s) corresponded to C-10 through C-1 of the capryl group; at 18.56 (q), 19.15 (q) to C-3 and C-3′, and at 34.18 (d) to C-2 of the isobutyryl group; at 11.41 (q) and 11.54 (q) to C-4, at 16.26 (q) and 16.35 (q) to C-3′, and at 40.92 (2d) to C-2 of the 2-methylbutyryl group. The signals at $\delta$ 174.75 (s), 176.72 (s) and 179.10 (s) were ascribed to the C-1 of the isobutyryl and 2-methylbutyryl groups. Acetylation of compound **4** also furnished a crystalline tetraacetate.

TABLE I

$^1$H-N.M.R. DATA ($\delta$) FOR THE SUCROSE ESTERS

| *Atom* | *6-O-Capryl-3,4-di-O-isobutyrylsucrose*[a] | **1** | **4** |
|---|---|---|---|
| H-1 | 5.52 d | 5.49 d | 5.49 d |
| ($J_{1,2}$) | (4.1) | (3.1) | (3.5) |
| H-2 | 3.80 dd | 3.70 dd | 3.70 dd |
| ($J_{2,3}$) | (9.8) | (9.8) | (9.8) |
| H-3 | 5.31 t | 5.23 t | 5.23 t |
| ($J_{3,4}$) | (9.7) | (9.9) | (9.9) |
| H-4 | 5.11 t | 5.11 t | 5.14 t |
| ($J_{4,5}$) | (9.7) | | |
| H-5 | 4.33 | 4.21 | 4.21 |
| H-6 | 4.15 | 4.18 | 4.19 |
| H-1′ | [b] | 3.70 | 3.70 |
| H-3′ | 4.22 d | 5.05 d | 5.06 d |
| ($J_{3',4'}$) | | (7.9) | (7.7) |
| H-4′ | 4.39 t | 4.63 t | 4.61 t |
| ($J_{4',5'}$) | | (8.0) | (8.1) |
| H-5′ | 3.88 | 3.94 | 3.94 |
| ($J_{5',6',a}$) | | (2.4) | (2.4) |
| H-6′ | [b] | 3.71, 3.87 | 3.71, 3.87 |
| ($J_{6'a,6'b}$) | | (12.9) | (12.9) |
| $\geq CHCO_2$ | 2.31 | 2.49, 2.54, 2.77 | 2.31, 2.40, 2.77 |
| $-CH_2CO_2-$ | 2.35 | 2.35 | 2.35 |
| $CH_3-\underset{\vert}{C}CO_2-$ | 1.08 (× 4) | 1.11, 1.12, 1.28<br>1.12, 1.14, 1.33 | 1.08, 1.09, 1.28, 1.33 |
| $-CH_2-$ | 1.23 | 1.26 | 1.26, 1.61 (× 2) |
| $CH_3-$ | 0.82 | 0.88 | 0.88, 0.90 (× 2) |

[a]Ref. 2. [b]Not resolved.

TABLE II

$^{13}$C-N.M.R. DATA ($\delta$) FOR THE CARBON ATOMS OF THE SUGAR SKELETON OF THE SUCROSE ESTERS

| *Carbon atom* | **1** | **4** |
|---|---|---|
| 1 | 92.16 | 92.12 |
| 2 | 70.67 | 70.68 |
| 3 | 72.75 | 72.66 |
| 4 | 66.96 | 66.91 |
| 5 | 69.41 | 69.28 |
| 6 | 60.98 | 61.02 |
| 1′ | 64.98 | 64.90 |
| 2′ | 103.74 | 103.73 |
| 3′ | 81.97 | 81.65 |
| 4′ | 71.95 | 71.91 |
| 5′ | 82.37 | 82.48 |
| 6′ | 60.18 | 60.25 |

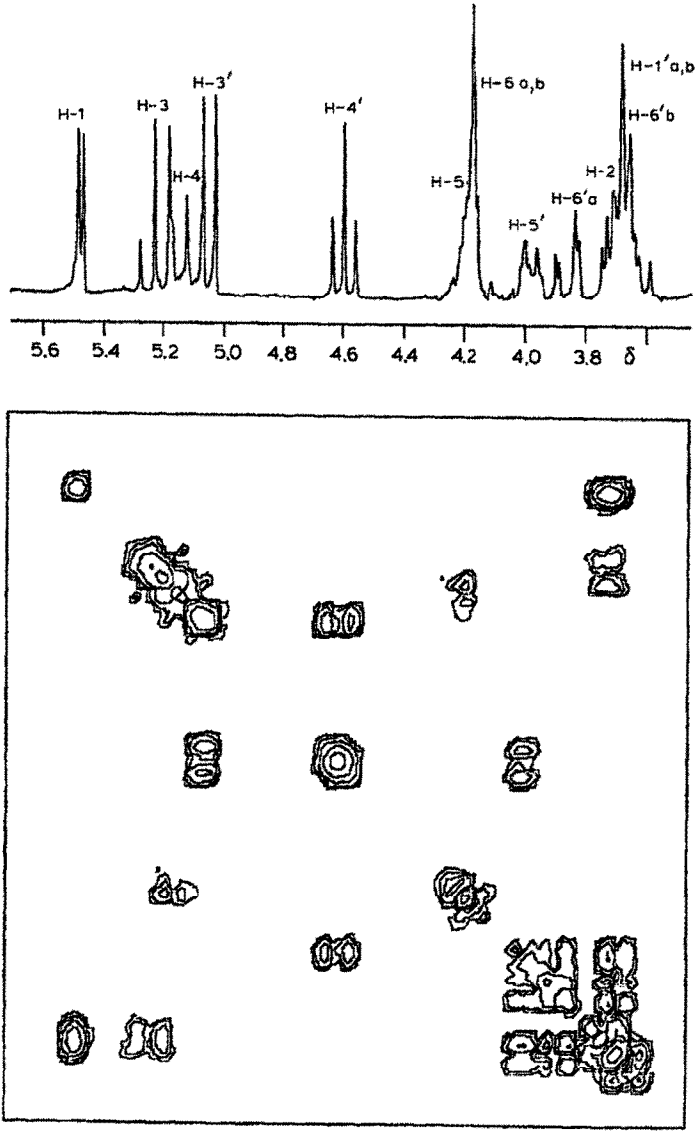



Fig. 1. Contour plot of the two-dimensional, $^1$H-shift-correlated (COSY) spectrum of the sugar skeleton of **4** with (top panel) the conventional $^1$H-n.m.r. spectrum.

Since completion of the present work, a comprehensive survey has revealed five other *S. berthaultii* introductions (*i.e.*, PI 218215, PI 265858, PI 310925, WRF 1727, and PI 473330) which have type B trichomes that contain a similar mixture of the sucrose esters described herein.

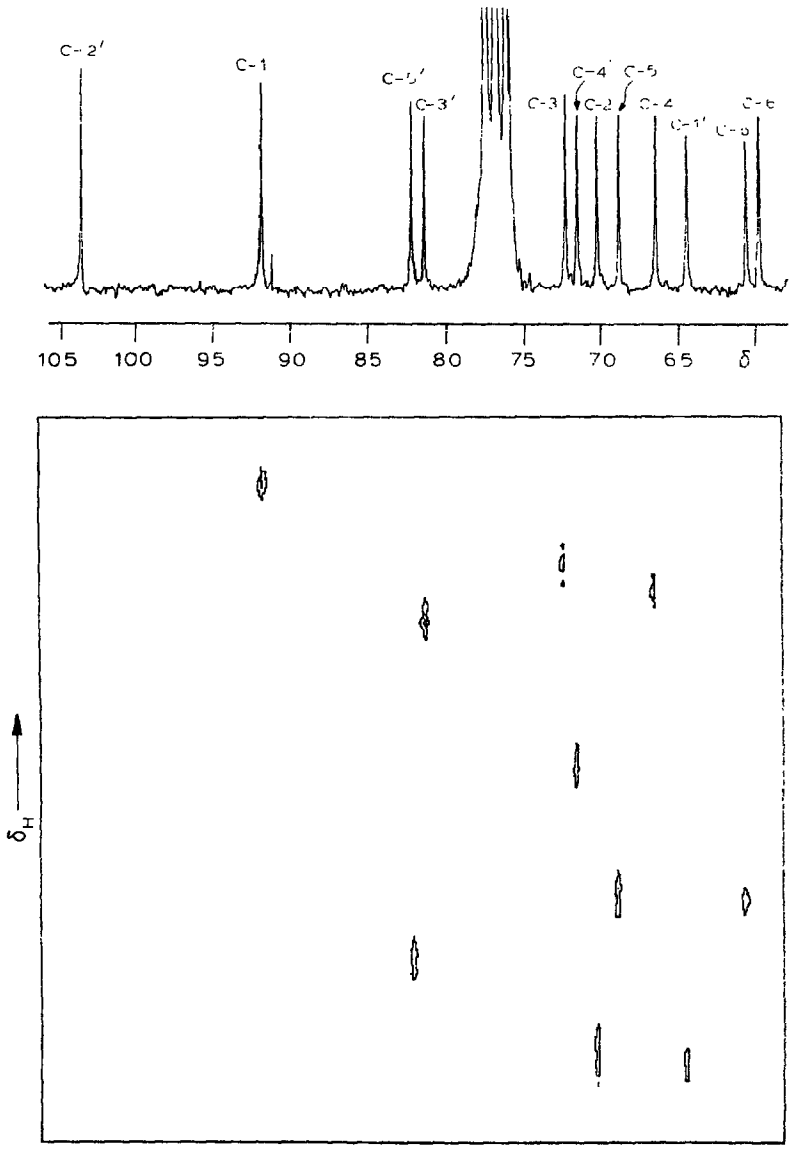



Fig. 2. Two-dimensional $^{13}C$–$^{1}H$ heteronuclear shift-correlated spectrum of the sugar skeleton of **4**.

## EXPERIMENTAL

*General methods.* — Melting points are uncorrected and were determined on a Kofler hot-stage microscope. I.r. spectra were recorded 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 with a precision of ±0.002, ±0.02, and ±0.03 p.p.m. for $^1H$, $^{13}C$, and two-dimensional $H^1$ shift-correlation (COSY)[5] spectra respectively. COSY spectra were obtained by use of spectral widths of 1300.1 Hz with 256 $\tau_1$ increments and one order of zero filling in $\tau_1$ to produce a 512 × 512 data-point matrix. A recycle delay time of 3 s was applied between each of 16 transients recorded per $\tau_1$ increment. Pseudo-echo processing[6] was used in both time domains. The $^{13}C$–$^1H$ heteronuclear, shift-correlation experiment[11] was performed with delays $\Delta_1$ and $\Delta_2$ of 3.6 and 2.4 ms, respectively, optimized for $J_{C,H}$ 140 Hz; 256 transients were recorded for each of 256 $\tau_1$ increments with a recycle delay time of 2 s per transient for a total experiment time of 44 h. After Fourier transformation of $\tau_2$ data using a 5-Hz line-broadening function, the transposed data were zero-filled to 512 points with 1-Hz line-broadening in $\tau_1$ to produce a final matrix of 512 × 2048 data points. Spectral widths were 1270 and 5574 Hz in $\tau_1$ and $\tau_2$, respectively. 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. The m.s. (e.i.) were determined with a Finnigan 4021 g.l.c.–m.s. coupled to an INCOS data-acquisition system.

*Isolation and identification of the sucrose ester complex.* — Plants were grown from seeds of *S. berthaultii* PI 265857, obtained from the Inter-Regional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin. Clone 26 originated from a single plant selected because of its sensitivity for indexing potato-spindle tuber viroid from dormant tubers[4]. Leaves (523 g) from mature plants were extracted by being dipped (~5 s) into 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 chloroform was removed *in vacuo* at room temperature. The residue (1.09 g) was taken up in acetone (50 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 yellowish, viscous residue (840 mg) which was then fractionated by t.l.c. (5 plates coated with 1.0-mm Silica gel 60, developed in 9:1 chloroform–methanol). The sucrose ester complex was detected (by use of a water spray) at $R_F$ 0.59 and eluted from the silica gel with acetone (300 mL). Removal of acetone *in vacuo* left an aqueous residue which was diluted to ~30 mL with water. The aqueous solution was extracted twice with chloroform (300 mL) and chloroform removed *in vacuo* at room temperature to yield the sucrose ester complex (506 mg) as a clear, viscous liquid.

*Transesterification of the sucrose ester complex.* — A portion of the sucrose ester complex (30 mg) was dissolved in anhydrous methanol (5 mL) and treated for 10 min at room temperature with 0.1M sodium methoxide (1.0 mL). The mixture was de-ionized with Amberlite IR-120 ($H^+$) cation-exchange resin and analyzed on a 30 M SP2330 capillary column at 35 kPa of He and splitless injection at 30°, held

for 2 min, then raised to 120° at 25°/min. The methyl esters were identified by comparative g.l.c. retention data of purchased or prepared standards and by g.l.c.–m.s. (e.i.). Removal of the methyl esters and acetylation of the sugar residue with acetic anhydride–pyridine yielded a compound with $^1$H-n.m.r., $^{13}$C-n.m.r., and g.l.c.–m.s. (e.i.) data identical to those of sucrose octaacetate.

*Acetylation and g.l.c.–m.s. analysis of the sucrose ester complex.* — A portion of the sucrose ester complex (10 mg) was treated with acetic anhydride (5 mL) and pyridine (1 mL) with stirring at room temperature overnight. The reaction was quenched with an excess of saturated $NaHCO_3$ solution, and the mixture extracted with chloroform (2 × 50 mL). Chloroform was removed *in vacuo* and the glassy residue dissolved in toluene and subjected to g.l.c.–m.s. (e.i.) analysis on a fused-silica capillary column (30 × 0.32 mm i.d.) containing a 0.25-$\mu$m film thickness of DB-5. The sample (2 $\mu$L) was injected *via* on-column injection at 90°, raised to 320° at 100°/min. The column oven was held at 90° for 2 min, raised to 250° at 25°/min, and then programmed at 8°/min to 300°. The mass spectrometer was operated at 70 eV (e.i.), 45–650 A at 1.5 s/scan. The data acquisition was started 13 min after injection.

*Purification and characterization of individual sucrose esters.* — The purified sucrose ester complex was fractionated on 0.2-mm RP-$C_{18}$ thin-layer plates developed in 3:1 acetone–water. Three zones could be distinguished by charring after spraying with 5% $H_2SO_4$ in ethanol. The sucrose esters were eluted with 9:1 acetone–95% ethanol. After rechromatography, the zone at $R_F$ 0.54 yielded 6-*O*-capryl-3,3′,4-tri-*O*-isobutyrylsucrose (**1**) as a viscous semisolid, $\nu_{max}^{CHCl_3}$ 3520 and 1735 cm$^{-1}$: for $^1$H-n.m.r., $^{13}$C-n.m.r., and m.s. data, see Results and Discussion section. Acetylation of **1** with acetic anhydride–pyridine and crystallization of the product from aqueous ethanol furnished the tetraacetate (purity >96% by g.l.c.) colorless needles, m.p. 60–61°; f.a.b.-m.s.: Calc. for $C_{42}H_{60}O_{19}$: $M^+$ 874.5648. Obs.: *m/z* 874.6. The zone at $R_F$ 0.51 could not be further resolved, but g.l.c. of the acetyl derivatives indicated the presence of acetylated components **2** and **3** in the original proportions. After rechromatography, the zone at $R_F$ 0.47 yielded 6-*O*-capryl-3′-*O*-isobutyryl-3,4-di-*O*-(2-methylbutyryl)sucrose (**4**) as another viscous semisolid, $\nu_{max}^{CHCl_3}$ 3520 and 1735 cm$^{-1}$; for $^1$H-n.m.r., $^{13}$C-n.m.r., and m.s. data, see Results and Discussion section. Acetylation of **4** with acetic anhydride–pyridine and crystallization of the product from aqueous ethanol furnished the tetraacetate (purity >98% by g.l.c.), colorless needles, m.p. 59–60°; f.a.b.-m.s.: Calc. for $C_{44}H_{70}O_{19}$: $M^+$ 902.5970. Obs.: *m/z* 902.6.

ACKNOWLEDGMENTS

The authors thank Alain Boucher for growing the *S. berthaultii* accessions and performing visual trichome ratings; See Hua Tan, New Brunswick Research and Productivity Council, Fredericton, New Brunswick for the g.l.c.–m.s. (e.i.) determinations; and P. Lafontaine, Chemistry and Biology Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, for the f.a.b. spectra.

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