Characterization of 2,3,4,3′-Tetra-O-acylated Sucrose Esters Associated with the Glandular Trichomes of *Lycopersicon typicum*

Russell R. King,*,† Larry A. Calhoun,‡ Rudra P. Singh,† and Alain Boucher†

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

A mixture of 2,3,4,3′-tetra-O-acylated sucrose esters was identified as the major nonvolatile constituent in the exudate from glandular trichomes of *Lycopersicon typicum* (PI LA 1777). The principal sucrose esters were resolved by reversed-phase TLC and characterized by a combination of hydrolysis studies and spectroscopic techniques.

The aerial surfaces of many Solanaceae genera (e.g., *Datura*, *Lycopersicon*, *Nicotiana*, *Petunia*, and *Solanum*) are endowed with glandular trichomes that utilize mucilaginous exudates to entrap or otherwise deter potential predators (Seithe, 1979). Extensive investigations involving the makeup of exudate from individual species have demonstrated that complexes of either glucose or sucrose esters (sometimes both) frequently constitute a majority of the nonvolatiles (Table I). Many combinations of short to medium fatty acids and a diversity of glucose and sucrose substitution patterns are involved. With the exception of possible taste differences, no significant variation in biological activity has yet been ascribed to these structural differences (Jackson et al., 1991).

As outlined in Table I, our previous investigations involving glandular exudate from the foliage of *Lycopersicon* accessions identified complexes of 2,3,4,1′-tetra-O- and 2,3,4-tri-O-acylated sucrose esters as the major trichome constituents from *L. hirsutum* and *L. hirsutum* f. sp. glabratum, respectively. A single 2,3,1′-tri-O-acylated sucrose ester was also found to be predominant in exudate from the glandular trichomes of *L. peruvianum* var. glandulosum. We now report the isolation and characterization of 2,3,4,3′-tetra-O-acylated sucrose esters from the trichome exudate of *L. typicum* (PI LA 1777). Sucrose esters containing this particular substituent pattern have previously been reported from *Nicotiana* and tuberous *Solanum* species (see Table I).

Table I

carbohydrate esters	Solanaceae species	reference
sucrose		
2,3,4-triacyl	*L. hirsutum* f.	King et al. (1990)
	N. glutinosa	Arrendale et al. (1990)
2,3,6-triacyl	*S. berthaultii*	King et al. (1986)
2,3,1′-triacyl	*L. peruvianum*	King et al. (1990)
2,3,4,6-tetraacyl	*N. tabacum*	Severson et al. (1985)
	P. multiflora nana	King et al. (1987a)
2,3,4,1′-tetraacyl	*L. hirsutum*	King et al. (1990)
2,3,4,3′-tetraacyl	*N. glutinosa*	Arrendale et al. (1990)
	S. neocardenasii	King et al. (1988b)
2,3,6,3′-tetraacyl	*S. berthaultii*	King et al. (1987b)
2,3,4,6,3′-pentaacyl	*N. acuminata*	Matsuzaki et al. (1991)
glucose		
2,3-diacyl	*D. metel*	King and Calhoun (1988)
	S. aethiopsicum	King et al. (1988a)
1,2,3-triacyl	*D. metel*	King and Calhoun (1988)
2,3,4-triacyl	*S. aethiopsicum*	King et al. (1988a)
	L. pennellii	Burke et al. (1987)
	N. acuminata	Matsuzaki et al. (1991)
1,2,3,4-tetraacyl	*N. acuminata*	Matsuzaki et al. (1991)
2,3,4,6-tetraacyl	*N. tabacum*	Severson et al. (1985)
	N. acuminata	Matsuzaki et al. (1991)
1,2,3,4,6-pentaacyl	*N. acuminata*	Matsuzaki et al. (1991)

MATERIALS AND METHODS

Materials. Plants were grown in a greenhouse from seeds obtained from C. M. Rick (College of Agriculture and Environmental Services, University of California, Davis, CA). A total of 10 seedlings of the species *Lycopersicon typicum* (PI LA 1777) were utilized in the study.

Thin-layer chromatography (TLC) was performed on Merck silica gel F_{254} plates and Whatman $KC_{18}F$ plates. All solvents used were of pesticide grade.

Analytical Methods. NMR spectra, unless otherwise stated, were recorded in $CDCl_3$ at 200 MHz for ^1H and at 50 MHz for ^{13}C. Chemical shifts were measured downfield from internal TMS, and selective INEPT spectra (Bax, 1984) were recorded using a decoupler field strength of 10 Hz (PP90 = 25 ms). Polarization transfer and refocusing delays were 110 ms for sugar methine–acyl carbonyl correlations, 71 and 36 ms, respectively, for acyl methylene–carbonyl correlations, and 102 and 41 ms, respectively, for acyl methyl–carbonyl correlations. Further details of the general procedures for NMR and MS (EI) determinations are outlined in a previous paper (King et al., 1987b). 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. Infrared spectra (IR) were recorded on a Perkin-Elmer 467 spectrophotometer.

Isolation and Purification of Sucrose Esters. Composite samples of freshly collected foliage (410 g) from mature plants were extracted with chloroform (5 mL/g) by soaking for 5 min. The $CHCl_3$ was subsequently removed in vacuo and the residue (1.2 g) taken up in a minimum volume of acetone, cooled in an ice bath, and filtered through Whatman No. 1 filter paper to remove coextracted plant waxes. After removal of acetone in vacuo, the residue was fractionated by TLC (1.0-mm silica gel, chloroform–methanol 9:1). The sucrose ester complex was detected (by charring a strip of the TLC plate after it was sprayed with 5% H_2SO_4 in ethanol) at R_f 0.32 and eluted from the silica gel with acetone. Removal of the acetone in vacuo yielded the sucrose ester complex as a clear viscous liquid with V_{max} ($CHCl_3$) 3420 and 1745 cm^{-1}.

The sucrose ester complex was fractionated on 0.2-mm RP-C_{18} TLC plates developed in acetone–water (3:1). Six distinct zones were defined (see Table II) by charring after a strip of the TLC was sprayed with 5% H_2SO_4 in ethanol. The sucrose esters were eluted from the plates with acetone–methanol (9:1).

Acetylation of the Sucrose Esters for GLC Analysis. 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

† Agriculture Canada.
‡ University of New Brunswick.

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Table II. Summary of Isolation Data for the Sucrose Esters

fraction	R_f	MW	formula	% of total
A	0.30	720	$C_{35}H_{60}O_{15}$	31
B	0.34	678	$C_{32}H_{54}O_{15}$	23
C	0.43	636	$C_{29}H_{48}O_{15}$	16
D	0.46	622	$C_{28}H_{46}O_{15}$	10
E	0.48	608	$C_{27}H_{44}O_{15}$	14
F	0.52	594	$C_{26}H_{42}O_{15}$	6

with chloroform (2 × 50 mL). The chloroform was removed in vacuo and the residue dissolved in toluene prior to GLC analysis.

RESULTS AND DISCUSSION

Sequential fractionation by silica gel TLC of the chloroform-soluble extracts of foliage from accessions of *L. hirsutum typicum* (PI LA 1777) isolated a complex of sucrose esters that constituted the bulk of recovered materials. The quantity of sucrose esters was directly related to the number of secretory glandular trichomes present in the progeny sampled and, on past evidence, was assumed to be representative of the trichome exudate (King et al., 1987a).

Capillary GC of the sucrose ester complex as their acetyl derivatives indicated the presence of at least six major components. Repetitive fractionation of the complex by RP C_{18} TLC yielded six distinct zones, but on the basis of ^{13}C NMR and/or GC–MS (EI) criteria, only fractions F and A–C (Table II) could be classified as homogeneous entities. Structural determinations of the fractionated materials were then undertaken by assessment of their respective ^1H NMR, ^{13}C NMR, and/or MS data.

Two-dimensional ^1H NMR spectra of the fractionated materials revealed that they were all composed of sucrose molecules acylated at the 2-O-, 3-O-, 4-O-, and 3′-O-positions. 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 pattern of the five methine protons can be anticipated on the basis of published data for acylated sucroses as follows: H-2 (dd, $J \sim 10$ and 2 Hz), H-3 (t, $J \sim 10$ Hz), H-4 (t, $J \sim 10$ Hz), H-3′ (d, $J \sim$ 5–8 Hz), and H-4′ (dd, both $J \sim$ 5–8 Hz) (Clode et al., 1985).

Since the fraction A sucrose ester constituted the major component of the total mix, its structural elucidation was undertaken first. Initial ^{13}C NMR spectral analysis revealed the presence of one acetyl, one isobutyryl, one isodecanoyl, and one 3-methylbutyryl group. In principle, the structure of this sucrose ester could be resolved by a combination of mass spectral and partial hydrolysis studies utilized in previous examples. However, since this compound represented the first sucrose ester obtained in quantity with different acyl groups in each substituted position, we undertook to utilize it as a test case for the systematic application of the selective INEPT (Bax, 1984) method to the problem of regiochemical assignment of the acyl groups. Previous use of this method was restricted to the assignment of acetyl grops by selective inversion of the appropriate methine hydrogen, resulting in polarization transfer (i.e., chemical shift correlation) to the carbonyl carbon of the acetyl group, which was easily recognized by a distinctive chemical shift of ca. 170 ppm. Extension of the selective INEPT technique to the other acyl groups involves regiochemical assignment of all carbonyl carbons by chemical shift correlation with the assigned sugar methine hydrogens. The carbonyl carbons are then correlated with the acyl group α- or β-hydrogens which have been identified from the COSY (Bax et al., 1981) spectrum. Results of the application of this technique to the fraction A sucrose ester are shown in Figure 1. The left portion of the figure depicts the carbonyl region of the ^{13}C NMR spectrum resulting from successive inversion of the methine hydrogen of each substituted position, thus showing the chemical shift of the carbonyl carbon of the acyl group attached to that position. The right portion of the figure shows the same region of the ^{13}C NMR spectrum resulting from successive inversion of the acyl α-hydrogens. Since chemical shift correlation of a methine and α-hydrogen to the same carbonyl carbon can only arise if these hydrogens are within three bonds of the carbon, observation of such correlations unambiguously assigns the positions of the acyl groups and establishes the structure of the fraction A sucrose ester as 2-*O*-acetyl-3-*O*-isobutyryl-3′-*O*-isodecanoyl-4-*O*-(3-methylbutyryl)-sucrose (1).

One important limitation of the selective INEPT techniques (shared with all selective NMR methods) is the requirement of sufficient chemical shift separation of the resonances which are to be selectively excited (in this case the hydrogen resonances in the ^1H NMR spectrum). If the chemical shift separation is too small, a selective pulse centered on one resonance will cause partial excitation of the other. Such a situation is observed with H-2 and H-4 of the sucrose ester from fraction A, where the separation of these resonances is only 8 Hz. Despite this small separation, the chemical shift correlation to the carbonyl carbons can be unambiguously determined by the relative amplitudes of the responses in the selective INEPT spectra. Thus, when the selective pulse is centered on H-2, the carbonyl carbon resonating at 170.08 ppm has a greater amplitude than the carbonyl carbon at 172.18 ppm, while a reversal in the relative amplitudes is observed in the H-4 spectrum (although the difference is significantly reduced in the latter case). It should be noted that in the special case of sucrose esters with two or more acyl groups of the same type this technique should work equally well since the chemical shift coincidence of the α-hydrogens of the acyl groups will not affect the process of regiochemical assignment. This technique will not be generally applicable to structures which incorporate similar acyl groups such as decanoyl and isodecanoyl unless a difference in the chemical shifts of the α-hydrogens is observed.

^{13}C NMR analysis of the fraction B sucrose ester indicated the presence of one acetyl, one isodecanoyl, and two isobutyryl groups. A selective INEPT spectrum obtained with the inversion pulse centered on H-2 showed correlation to carbonyl carbons at δ 170.08 and 176.17, corresponding to one acetyl and one isobutyryl group, respectively. The aforesaid isobutyryl carbonyl carbon was then assigned to the 3-O-position due to the similarity of its chemical shift with the isobutyryl group rigorously assigned to the 3-O-position in compound 1 from fraction A. To provide a precise structural assignment for the fraction B compound, it was subjected to mild hydrolysis conditions (methanolic ammonium hydroxide at room temperature). As observed in several previous examples (King et al., 1990), these conditions catalyzed initial migration of the 4-O-substituent to the 6-O-position, yielding a 2,3,6,3′-tetra-O-acylated derivative (3) (for the H′ NMR evidence of this migration, see Table IV). Subsequent partial hydrolysis of the rearranged product yielded a 2,3,6-tri-O-acylated derivative (4), the ^1H NMR of which indicated it was now devoid of an isodecanoyl group. From these observations it was concluded that in the original compound the isodecanoyl substituent was

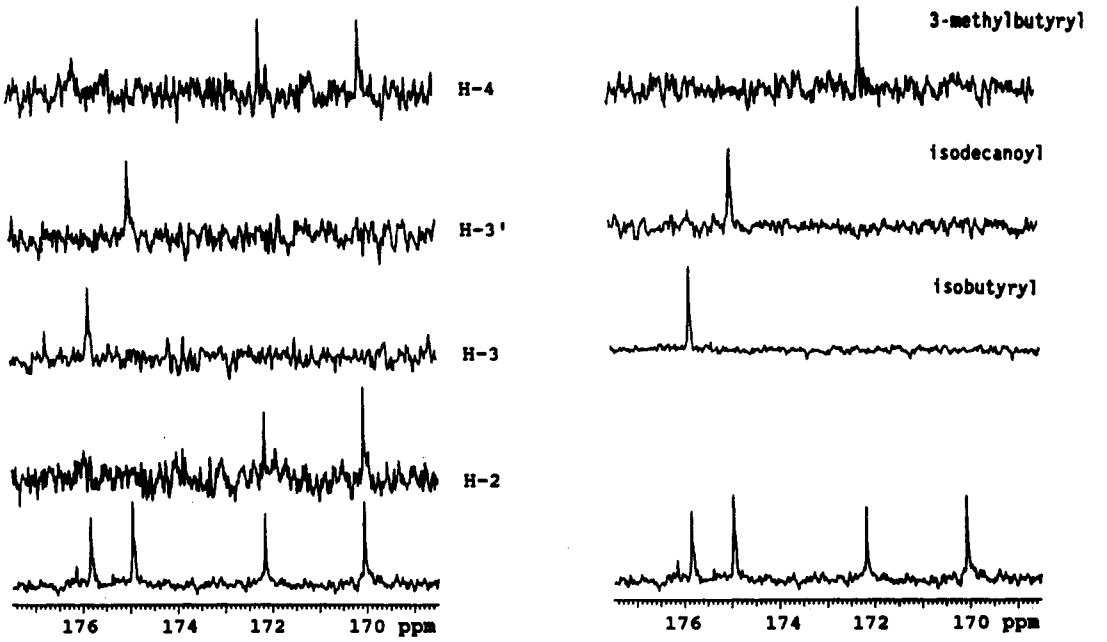
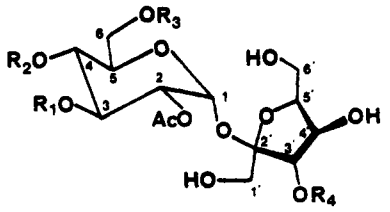


Figure 1. Selective INEPT spectra of the fraction A sucrose ester.



	R_1	R_2	R_3	R_4
1	isobutyryl	3-methylbutyryl	H	isodecanoyl
2	isobutyryl	isobutyryl	H	isodecanoyl
3	isobutyryl	H	isobutyryl	isodecanoyl
4	isobutyryl	H	isobutyryl	H
5	2-methylbutyryl	3-methylbutyryl	H	3-methylbutyryl
6	isobutyryl	isobutyryl	H	isobutyryl

Figure 2. Structural formulas of sucrose esters 1–6.

contained in the 3′-O-position, and its overall structure could then be assigned as 2-acetyl-3,4-di-*O*-isobutyryl-3′-*O*-isodecanoyl sucrose (**2**).

^{13}C NMR analysis of the fraction C sucrose ester indicated the presence of one acetyl, one 2-methylbutyryl, and two 3-methylbutyryl groups. A HCCOSY experiment (Bax, 1983) allowed assignment of the sucrose carbons from the proton spectrum (Table III) and by analogy the corresponding carbons of compounds **1** and **2**. The acetyl and one 3-methylbutyryl groups were assigned to the 2-O- and 4-O-positions, respectively, on the basis of a selective INEPT experiment with the soft pulse again centered on H-2. The resulting spectrum showed correlations to carbonyl carbons at δ 170.05 and 172.17, corresponding to the acetyl and 3-methylbutyryl groups, respectively, as determined by the analysis of compound **1**. The stronger response of the upfield carbon was taken to indicate substitution of the acetyl group at C-2 as previously discussed. Assignment of the other 3-methylbutyryl group to the 3′-O-position was facilitated by the observation of a 0.2 ppm chemical shift dependence between the H-2 signals of the two 3-methylbutyryl groups (Table IV) (i.e., an analysis of the ^1H NMR data for sucrose esters whose structures were previously determined by independent

Table III. ^{13}C NMR Spectral Assignments for Sucrose Esters 1, 2, and 5

1			2			5		
carbon	δ_c	mult	carbon	δ_c		carbon	δ_c	mult
1	89.49	d	1	89.48		1	89.38	d
2	70.47	d	2	70.41		2	70.41	d
3	68.84	d	3	68.79		3	68.78	d
4	68.28	d	4	68.21		4	68.39	d
5	72.03	d	5	72.08		5	72.11	d
6	61.63	t	6	61.62		6	61.61	t
1′	64.52	t	1′	64.58		1′	64.54	t
2′	104.01	s	2′	103.98		2′	103.96	s
3′	79.83	d	3′	79.77		3′	79.64	d
4′	71.38	d	4′	71.33		4′	71.21	d
5′	82.43	d	5′	82.43		5′	82.46	d
6′	59.71	t	6′	59.67		6′	59.65	t
isodecanoyl			isodecanoyl			3-methylbutyryl		
1	174.99	s	1	174.95		1	174.29	s
2	34.03	t	2	34.02		2	43.08	t
3	24.85	t	3	24.84		3	25.86	d
4	29.54	t	4	29.51		4	22.41	q
5	29.19	t	5	29.17		4′	22.33	q
6	27.32	t	6	27.30				
7	39.00	t	7	38.98				
8	27.98	t	8	27.95				
9	22.66	q	9	22.65				
9′	22.66	q	9′	22.65				
3-methylbutyryl			isobutyryl			3-methylbutyryl		
1	172.18	s	1	176.17		1	172.18	s
2	42.97	t	2	33.89		2	42.97	t
3	25.42	d	3	18.88		3	25.37	d
4	22.35	q	3′	18.81		4	22.41	q
4′	22.35	q				4′	22.41	q
isobutyryl			isobutyryl			2-methylbutyryl		
1	175.86	s	1	175.82		1	175.38	s
2	33.90	d	2	33.89		2	40.92	d
3	19.00	q	3	18.97		3	26.51	t
3′	18.62	q	3′	18.67		3′	16.42	q
						4	11.47	q
acetyl			acetyl			acetyl		
1	170.08	s	1	170.08		1	170.05	s
2	20.59	q	2	20.59		2	20.62	q

chemical means revealed a consistent correlation between substitution of the ester in the 3′-O-position of the sucrose moiety and an approximate 0.2 ppm downfield shift of the related α- and β-acyl group hydrogens). Hence, the structure 2-*O*-acetyl-4,3′-di-*O*-(3-methylbutyryl)-3-*O*-(2-

Table IV. ^{1}H NMR Spectral Assignments for Sucrose Esters 1–6

1			2			3		4		5			6		
H	δ_H	m, J (Hz)	H	δ_H	m, J (Hz)	H	δ_H	H	δ_H	H	δ_H	m, J (Hz)	H	δ_H	m, J (Hz)
1	5.61	d, 3.6	1	5.61	d, 3.7	1	5.55	1	5.68	1	5.62	d, 3.6	1	5.60	d, 3.7
2	4.90	dd, 3.7, 10.4	2	4.90	dd, 3.6, 10.4	2	4.88	2	4.91	2	4.90	dd, 3.8, 10.4	2	4.94	d, 3.7, 10.3
3	5.48	dd, 9.4, 10.4	3	5.48	dd, 9.5, 10.5	3	5.26	3	5.37	3	5.49	dd, 9.4, 10.5	3	5.50	dd, 9.7, 10.1
4	4.93	dd, 9.4, 10.5	4	4.94	dd, 9.5, 10.5	4	3.50	4	3.58	4	4.94	dd, 9.4, 10.4	4	4.94	dd, 9.5, 10.4
5	4.13	m	5	4.13	m	5	4.04	5	4.18	5	4.13	m	5	4.17	m
6	3.60	m	6	3.58	m	6	4.59	6	4.45	6	3.59	m	6	3.60	m
1′	3.63	m	1′	3.60	m	1′	3.55	1′	3.60	1′	3.64	m	1′	3.62	m
	3.45	m		3.50	m						3.59	d, 12.5		3.49	d, 12.4
3′	5.16	d, 7.9	3′	5.19	d, 7.8	3′	5.20	3′	4.22	3′	5.20	d, 8.0	3′	5.17	d, 8.1
4′	4.58	t, 8.0	4′	4.55	t, 8.2	4′	4.53	4′	4.32	4′	4.57	t, 8.2	4′	4.60	t, 8.3
5′	3.91	m	5′	3.90	m	5′	3.95	5′	3.85	5′	3.93	m	5′	3.95	m
6′	3.90	m	6′	3.90	m	6′	3.90	6′	3.65	6′	3.89	m	6′	3.95	m
	3.70	m	6′	3.70	m		3.70				3.71	m		3.72	m
	isodecanoyl			isodecanoyl			isodecanoyl				3-methylbutyryl			isobutyryl	
2	2.52	t, 7.0	2	2.51	t, 7.0	2	2.52			2	2.41	m	2	2.77	h, 7.0
3	1.70	m	3	1.70	m	3	1.68			3	2.33	m	3	1.33	d, 7.0
4	1.30	m	4	1.30	m	4	1.30			4′	1.05	d, 7.0	3′	1.30	d, 7.0
										4′	1.05	d, 7.0			
5	1.30	m	5	1.30	m	5	1.30								
6	1.30	m	6	1.30	m	6	1.30								
7	1.30	m	7	1.30	m	7	1.30								
8	1.52	m	8	1.50	m	8	1.50								
9	0.86	d, 7.0	9	0.86	d, 7.0	9	0.86								
9′	0.86	d, 7.0	9′	0.86	d, 7.0	9′	0.86								
	3-methylbutyryl			isobutyryl			isobutyryl		isobutyryl		3-methylbutyryl			isobutyryl	
2	2.18	m	2	2.50	m	2	2.64	2	2.64	2	2.16	m	2	2.52	m
3	2.10	m	3	1.15	d, 7.0	3	1.20	3	1.20	3	2.09	m	3	1.14	d, 7.0
			3′	1.12	d, 7.0	3′	1.19	3′	1.19				3′	1.12	d, 7.0
4	0.93	d, 7.0								4	0.94	d, 7.0			
4′	0.93	d, 7.0								4′	0.94	d, 7.0			
	isobutyryl			isobutyryl			isobutyryl		isobutyryl		2-methylbutyryl			isobutyryl	
2	2.48	m	2	2.48	m	2	2.58	2	2.58	2	2.30	m	2	2.48	m
3	1.09	d, 7.0	3	1.09	d, 7.0	3	1.15	3	1.15	3	1.40	m	3	1.09	d, 7.0
											1.57	m			
3′	1.09	d, 7.0	3′	1.08	d, 7.0	3′	1.17	3′	1.17	3′	1.06	d, 7.1	3′	1.08	d, 7.0
										4	0.85	d, 7.2			
	acetyl			acetyl			acetyl		acetyl		acetyl			acetyl	
2	2.03	s	2	2.03	s	2	2.05	2	2.05	2	2.02	s	2	2.04	s

methylbutyryl)sucrose (5) could be assigned to the fraction C component.

Although ^{13}C NMR spectra of fractions D and E indicated various degrees of inhomogeneity, GC–MS (EI) of the components as their tetraacetylated derivatives did elicit relevant structural information. For fraction D GC–MS (EI) of the components in derivatized form yielded high mass ions at m/z 401 corresponding to tetraacetylated glucopyranosyl units containing two acetyls, one C_4, and one C_5 acyl group. Also prominent in the mass spectra were fragment ions at m/z 373 which could be assigned to tetraacylated fructofuranosyl residues containing three acetyls and one C_5 acyl group. On the basis of this information the fraction D components were assumed to be isomeric mixtures of sucrose esters containing one acetyl, one butyryl, and two methylbutyryl groups.

GC–MS (EI) of the fraction E components in derivatized form yielded high mass ions at m/z 401 and 387 corresponding to tetra-O-acylated glucopyranosyl units containing two acetyls, one C_4, and one C_5 acyl group in the former and then two acetyls and two C_4 acyl groups in the latter. Also prominent in the mass spectra were fragment ions at m/z 373 and 359 which could be ascribed to tetraacylated fructofuranosyl residues containing three acetyls and one C_5 acyl group. Thus, the fraction E components again appeared to be isomeric mixtures of sucrose esters containing one acetyl, two butyryl, and one methylbutyryl group.

A ^{1}H NMR spectrum of the fraction F component revealed the presence of one acetyl and three isobutyryl groups. GC–MS (EI) of its pentaacetate derivative yielded a high mass ion m/z 387 corresponding to a tetra-O-acylated glucopyranosyl unit containing two acetyls and two C_4 groups. Also prominent in the mass spectrum was a fragment ion at m/z 359 that could be assigned to a tetraacylated fructofuranosyl residue containing three acetyls and one C_4 acyl group. We did not purify sufficient material for a selective INEPT experiment because it appeared reasonable to assume by analogy with other compounds in this series that the acetyl group is situated in the 2-O-position. On the basis of this consideration, the structure of the fraction F component was finalized as 2-*O*-acetyl-3,4,3′-tri-*O*-isobutyrylsucrose (6).

Signifying perhaps a potential kinship, the acyl group substituent pattern in these *L. typicum* sucrose esters is akin to those previously elaborated for *S. neocardenasii* (King and Calhoun, 1988); i.e., both complexes have acetyl substituents at the 2-O-position, and when present, long-chain acyl groups were relegated to the fructose portion of the molecule.

A surprising feature and one not detected in previous investigations of other genera was the discovery that as some *L. typicum* accessions utilized in this study reached the senescence stage, another pattern of sucrose esters started to emerge. Subsequent analysis of the new esters determined that these were comparable in all respects to the 2,3,4-tri-O- and 2,3,4,1′-tetra-O-acylated sucrose esters previously identified in the exudate from glandular trichomes of *L. hirsutum*, etc. (King et al., 1990).

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