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Isolation and Characterization of the Sucrose Esters of the Cuticular Waxes of Green Tobacco Leaf

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The cuticular waxes of a tobacco budworm resistant tobacco, TI-165, contain a series of polar, high molecular weight compounds, which were separated from other components by solvent partitioning and Sephadex LH-20 gel chromatography. Glass capillary gas chromatography (GC-2) of these constituents as trimethylsilyl ethers and GC-2/mass spectrometry indicated that there were six groupings of isomers differing in mass, each from the next by 14 amu. Saponification of the total mixture of compounds yielded sucrose and a series of C_2- to C_8-aliphatic acids. The major acids were acetic, 2-methylbutyric, and 3-methylvaleric acids. Repeated gel chromatography resulted in the isolation of 6-*O*-acetyl 2,3,4-tri-*O*-[3-methylvaleryl]-α-D-glucopyranosyl-β-D-fructofuranoside, the major isomer, as defined by NMR and MS data. Other sucrose esters with similar molecular weights were isolated by preparative gas chromatography and saponified, and their acid compositions were determined. Partial hydrolysis of the SE yielded known tetraacylglucopyranosides.

INTRODUCTION

During our investigation of the cuticular leaf waxes of green tobacco from budworm-resistant and budworm-susceptible genotypes, several tobacco introductions (TI) were found to produce a series of polar, high molecular weight (MW) components. The observed field resistance of these tobaccos was postulated to be due to an antibiosis factor (Johnson and Severson, 1984). These high MW components were first detected when the glass capillary gas chromatographic (GC-2) profile of the cuticular waxes from the green leaf of a budworm-susceptible, flue-cured tobacco, NC 2326, was compared to that of the waxes from a resistant tobacco, TI-165 (Figure 1). Major components common to both tobaccos were the diterpenes, α- and β-4,8,13-duvatriene-1,3-diols (diols), docosanol ($C_{22}OH$), and the C_{25}–C_{36} aliphatic hydrocarbons. The high MW components were very apparent in the GC-2 chromatogram of the TI-165 cuticular waxes. A crude isolate of the high MW components from TI-165 was obtained by column chromatography on basic alumina (Severson et al., 1981). NMR analysis of the isolate indicated the presence of a sucrose ring system with the glucose moiety fully esterified and with an acetate group on the C_6 position, while the fructose portion showed four free hydroxy groups (Figure 2). Alkaline hydrolysis of the isolate confirmed the presence of sucrose. GC-2 analysis of the hydrolyzate, after acidification, confirmed the presence of C_2–C_8 acids, with the major acids being acetic, 3-methylbutyric, and 3-methylvaleric acids. We postulated that these green leaf sucrose esters (SE) were the precursors of the 6-*O*-acetyl triacylglucopyranosides (glucose esters, GE), previously isolated from a hexane-soluble fraction of cured Turkish tobaccos (Schumacher, 1970; Rivers, 1981). In addition, we have found SE in the cuticular extracts of the green leaf of Turkish cultivars (Severson et al., 1984). These SE and GE are believed to be the precursors of the important Turkish tobacco smoke flavor components, 3-methylbutyric and 3-methylvaleric acids (Kallianos, 1976; Chu-

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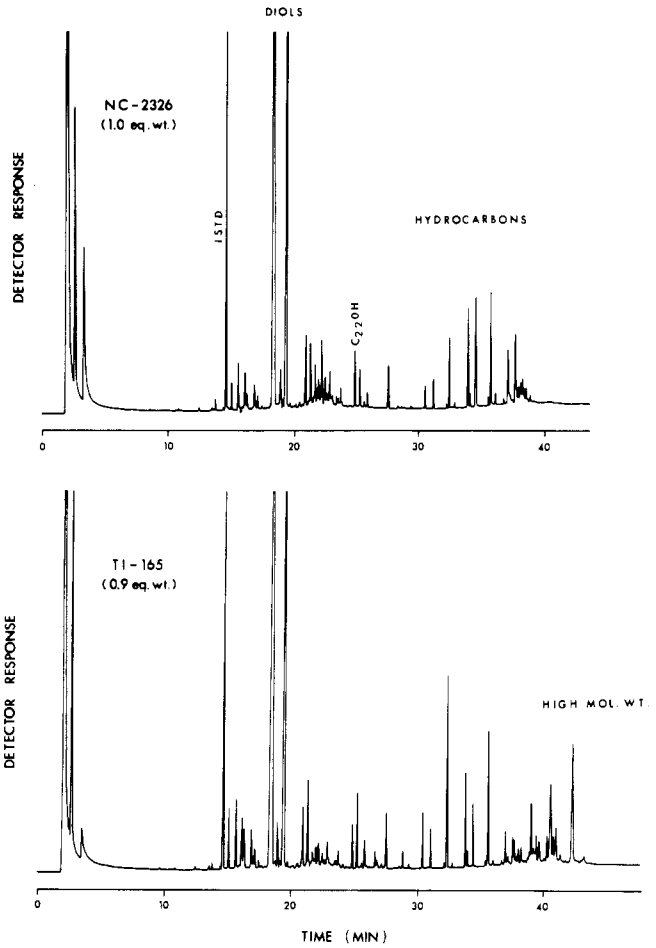

Figure 1. Glass capillary gas chromatography (GC-2) profiles of the cuticular waxes of NC 2326 and TI 165 tobaccos.

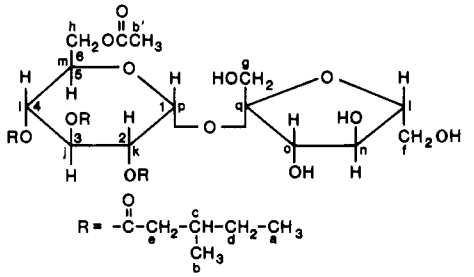

Figure 2. Structure of isolated sucrose esters (R = C_3–C_8), letters refer to NMR chemical shifts shown in Table III.

man and Noguchi, 1977; Matsushima et al., 1979). In agreement with these assumptions, the smoke of cured TI-165 yielded a flavor characteristic of Turkish tobaccos.

In this paper, we will describe methods for the isolation of the SE and will present data to confirm the basic structure of the isolated sucrose esters.

EXPERIMENTAL SECTION

Materials and Methods. Solvents were Baker "Resi-analyzed" grade and were used as received. The chloroform contained 0.75% by volume of ethanol. Dimethylformamide (DMF) and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) were silylation grade (Pierce Chemical Co., Rockford, IL). Aliphatic acid standards were obtained from Aldrich Chemical Co. and Sigma Chemical Co. (99+%).

Whole leaf wash and SE samples were analyzed as their trimethylsilyl (Me_3Si) ethers with a Hewlett Packard 5840 reporting gas chromatograph on a thin film SE-54 fused silica glass WCOT column (0.3 mm i.d. × 25 m), as described by Severson et al. (1984).

The gel chromatographic system consisted of glass Cheminert LC columns (2.54 i.d. × 76 cm and 1.37 i.d. × 122 cm) packed with Sephadex LH-20 in $CHCl_3$, loop injection valves, an Altex Model 110 pump, a Gilson Mini-Escargot fraction collector, and a Laboratory Data Control Refracto Meter III. A flow rate of 2 mL/min was used and 5-mL fractions were collected. Electron ionization (EI) and chemical ionization (CI) mass spectral (MS) data and EI/GC-2/MS data were obtained on an HP 5985 system modified for GC-2/MS (Arrendale et al., 1984). The direct chemical ionization (DCI) spectra were obtained on a Nermag R10-10 system at Columbia University. The field desorption (FD) and fast atom bombardment (FAB) spectra were taken on a MAT 731 instrument at the University of Illinois.

NMR data were obtained with a Varian CFT-20 ^{13}C spectrometer operating at ambient temperature (25 °C). Samples were dissolved in 100% deuterated $CHCl_3$ and run in the micro mode in a 1.7-mm o.d. capillary tube.

Extraction of Cuticular SE. Cuticular waxes, containing the SE, were collected from TI-165, grown at the Clemson University Pee Dee Research and Education Center, Florence, SC, under conditions normally used for the production of flue-cured tobacco. Whole plants, 50–60 cm in height, were cut off 20–25 cm above the ground and the cuticular components were extracted by dipping the whole plant tops four times (2 s/dip) into a 4-L beaker containing 3 L of methylene chloride. The plants were then reextracted (as above) in a second beaker. After washing 50 plants, the extract in the first beaker was filtered through anhydrous Na_2SO_4 into an amber glass solvent bottle. The beaker was refilled to the 3-L mark and became the second wash beaker, and another 50 plants were extracted. The process was repeated until the desired quantity of cuticular components was obtained. Extracts were cooled (dry ice), transferred to the laboratory, and stored at −18 °C. The above procedure was found to recover 95+% of the extractable SE.

Isolation of Sucrose Esters. After warming to room temperature, the whole leaf wash (WLW) was filtered through anhydrous Na_2SO_4 and the CH_2Cl_2 was removed on a rotary evaporator at 40 °C under 80–100 mmHg of vacuum. The residue was placed in a vacuum desiccator for 2 h to yield the WLW fraction. A 3-g portion of WLW was partitioned between 150 mL each of hexane and 80% MeOH–H_2O (Figure 3). The MeOH–H_2O solubles were extracted with hexane (2 × 50 mL). A mixture of 200 mL of chloroform ($CHCl_3$), 100 mL of H_2O, and 50 mL of saturated KCl was then added to the MeOH–H_2O solution and the mixture was shaken and partitioned. The $CHCl_3$ solubles were removed and the aqueous fraction was extracted with $CHCl_3$ (2 × 50 mL). The combined $CHCl_3$ fractions were washed with H_2O (2 × 100 mL) and filtered through anhydrous Na_2SO_4, benzene (50 mL) was added, and the solvent was removed in vacuo as above to yield fraction A (2.1–2.3 g).

Approximately 3 g of fraction A in $CHCl_3$ (total volume 10 mL) were added to the top of a 2.54 cm i.d. Sephadex LH-20 column (bed length 58 cm). Elution with $CHCl_3$ produced a crude SE fraction in gel fractions (GF) 75–140 (fraction B, Figure 3). After elution of fraction B, the column was eluted with 10% MeOH–$CHCl_3$ for 3 h and then reequilibrated overnight with $CHCl_3$ (flow rate 0.5 mL/min).

Fraction B was dissolved in 3 mL of $CHCl_3$ and rechromatographed on a 1.37 cm i.d. column (bed length 110 cm). Elution with $CHCl_3$ produced fraction C (GF 45–65,

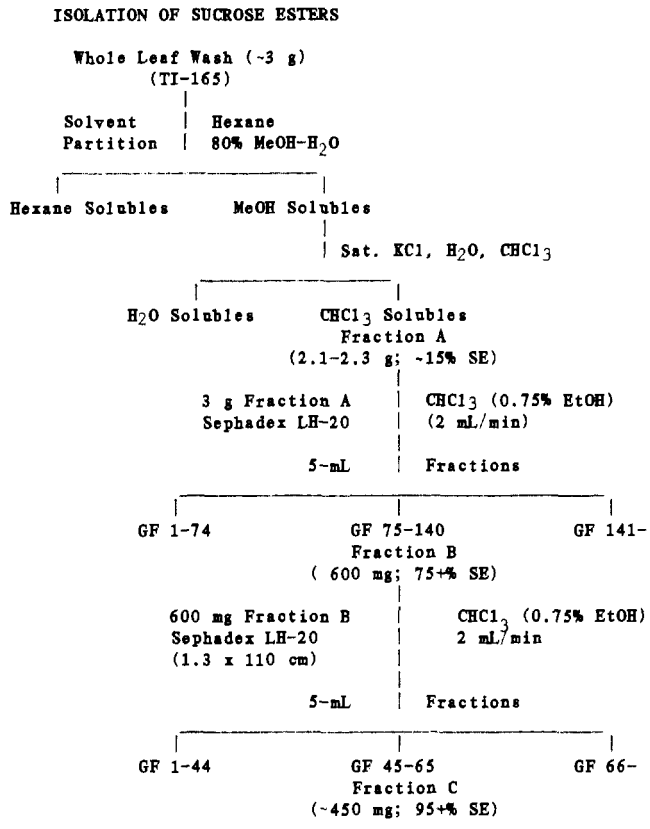

Figure 3. Scheme for the isolation of SE from cuticular waxes of TI 165 tobacco (GF = gel fractions).

95+% SE). The column was reconditioned as above.

Isolation of Group V Sucrose Esters. The isomer in group V of fraction C (Figure 3) was isolated by Sephadex LH-20 chromatography on three 1.37 cm i.d. columns (bed length 109 cm) connected in series (Figure 4). The material from GF 45–50 of fraction C (high in group V SE) and isolation run I and II isolates were placed on the column with a 2-mL injection loop and eluted with 1% MeOH–$CHCl_3$ (1% EtOH preservative in $CHCl_3$, Burdick and Jackson, distilled-in-glass) at a 2 mL/min flow rate. The elution of SE was monitored by GC-2 on an SE-54 column. About 36 mg of group V SE (93+% purity) was obtained in isolation run III.

Analysis of Sucrose Ester Acids. About 30 mg of SE isolate (fraction C, Figure 3) were dissolved in 3.00 mL of 1.0 N KOH in 80% MeOH–H_2O. After 24 h at room temperature, 30 μL of the saponificate was transferred to a 100-μL injector vial, the vial was capped, 10 μL of 6 N HCl was added, and the vial was gently agitated. (A precipitate of KCl formed immediately.) The mixture was analyzed immediately on a 0.2 mm × 10 m SP-1000 fused silica glass capillary column (Arrendale et al., 1983a), with a temperature program of 80–170 °C at 8 °C/min, injector temperature 200 °C, detector temperature 300 °C, and hydrogen carrier gas.

For determination of chromatographic response, 30 μL of a standard mixture, containing 2 μg/μL of each acid in 1 N KOH 80% MeOH–H_2O, was treated with 10 μL of 6 N HCl and analyzed as above. Free acids were identified by GC-2 retention data, by GC-2/MS, and by GC-2 retention data of their butyl esters. To convert the acid salts to their butyl esters, 0.5 mL of the SE saponificate was taken to dryness under a stream of N_2 at 40 °C. The residue was dissolved in 0.5 mL of *n*-butanol, 3–4 drops of concentrated H_2SO_4 was added, and the mixture was heated at 110 °C for 1 h. After cooling, 2 mL of hexane was added and the mixture was extracted with H_2O until

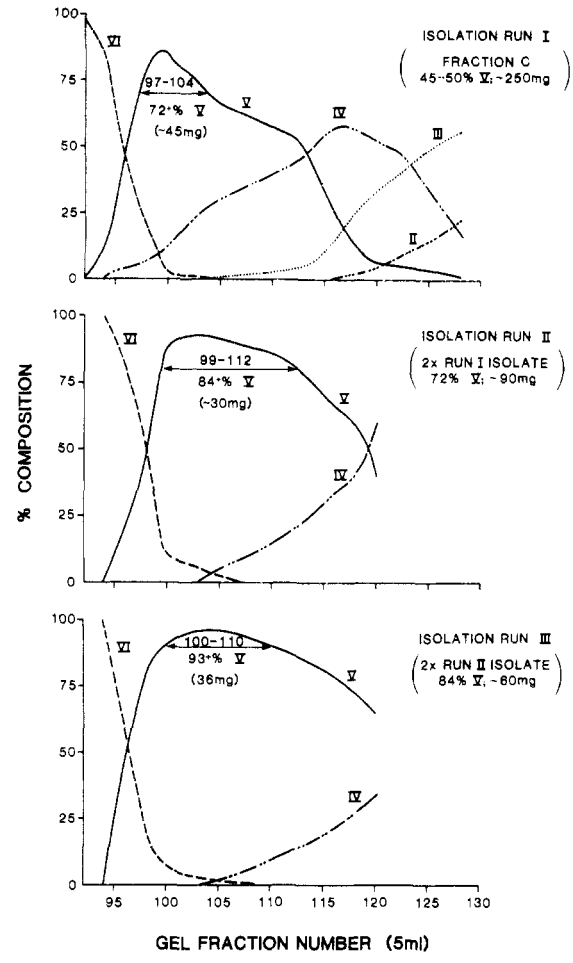

Figure 4. Compositions of individual gel fractions in gel chromatographic isolations (runs I–III) to obtain purified group V sucrose ester.

neutral. The hexane solubles were directly analyzed on a thick film 0.2 mm i.d. × 25 m SE-54 glass capillary column (Arrendale et al., 1983b), with a temperature program of 70–100 °C at 4 °C/min, injector temperature 200 °C, detector temperature 300 °C, and hydrogen carrier gas. When the standard acid salts were converted to their butyl esters and analyzed as above, unitary chromatographic responses were found for the butyl esters of the methylbutyric acids.

Preparative GC Isolation of SE Groups I–VI. Selected fractions from the isolation of group V SE (Figure 4) were converted to their Me_3Si derivatives and subjected to preparative GC with an HP 5750 gas chromatograph, equipped with a 3.2 mm × 76 cm stainless steel column of 5% Dexsil 300 GC on Chromosorb W/AW (100–120 mesh). The temperature program was 200–300 °C at 4 °C/min, injector and thermal conductivity detector temperatures were 275 and 310 °C, respectively, and the flow rate of He was 40 mL/min. After several collections, each group was dissolved in BSTFA/DMF and rechromatographed. A portion of the final preparative isolate for each group was analyzed by GC-2 (Figure 5). The remaining sample was subjected to microsaponification, as above, and the acid salts were converted with HCl to free acids, which were analyzed as described.

Conversion of Sucrose Esters to Glucose Esters. About 10 mg of SE fraction C (Figure 5) were dissolved in 500 μL of 80% MeOH in an 8-mL stoppered test tube. To this mixture were added 5 mL of hexane and 250 μL of 0.05 N HCl. After 48 h at room temperature, the hexane portion was removed and washed with water. It yielded

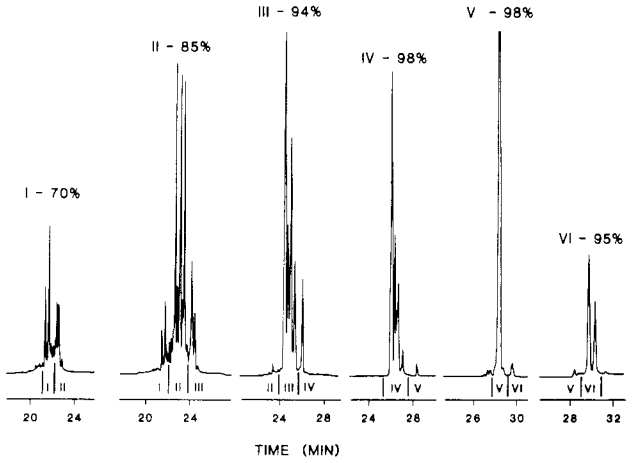

Figure 5. GC-2 profiles of material from preparative GC separations to obtain SE group I–VI isolates.

a crude mixture of SE and GE.

RESULTS AND DISCUSSION

Isolation of Sucrose Esters. The flow chart for the isolation of the SE from the whole leaf wash (WLW) of TI-165 tobacco is given in Figure 3. WLW concentrate was partitioned between hexane and 80% MeOH–H_2O. The SE and other polar components were soluble in the MeOH–H_2O fraction. After the addition of water and saturation with KCl, the SE were quantitatively extracted with $CHCl_3$. The majority of the water-soluble nicotine in the WLW was retained in the water. The GC-2 chromatogram of the $CHCl_3$ soluble material (Fraction A) is shown in Figure 6. The major components were the α- and β-4,8,13-duvatriene-1,3-diols (α- and β-diol) and the SE. Six groupings of ester isomers, differing by 14 amu, were observed. In the chromatogram of the total WLW extract (Figure 1), SE groups I and II coeluted with the C_{33} and C_{34} paraffinic hydrocarbons.

Preparative gel chromatography was used to separate the SE from the α- and β-diols and other polar components. GC monitoring of the resulting gel fractions (GF) showed that the SE eluted in GF 75-140 (fraction B). The major contaminants in fraction B were oxidative degradation products of α- and β-diols (oxydihydroxy- and trihydroxyduvanes). Subsequent gel chromatography on a more efficient Sephadex LH-20 system resulted in the isolation of 95+% pure SE isolate (fraction C), as determined by GC-2 (Figure 6).

During the gel chromatographic isolation of the SE, we observed some separation of the SE groups. Group VI eluted slightly earlier than group V and so on. The data indicated that a higher efficiency gel system should be able to separate a fraction greatly enriched in group V, which appeared to consist of a single isomer. Three columns (connected in series) were used to rechromatograph fraction C. To obtain reasonable elution volumes, a 1% MeOH–$CHCl_3$ (1% EtOH preservative in $CHCl_3$) solvent mixture was used as eluting solvent. Figure 4 shows the separations obtained for consecutive isolation runs. The elution data were calculated from GC-2 analyses of each GF, assuming unitary detector response for each group. Since these data are based on composition and not weight, elution volumes of each group of esters were not determined. However, the upper chromatogram in Figure 4 clearly shows that different elution volumes are obtained for the different SE groups and that more than one run would be required to isolate a single SE group. It also shows that it was only practical to isolate the single isomer in group V. The first isolation run yielded material containing 72+% of group V, which was essentially free of groups II and III. Materials from two run I isolates (GF 97–104) were combined and chromatographed in isolation run II. The combination of GF 99–112 produced an isolate which was 84+% pure group V. As shown on the bottom chromatogram, two run II isolates were chromatographed to give a 93+% purity isolate of group V in combined GF 100–110 material.

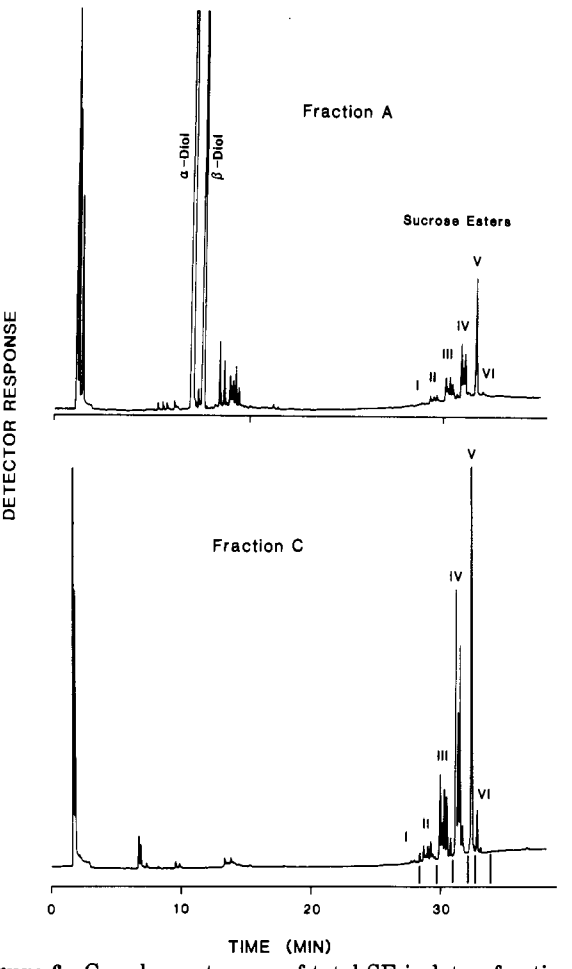

Figure 6. Gas chromatogram of total SE isolates, fractions A and C.

Table I. The Composition and GC-2 Response Data on Fraction C Acids[a]

acid	relative molar[b] response (± rel S/D)	moles acid[c]/ sucrose moiety
acetic	0.19 ± 0.2	1.00
propionic	0.41 ± 0.04	0.01
isobutyric	0.64 ± 0.03	0.08
butyric	0.60 ± 0.03	0.03
2-methylbutyric	0.84 ± 0.01	0.65[d]
valeric	0.77 ± 0.02	0.02
3-methylvaleric	1.00 ± 0.00	2.10[e]
caproic	0.98 ± 0.04	
methylcaproic		0.06[f]
heptanoic	1.10 ± 0.04	
methylheptanoic		0.01[f]
octanoic	1.23 ± 0.02	
Moles Acetic/Moles C_3–C_8 Acids 1.0:3.0		

[a] Determined as free acids by GC-2 analysis on an SP-1000 column. [b] Molar response of acid X/molar response of 3-methylvaleric; average of three determinations. [c] Calculated assuming 1 mol of acetic acid per sucrose moiety; average of three determinations. [d] 63% 3-methylbutyric; 37% 2-methylbutyric; determined after conversion to butyl esters and GC-2 analysis on thick film SE-54 column. [e] 97% 3-methylvaleric; 3% 4-methylvaleric; determined as above. [f] Calculated assuming response identical with that of normal chain acid.

Table II. Mass Spectral Analyses of Sucrose Esters

group (mol wt)	high mass ion type of MS analyses					
	EI[a] (neat)	EI/GC–MS[a] (4 Me_3Si)	CI (CH_4)[b] (4 Me_3Si) (M + 288 + H^+)	DCI (NH_3)[c] (M + NH_3 + H^+)	FAB/positive ion[d] (NaCl, M + 23)	FD/MS[e]
I (622)	443	443		640	645	622
II (636)	457	457		654	659	636
III (650)	471	471		668	673	650
IV (664)	485	485		682	687	664
V (678)	499	499	967	696	701	678
VI (692)	513	513				

[a] Electron impact ionization, HP 5985. [b] Chemical ionization, HP 5985. [c] Direct chemical ionization, Columbia University. Nermag R10-10, Vinca Parmakovich. [d] Fast atom bombardment, University of Illinois, J. C. Cook. [e] Field desorption, University of Illinois, MAT 731, J. C. Cook.

Characterization of the Sucrose Esters. To determine acid composition of SE, fraction C was saponified. The acid salts were converted to free acids, and the mixture was analyzed by GC-2 on an SP-1000 column (Table I). It should be noted that peak areas were not representative of component levels. The relative molar response increased dramatically from C_2 to C_8 acids, with the response of octanoic acid being about six times that of acetic acid. The methyl branched isomers showed a higher response than the normal isomers. However, the difference in response between branched and normal chains decreased with increasing carbon number. The major acids identified in fraction C were acetic, 3-methylbutyric, 2-methylbutyric, and 3-methylvaleric acids.

Molecular ions from the SE in fraction C could not be obtained in direct probe MS analyses in the electron impact mode (EI). Instead, ions for the glucose and fructose moieties, with the bridge oxygen atom being transferred to the neutral fragment moieties, were observed. Six high mass ions, differing by 14 amu and corresponding to the different glucose moieties (Table II), were observed for the neat sample. Relative intensities of these ions were in agreement with the relative amounts of the six SE groups, as determined by GC-2. Ions from the major acids were also present: acetyl, *m/e* 43; valeryl, *m/e* 85; caproyl, *m/e* 99.

The fragmentations of the SE tetramethylsilyl ethers via EI/GC-2/MS were similar. For the fructose portion of the dissacharide, all isomers yielded weak ions at 451 (4 Me_3Si) and a stronger ion of 361 (3 Me_3Si). The glucose moiety produced a series of ions identical with those of the neat sample. Selected single ion profiles of GC–MS data provided additional valuable information on the chemical composition of each SE group. The fragments related to the glucose moiety of each SE group were unambiguously determined, and each group yielded identical fructose ions. This indicated that the SE possess a structure with a completely esterified glucose portion and a fructose moiety with four free hydroxyl groups. The composition of the major acids of each group could also be determined. In comparing MS profiles, the acetyl (*m/e* 43) selected ion chromatogram was identical with the total ion chromatogram. This is consistent with one acetyl moiety per SE molecule. The C_6 acid ion (*m/e* 99, 3-methylvaleryl) was abundant in groups III–VI. The valeryl ion (*m/e* 85, from methylbutyryl groups) was abundant in groups I–IV. The abundant C_3H_7Si ion (*m/e* 71) from the Me_3Si moieties did not permit meaningful selected ion monitoring of the butyryl substituents.

Mild ionization methods were required to obtain molecular weight data. Only in DCI, FAB, and FD MS modes, where thermal energy is not used to vaporize the SE, was molecular ion information obtained on fraction C. In the DCI, it was possible to detect the molecular ion of the Me_3Si derivative of group V only after the MS source temperature was decreased to 100 °C.

Table III. Carbon-13 Chemical Shifts and Assignments for 6-*O*-Acetyl 2,3,4-Tri-*O*-[3-methylvaleryl]-α-D-glucopyranosyl-β-D-fructofuranoside (Group V SE)

carbon[a]	chemical shift[b]	carbon	chemical shift
a	11.2	k	69.4
b	19.2	l	70.6
b′	20.8	m	74.1
c	29.2	n	77.8
d	31.6	o	82.2
e	41.0	p	89.3
f	61.4	q	104.3
g	61.8	C=O	171.1
h	63.0		171.8
i	68.0		172.6
j	68.6		172.9

[a] See Figure 2 for carbon designations. [b] δ values in $CDCl_3$ relative to Me_4Si.

NMR data on a crude SE isolate also supported the presence of a completely esterified glucose ring with an acetyl moiety at the 6 position. Group V, isolated as above, was analyzed by ^{13}C NMR and the data are given in Table III. The data confirmed that the major isomer in group V of the SE fraction of TI-165 tobacco was 6-*O*-acetyl 2,3,4-tri-*O*-[3-methylvaleryl]-α-D-glucopyranosyl-β-D-fructofuranoside. There were 22 different resonances for the 32 carbon atoms in the molecule; 12 for the sucrose carbons, 5 for the saturated carbons in the three 3-methylvaleric moieties, 4 for the carbonyl carbons, and 1 for the acetyl methyl carbon.

Thus MS, NMR, and fraction C acid composition data indicated that each SE molecule contains one acetate moiety at the C_6 carbon and different combinations of C_3 to C_8 acids attached to the oxygen atoms at the C_2, C_3, and C_4 positions of glucose to yield the six groups (group I–VI) of SE, differing by 14 amu. To confirm this by chemical analysis, specific GF from isolation run I (Figure 4) were selected for their high contents of individual groups. These were converted to their Me_3Si derivatives and subjected to repetitive, preparative GC. The GC-2 chromatograms of the SE groups are shown in Figure 5. High purity isolates were obtained for groups III–VI. Collected GC fractions were then hydrolyzed and analyzed for their acid contents (Table IV). In agreement with NMR and MS data, group V was essentially a single SE, containing an acetyl and three 3-methylvaleryl acid moieties. All SE groups yielded ratios of one acetic acid to three C_3–C_8 acids. For SE groups with mixed acids (I–IV, VI), several arrangements of the acids at the C_3, C_4, and C_5 hydroxyls of the glucose molecule are possible. This would account for the numerous peaks observed on GC-2 analysis. The probable major acid constituents for each SE group are given at the bottom of the table.

It is postulated that the GE of Turkish tobaccos, reported by Schumacher (1970) and Rivers (1981), were

Table IV. Fatty Acid Composition of Preparative GC Sucrose Ester Groups

acid	SE group (% purity[a]) I (70)	II (85)	III (94)	IV (98)	V (98)	VI (95)
			Mol/Sucrose Moiety[b]			
acetic	1.00	1.00	1.00	1.00	1.00	1.00
propionic	0.11	0.03	0.01	0.01		
isobutyric	0.60	0.42	0.16	0.06	0.02	
butyric	0.07	0.02	0.02	0.02		
methylbutyric[c]	2.21	1.74	1.52	0.92	0.05	0.03
valeric	0.08	0.02	0.03	0.01		
methylvaleric[d]	0.32	0.82	1.35	1.85	2.89	1.83
caproic			0.01	0.02	0.04	0.04
methylcaproic			0.03	0.04	0.03	0.89
heptanoic					0.01	0.03
methylheptanoic					0.01	0.03
			Mol Acetic Acid/Mol C_3–C_8 Acids			
	1/3.3	1/3.1	1/3.1	1/2.9	1/3.1	1/2.9
			Major Acid Moieties			
	$C_2C_4$2C_5	$C_2$3C_5	$C_2$2C_5C_6	$C_2C_5$2C_6	$C_2$3C_6	$C_2$2C_6C_7
	$C_2$2C_4C_6	$C_2C_4C_5C_6$	$C_2C_4$2C_6			

[a] Determined by GC-2 on $(Me_3Si)_4$ derivatives assuming unitary detector response. [b] Determined by GC-2 on free acids assuming 1 mol of acetic acid per sucrose moiety, corrected for differences in detector response. [c] 2- and 3-methylbutyric. [d] 3- and 4-methylvaleric.

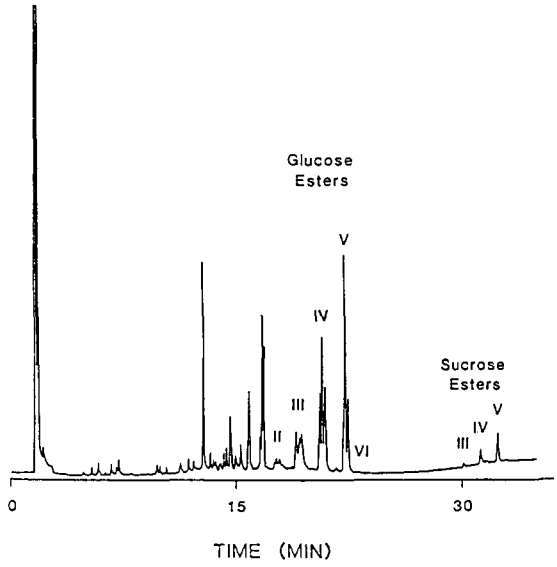

Figure 7. Gas chromatogram of the crude glucose esters isolate from the partial hydrolysis of SE.

formed by hydrolysis from the SE. Under normal laboratory acid hydrolysis conditions, SE are hydrolyzed directly to free acids, glucose, and fructose. However, preferential solubility of the GE in hexane, compared to SE, has permitted the initially formed GE to be trapped before significant hydrolysis of their ester linkages occurred. Fraction C was hydrolyzed in a mildly acidic MeOH–H_2O–hexane system. The MeOH–H_2O insoluble GE were partitioned into the hexane as they formed and were thus protected from further hydrolysis. GC-2 analysis of the hexane solubles (after conversion to Me_3Si derivatives) indicated that a series of GE, with a distribution similar to the parent SE, was formed (Figure 7). This was confirmed by GC-2/MS data. As for many Me_3Si ethers, a molecular ion was not obtained. The high mass peaks were at M – 15 and M – 90, resulting from the loss of a methyl group from the Me_3Si moiety and the elimination of hydroxytrimethylsilane. The mass spectrum of group V Me_3Si-GE was identical with that of the Me_3Si derivative of an authentic sample of 6-*O*-acetyl 2,3,4-tri-*O*-[3-methylvaleryl]glucopyranoside (Schumacher, 1970).

With this last piece of information, we have chemically and spectrometrically confirmed the basic structure of the SE. We are currently conducting studies to determine the effect that SE have on tobacco smoke flavor and on larval mortality and development of the tobacco budworm.

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