Isolation and Characterization of Sucrose Polyesters

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Various chromatographic techniques for isolation and separation of highly esterified sucrose polyesters (SPE) of olive oil are described. High-performance size-exclusion chromatography was used to check the purity of the samples, particularly to show that SPE were free from unreacted fatty acid methyl esters. Thin-layer chromatography (TLC), Iatroscan TLC flame-ionization detection (FID) and high-performance liquid chromatography (HPLC) on reversed-phase were applied for separation of octa-, hepta- and hexaesters of sucrose. Pure fractions from the total mixture, obtained by column chromatography, were identified by infrared and nuclear magnetic resonance spectroscopy. When necessary, specific reactions were applied; particularly, silylation and lead acetate-dichlorofluorescein (in toluene) spray were used to ascertain the degee of esterification of sucrose. Finally, octa-, hepta- and hexaesters of sucrose were quantitated by silica column chromatography, TLC/FID and HPLC on reversed-phase.

KEY WORDS: ^{13}C NMR, HPLC, HPSEC, infrared spectroscopy, sucrose polyesters (SPE), TLC/FID.

Reaction of sucrose with fatty acid methyl esters (FAMEs) leads to a mixture of great complexity, which includes sucrose esters containing from one to eight fatty acyl groups and remaining quantities of reaction substrates. The methanol-insoluble fraction is composed of sucrose esters with a high degree of substitution and is commonly termed sucrose polyesters (SPE) (1,2).

Physical and chemical properties of SPE closely resemble those of triglycerides (TG) with similar fatty acid composition (3), but SPE differ in their resistance to pancreatic lipase and, hence, nonabsorbability (4,5). Therein, SPE could be used as fat substitutes of low digestibility, either alone or in mixtures with edible fats and oils. In the latter cases, the properties of the final product would be determined by the initial composition of the mixture SPE/TG (2).

Given that SPE are of growing importance in food technology for their potential use in baking and frying, some interesting studies regarding their behavior at high temperature have been published (6–8). Also, characterization of SPE and related noncaloric fats by nuclear magnetic resonance (NMR), infrared (IR) and mass spectrometry has been reported (9–14). Nevertheless, little information is available regarding the application of chromatographic techniques for a better knowledge of SPE mixtures (14–16). In this paper, various chromatographic techniques were applied for isolation, characterization and quantitation of the mixture obtained by reacting sucrose with methyl esters from olive oil by means of a solvent-free catalyzed method (17). NMR and IR spectroscopies were used for identification purposes.

EXPERIMENTAL PROCEDURES

Synthesis and purification of SPE. SPE were prepared according to the solvent-free sucrate-catalyzed method of Rizzi and Taylor (17), starting from sucrose and FAMEs derived from olive oil. The crude product was treated with acetic acid to neutralize the reaction mixture and washed with methanol several times to remove FAMEs and low-substituted sucrose esters. Isolation and purification of SPEs were carried out by means of silica column chromatography. Then, 50 g of sample were dissolved in petroleum ether/ethyl ether (95:5, vol/vol) and transferred to a column (4.5 cm i.d. × 42 cm) containing 320 g of Silica Gel 60, particle size 0.063–0.200 mm (70–230 mesh ASTM, Merck No. 7734; Darmstadt, Germany), adjusted to an H_2O content of 5% and suspended in the same mixture of solvents. The first fraction, eluted with 1.25 L petroleum ether/ethyl ether (95:5, vol/vol), contained minor nonpolar impurities and was discarded. A second fraction, eluted with 1.25 L petroleum ether/ethyl ether (50:50, vol/vol), contained pure SPE. Pure sucrose octaoleate was prepared starting from sucrose and excess of oleyl chloride to form the complete ester (18). Briefly, a molar ratio of one sucrose to twelve fatty acid chloride in pyridine and chloroform was refluxed for 4 h at 40°C under a nitrogen stream. Sucrose octaoleate was isolated and purified by silica column chromatography. After washing thoroughly, the sample was dissolved in petroleum ether/ethyl ether (92:8, vol/vol) and transferred to a column containing silica (20 g/g of sample) suspended in petroleum ether. Pure sucrose octaoleate was obtained by elution with petroleum ether/ethyl ether (92:8, vol/vol) (150 mL/g of sample) (19).

Chromatographic techniques for separation and quantitation of SPE. (i) Classical thin-layer chromatography (TLC) and thin-layer chromatography/flame ionization detection (TLC/FID): SPEs were resolved by TLC on plates coated with 0.25-mm layers of silica gel 60 and developed with petroleum ether/ethyl ether/acetic acid (65:35:1, vol/vol/vol). Spots were made visible by exposure to iodine vapor and/or spraying with 25% sulfuric acid and heating at 250°C. Glycol groups were detected with lead (IV) acetate as reagent (20). Additionally, samples were analyzed by TLC/FID. Sample solutions (1/μL of 15–20 mg/mL hexane) were spotted on Chromarods S-III (Iatron Laboratories, Tokyo, Japan). The rods were developed in petroleum ether/ethyl ether/acetic acid (90:10:2, vol/vol/vol) for 35 min and scanned in an Iatroscan MK-5 analyzer (Iatron Laboratories).

(ii) High-performance size-exclusion chromatography (HPSEC): Samples were analyzed in a Konik 500 A chromatograph (Konik, Barcelona, Spain) fitted with a Rheodyne 7125 injector (Hewlett-Packard, Palo Alto, CA) and a 10 μL sample loop. A Hewlett-Packard 1040 A refractive index detector and two 100 Å and 500 Å PLgel columns (particle size: 5 μm) connected in series were used (Hewlett-Packard). The columns were 30 cm × 0.75 cm i.d., with polystyrene–divinylbenzene highly cross-linked macroporous spherical packing. High-performance liquid (HPLC)-grade tetrahydrofuran (THF) served as the mobile phase at a flow rate of 1.0 mL/min, and the sample concentration was 15–20 mg/mL in THF.

(iii) HPLC on reversed-phase: Samples were analyzed as described above for HPSEC but using a 5-μL sample loop and a packed (250 × 2.4 mm id.) column, filled with

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5 μm Spherisorb Octadecyl-1 (ODS; Beckman, Berkeley, CA). The mobile phase consisted of a mixture of THF/methanol (45:55, vol/vol) at a flow rate of 1.0 mL/min. Sample concentration was 40 mg/mL in THF.

(iv) Adsorption chromatography on silica column: 1 g of purified SPE was dissolved in hexane/ethyl ether (92:8, vol/vol), transferred to a column that contained silica (20 g/g SPE) suspended in petroleum ether, and eluted sequentially with 150 mL hexane/ethyl ether (92:8, vol/vol), 150 mL hexane/ethyl ether (80:20, vol/vol) and 150 mL ethyl ether. The fractions obtained were determined gravimetrically.

Identification techniques: NMR and IR spectra. IR spectra were taken on the pure SPE samples, preparatively isolated by adsorption chromatography columns. When necessary, a further purification by TLC was carried out. NMR spectra were obtained on a Bruker AC-300P spectrometer (Karlsruhe, Germany) at a carbon frequency of 75.5 MHz. Samples were dissolved in deuterated chloroform. All resonances were referenced to internal tetramethylsilane (21). IR spectra were recorded in capillary films with a Perkin-Elmer 782 IR spectrophotometer (Norwalk, CT).

RESULTS AND DISCUSSION

Figure 1A shows the HPSEC chromatogram of the pure SPE sample used in this study. A single peak could be observed following the conditions described in the Experimental Procedures section. Those conditions were selected with the aim of checking the purity of the sample, particularly the absence of remaining FAMEs, which elute at longer retention times than SPE. A clear separation between SPE and TG was also obtained under these conditions, as illustrated in Figure 1B, which shows the efficacy of the separation achieved among SPE, TG and FAME. A quantitative evaluation of SPE and TG in mixtures is possible. Such analysis is important considering a recent claim concerning the use of SPE in mixtures with edible fats and oils (22,23).

Analyses by TLC in Chromarods are presented in Figure 2. As expected, the chromatogram of SPE (Fig. 2A) showed a complex mixture of compounds with a wide range of polarity. Silylation with N,O-*bis*-(trimethylsilyl)-trifluoroacetamide (BSTFA) (18) was used to convert unesterified free OH groups in sucrose to their BSTFA derivatives. After silylation, only one peak of low polarity was present (Fig. 2B). These results indicated that the polar compounds were partially esterified sucrose esters and that compounds other than SPEs were not present in appreciable amounts.

Valuable information was obtained by HPLC on reversed-phase, as shown in Figure 3A. The total mixture of sucrose polyesters gave only three significant peaks. It appears that this technique enables the separation of compounds with differences in polarity but cannot distinguish among isomers with the same degree of esterification. Three fractions were cut from the eluent after several injections, and chromatograms obtained by TLC/FID of the fractions isolated are also shown in Figure 3B. The fraction of lower polarity, numbered I, contained a unique peak whereas fractions II and III were, in contrast, comprised of more than one peak. Accordingly, isolation of hexa-, hepta- and octaesters seems to have been achieved. Several attempts were made to obtain significant amounts of these three fractions by silica column chromatography, as outlined in the Experimental Procedures section, for further identification by NMR and IR spectroscopies. Fractions were then labeled FI, FII and FIII, from lower to higher polarity, and corresponded to those obtained by HPLC. FI, FII, FIII and sucrose octaoleate were used in the spectroscopic analysis for identification of the main components of the mixture obtained.

Figure 4 shows the ^{13}C NMR spectrum of pure sucrose octaoleate. The signals corresponding to methyl and

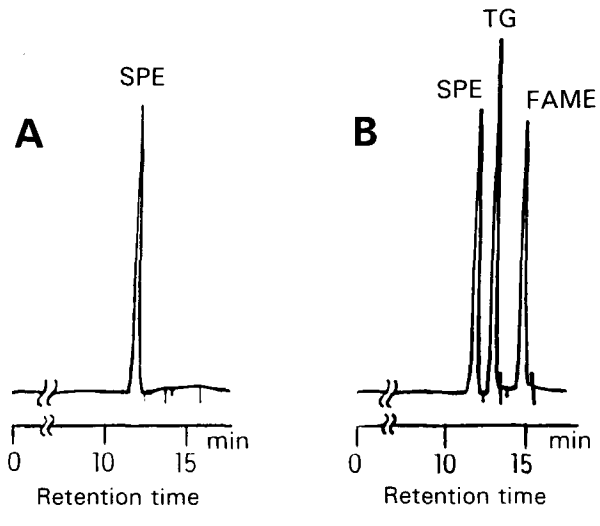

FIG. 1. High-performance size-exclusion chromatograms of (A): sucrose polyesters (SPE) and (B): mixture of SPE, triglycerides (TG) and fatty acid methyl esters (FAME).

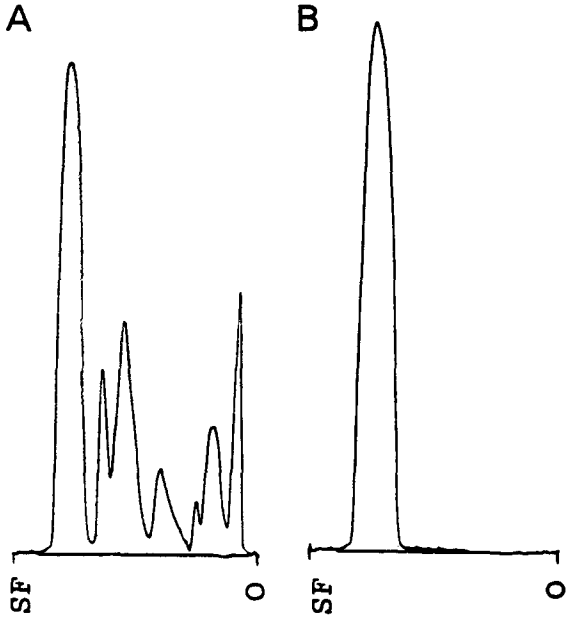

FIG. 2. Efficacy of the separation of SPE by thin-layer chromatography–flame ionization detection. (A): SPE; (B): SPE after silylation; O = origin, SF = solvent front. See Figure 1 for abbreviation.

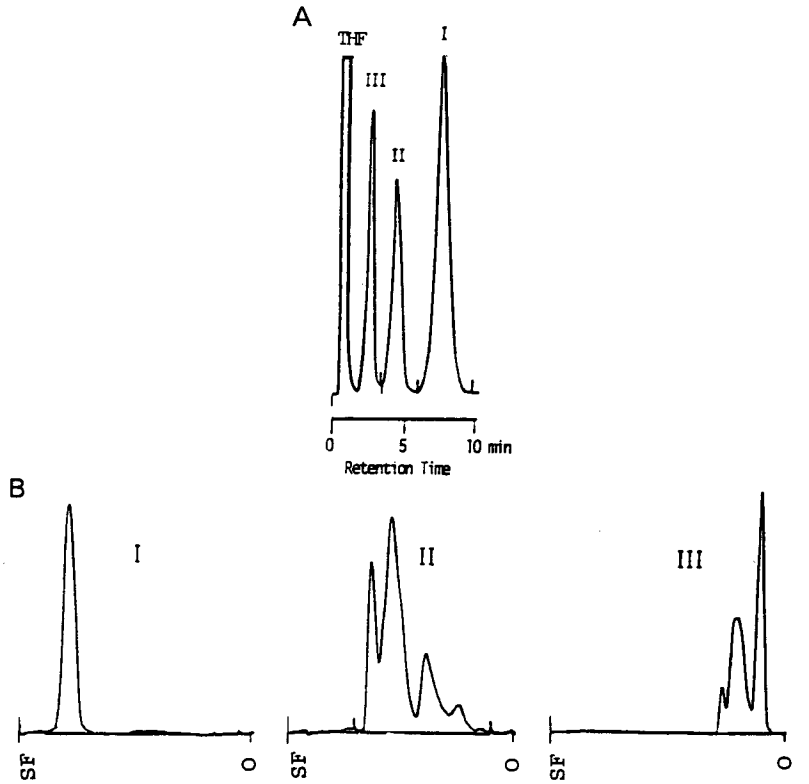

FIG. 3. Efficacy of the separation of sucrose polyesters by reversed-phase high-performance liquid chromatography (A) and thin-layer chromatography–flame-ionization detection (B) chromatograms corresponding to the isolated fractions I, II and III (three fractions cut from the eluent after several injections: I, a fraction of lower polarity containing a unique peak; II and III, fractions comprised of more than one peak). See Figure 2 for abbreviations; THF, tetrahydrofuran.

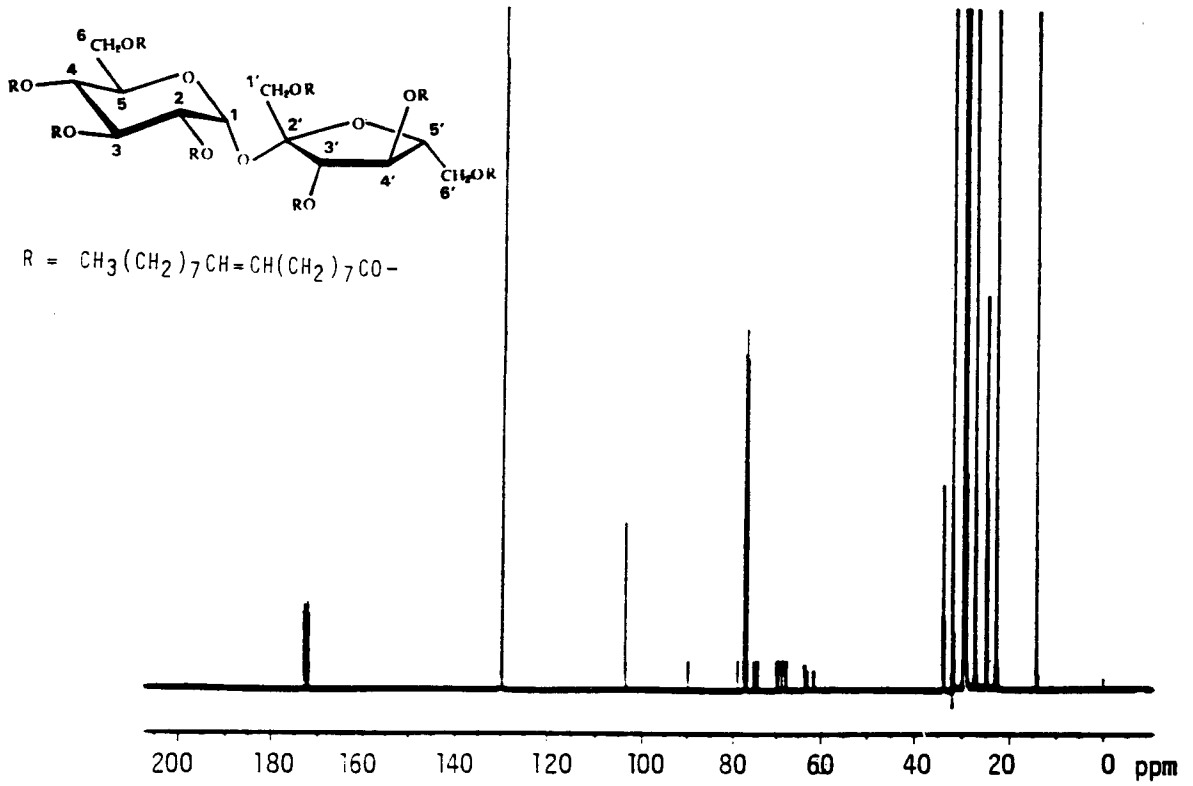

FIG. 4. Structure and ^{13}C nuclear magnetic resonance spectrum of sucrose octaoleate taken in $CDCl_3$.

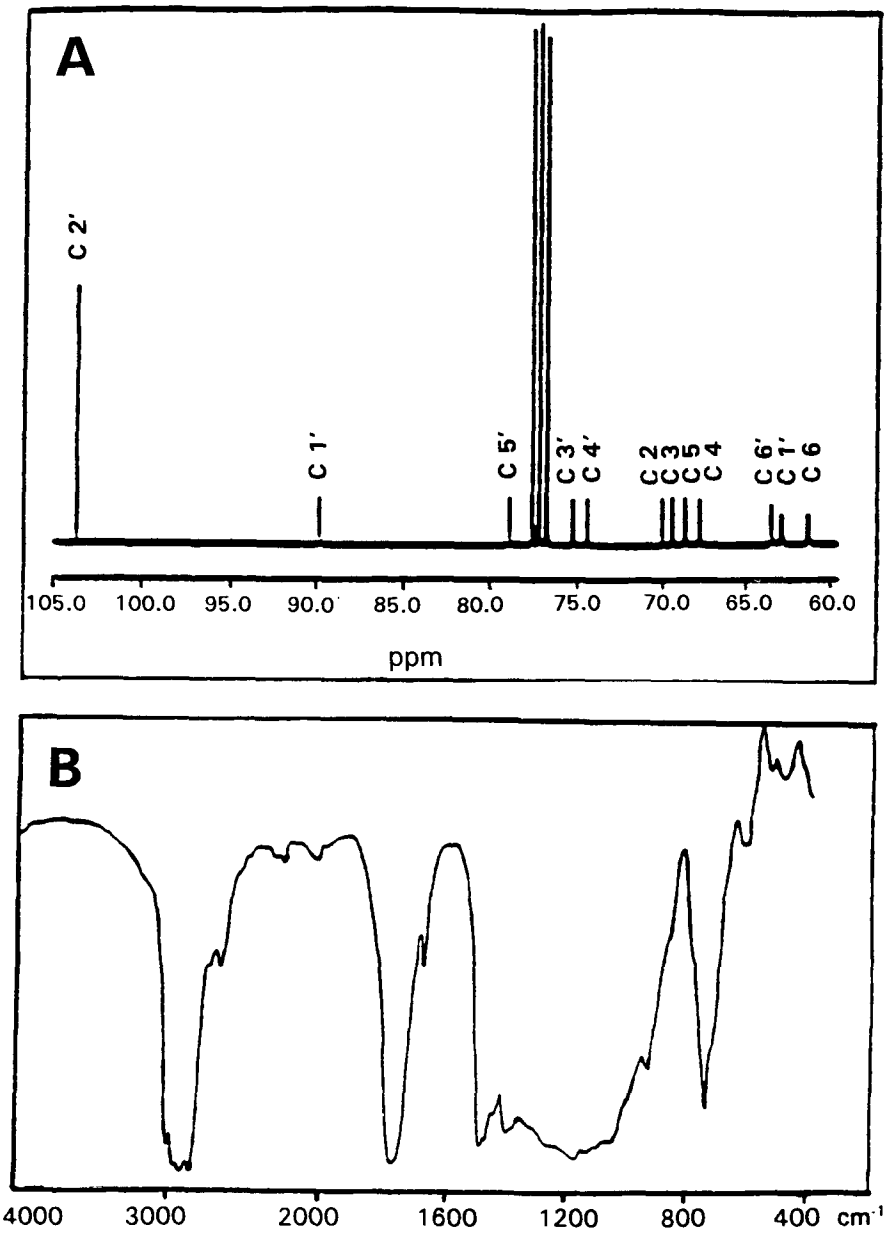

FIG. 5. Fraction of low polarity isolated from sucrose polyesters (FI): (A) sugar portion of the ^{13}C nuclear magnetic resonance spectrum taken in $CDCl_3$ and (B) infrared spectrum. FI, fraction I; see Figure 3 for explanation.

methylene carbons of the oleyl group (14–35 ppm), carbons of the sucrose skeleton (62–110 ppm), double-bonded carbons (129.2–130.0 ppm) and carbonyl carbons (172.7–173.5 ppm) were observed. These signals were also observed in the spectra of FI, FII and FIII.

The ^{13}C NMR spectrum of FI was virtually identical to that of sucrose octaoleate, suggesting that FI corresponded to sucrose octaesters. Figure 5A shows the significant part of the spectrum corresponding to the sucrose carbon atoms (62–110 ppm). Only one signal for each sucrose carbon atom was observed, and the chemical shifts data are presented in the first column of Table 1. In addition, its IR spectrum, Figure 5B, did not show any band of hydroxyl groups, thus confirming that FI corresponded to sucrose octaesters.

The spectrum of fraction FII presented numerous signals in the sucrose carbon atom zone, among which those corresponding to C2′ (103–105 ppm) stood out. This zone is shown in Figure 6A, where seven isomers were observed, three of which added up to more than 75% of the total area. This spectrum corresponded to a mixture of esters of sucrose, presumably heptaesters, according to previous results obtained by chromatographic techniques and to the IR spectrum, given in Figure 6B, which clearly

TABLE 1

Chemical Shifts for Sucrose Carbon Atoms

Carbon	Sucrose octaester	Isomer isolated from FII[a]
C2′	103.2	102.8
C1	89.4	89.0
C5′	78.6	80.7
C3′	74.9	78.4
C4′	74.2	77.4
C2	69.5	69.9
C3	68.9	69.6
C5	68.2	68.6
C4	67.4	68.4
C6′	62.8	63.8
C1′	62.2	63.6
C6	61.0	62.2

[a]FII is the sucrose polyester fraction of medium polarity, isolated by adsorption chromatography.

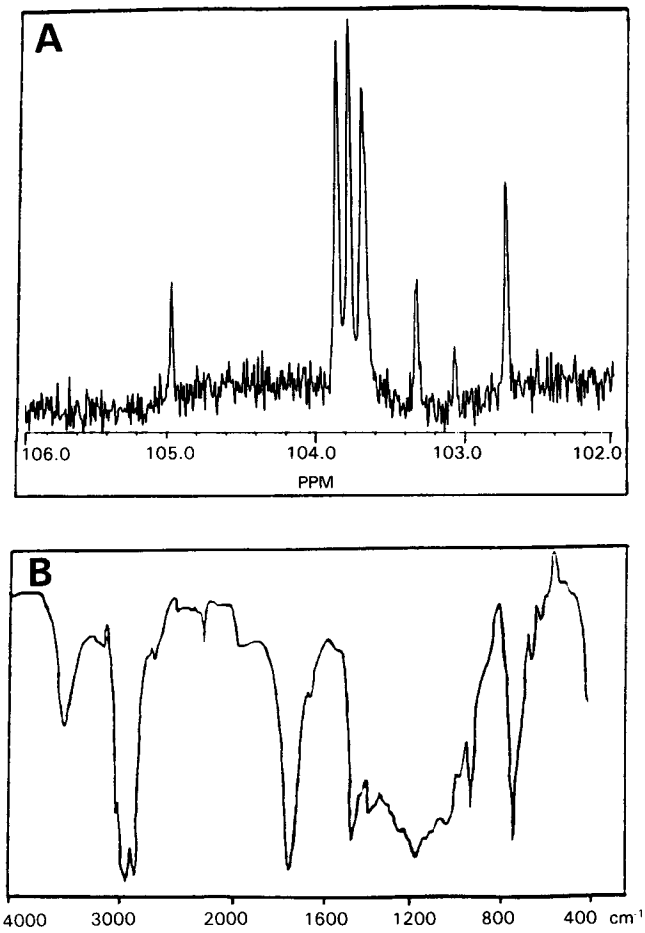

FIG. 6. Fraction of medium polarity isolated from sucrose polyesters (FII): (A) expanded plot of the ^{13}C nuclear magnetic resonance spectrum showing the resonances of C2′ and (B) infrared spectrum. FIII, fraction III; see Figure 3 for explanation.

shows an absorption band at 3420 cm^{-1} corresponding to hydroxyl groups.

The isomer of greatest polarity was isolated from FII by TLC, and its ^{13}C NMR spectrum was obtained. Figure 7 shows the signal corresponding to C2′, and Table 1 lists the chemical shifts for its sucrose carbon atom resonances. By comparing the chemical shifts of sugar carbons of this isomer with those from the octaester, the chemical shifts of C3′ and C4′ were the most affected, which suggests that the free hydroxy group was in the fructose ring. Therefore, the structure of this isomer has been tentatively assigned as the 3′-hydroxy or 4′-hydroxy heptaester of sucrose, in accordance with the above and the changes in chemical shifts expected when changing a hydroxy group by an alkoxycarbonyl group.

The spectrum of FIII (not shown) was complex, denoting the presence of numerous isomers, which could not be isolated. However, occurrence of vicinal diols was checked by TLC through a specific reaction with lead (IV) acetate (24), whereby it was deduced that FIII included sucrose hexaesters. The reaction was negative when applied to FI and FII.

Overall, the results obtained in this study indicated that adsorption chromatography, either classical silica column or TLC/FID, and HPLC on reversed-phase, are appropriate techniques for quantitation of SPE mixtures. Previous evaluation of SPE has been achieved by a standard hydroxyl value determination, from which an approximate measurement of the average number of free OH groups in the global sample was obtained (17,25). Alternatively, SPE have been determined by the quantitative TLC method of Weiss *et al.* (16), although some degree of uncertainty was associated with the identity of specific spots for the tetra- through heptaesters (25). Table 2 summarizes the composition of SPE analyzed by silica column chromatography, TLC/FID and HPLC on reversed-phase. Sucrose octa-, hepta- and hexaesters, as isolated by silica column chromatography, showed similar response factors in TLC/FID and HPLC analyses; hence, correction factors were not applied. No significant differences were found between the values obtained by the three techniques used. Reproducibility was excellent for sucrose octaesters, and the maximum coefficient of variation was 7% in the case of sucrose hexaesters analyzed by TLC/FID. Finally, the chromatographic techniques applied in this study could also be of great use for characterization of SPE/TG

TABLE 2

Comparison of Silica Column Chromatography, TLC/FID and HPLC on Reversed-Phase in Analyses of Sucrose Polyesters (wt% on sample)[a]

	Adsorption chromatography		HPLC on reversed-phase
	Silica column	TLC/FID	
Sucrose octaesters	54.0 ± 0.42	53.7 ± 0.82	55.1 ± 0.61
Sucrose heptaesters	25.3 ± 0.75	24.6 ± 0.72	23.6 ± 0.77
Sucrose hexaesters	20.7 ± 0.45	21.7 ± 1.53	20.3 ± 0.81

[a]Values are means ± SEM of three determinations. Conditions as described in Experimental Procedures section. Abbreviations: TLC/FID, thin-layer chromatography/flame-ionization detection; HPLC, high-performance liquid chromatography.

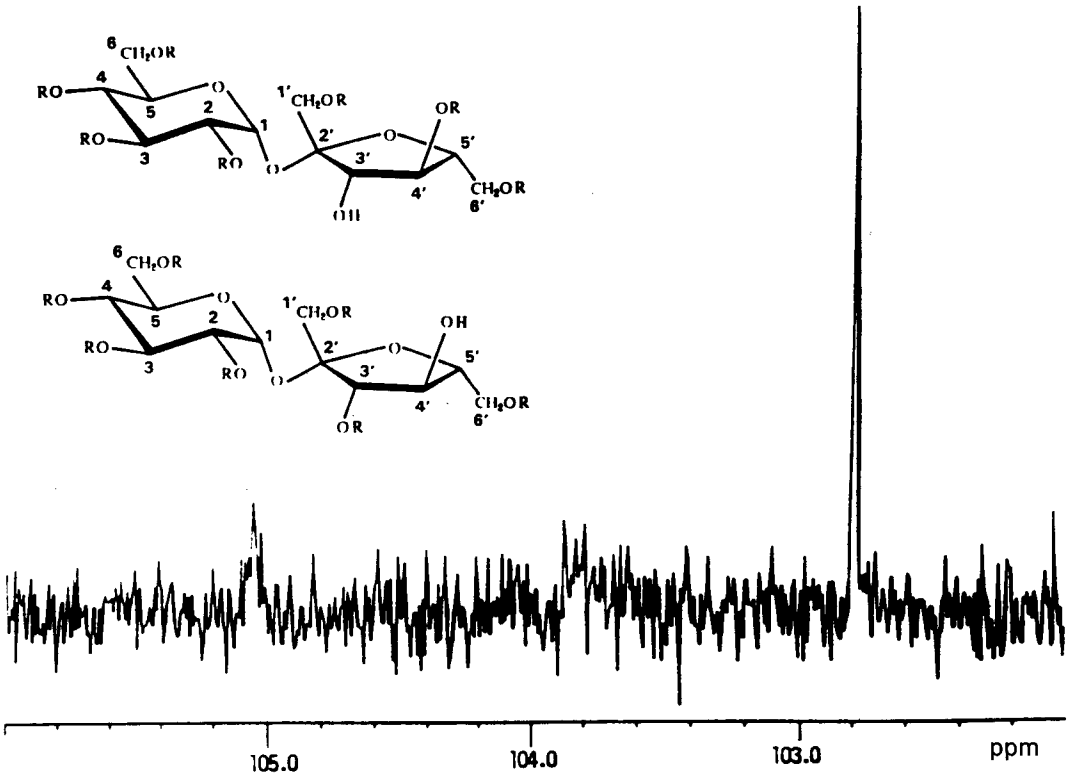

FIG. 7. Expanded plot of the ^{13}C-nuclear magnetic resonance spectrum of the isomer isolated from FII, showing the C2′ resonance and the structures tentatively assigned. See Figure 6 for abbreviation and explanation.

mixtures. Studies focussed on this subject are currently underway in our laboratory, and results will be communicated shortly.

ACKNOWLEDGMENTS

This work was supported by CICYT (Project ALI 91-0544). The authors thank Drs. J. Hidalgo, R. Zamora and R. Maestro for recording the spectra and helpful discussion, and Giménez for assistance.

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[Received May 14, 1993; accepted January 26, 1993]