J. Agric. Food Chem. 1985, 33, 213–217.
Lohan, O. P.; Lall, D.; Vaid, J.; Negi, S. S. "Utilization of Oak Tree (*Quercus incana*) Fodder in Cattle Rations and Fate of Oak Leaf Tannins in Ruminant System". *Indian J. Anim. Sci.* 1983, *53*, 1057–1063.
Makkar, H. P. S.; Singh, B.; Dawra, R. K. "Can Oak Leaves Be Fed to Animals?" *Farmers and Parliament* 1986, *24*, 17–18.
Makkar, H. P. S.; Singh, B.; Dawra, R. K. "Tannins-Nutrient Interaction—A Review". *Int. J. Anim. Sci.* 1987, *2*, 127–140.
Martin, J. S.; Martin, M. M. "Tannin Assay in Ecological Studies. Lack of Correlation between Phenolics, Proanthocyanidins and Protein-Precipitating Constituents, in Mature Foliage of Six Oak Species". *Oceologia* 1982, *54*, 205–211.
Nastis, A. S.; Malechek, J. C. "Digestion and Utilization of Nutrients in Oak Browse by Goats". *J. Anim. Sci.* 1981, *53*, 283–290.
Negi, S. S.; Pal, R. N.; Ehrich, C. *Tree Fodders in Himachal Pradesh*; German Agency for Technical Cooperation: Eschborn, FRG, 1979.
Neser, J. A.; Coetzer, J. A. W.; Boomker, J.; Cable, H. "Oak (*Quercus robur*) Poisoning in Cattle". *J. S. Afr. Vet. Med. Assoc.* 1982, *53*, 151–156.

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Carbon-13 Nuclear Magnetic Resonance for the Qualitative and Quantitative Analysis of Structurally Similar Disaccharides

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The use of carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy for both the qualitative and quantitative analyses of structurally similar glucose–glucose and glucose–fructose disaccharides in honey was investigated. Two of the main problems associated with the quantitative use of ^{13}C NMR, which are differences in carbon relaxation times and the variable nuclear Overhauser effect (NOE), were overcome by employing a relaxing reagent (chromium acetylacetonate). ^{13}C NMR was applied to the analysis of the complex mixture of minor disaccharides found in honey.

Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy has been used extensively as a method for the structural determination of carbohydrates. A number of recent reviews have been written on the application of this methodology to the study of carbohydrates both in solution and in the solid state (Bock and Thoegersen, 1982; Coxon, 1980; Gorin, 1981; Inch, 1972; Pfeffer, 1984; Vliegenthart et al., 1983).

Coxon (1980) and Rathbone (1985) have recognized that ^{13}C NMR spectroscopy could be used for the quantitative analysis of carbohydrates. Some of the problems associated with the use of ^{13}C NMR for quantitation of carbohydrates have been documented by Wehrli and Wirthlin (1976). These problems include differences in carbon relaxation times (T_1 and T_2) and nuclear Overhauser effects (NOE), viscosity effects, temperature effects, solubility, digital resolution, and the low sensitivity of the ^{13}C nuclei. It has been recognized that the most serious of these effects are due to the long relaxation times and the variable nuclear Overhauser effects (Wehrli and Wirthlin, 1976; Levy and Nelson, 1972). Berry et al. (1977) and Czarniecki and Thornton (1977) have shown that the NOE for the carbon nuclei in carbohydrates vary dramatically, which introduces errors into quantitative measurements. Coxon (1980) has also found that the T_1 for most carbon nuclei in carbohydrates is less than 1 s. In order for carbon nuclei to relax completely after a 90° pulse it is necessary to wait a period of at least $5T_1$ (or 5–6 s) before another pulse is applied.

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Cerbulis et al. (1978) noted that, in order to quantitate carbohydrates by ^{13}C NMR, the NOE needed to be suppressed. This may be accomplished by employing the gate-decoupling technique (Freeman et al., 1972), which requires turning off the decoupler for long periods (or 5–7 T_1) between data acquisition if a 90° pulse is used.

Due to the inherent lack of sensitivity of ^{13}C nuclei to NMR detection when compared to ^{1}H nuclei (approximately 6000 times less sensitive), either concentrated carbohydrate solutions or long accumulation times are required to obtain reasonable spectra. Use of the gated-decoupling technique or waiting $5–6T_1$'s makes the acquisition time necessary to obtain a ^{13}C spectrum lengthy and expensive. In addition, the use of concentrated carbohydrate solutions may be very difficult or impossible with rare or expensive carbohydrates.

Applications of ^{13}C NMR to the quantitation of carbohydrates in food are few. Blunt and Munro (1976) applied ^{13}C NMR to the quantitation of carbohydrates extracted from various tissues of *Pinus radiata*. When relaxation delays of $>4T_1$ (>4 s) were employed, the levels of fructose, glucose, and sucrose were determined with standard deviations ranging from 3 to 8%. Tamate and Bradbury (1985) applied ^{13}C NMR spectroscopy to the analysis of carbohydrates in tropical root crops. Comparisons of the results obtained by ^{13}C NMR to those obtained by HPLC showed deviations of 3–30%.

In this work we investigated the use of ^{13}C NMR spectroscopy for the qualitative and quantitative analyses of the oligosaccharides found in honey. ^{13}C NMR analyses were carried out on a Bruker 400-MHz spectrometer operating in the Fourier transform (FT) mode. These analyses were performed on standard solutions of reduced disaccharides prepared by weighing a fixed amount of the reduced disaccharide and dissolving it in 0.5 mL of DMSO-d_6 with the addition of a fixed concentrations of

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internal standard (*O*-methyl β-D-ribofuranoside) and relaxation agent (chromium acetylacetonate). From these solutions standard curves were generated by comparing the anomeric carbon area ratios of the standard/internal standard. This ratio was found to be linear for the concentrations of standard carbohydrates studied (3–70 mg).

We extended this analysis to the identification and quantitation of the complex oligosaccharide mixture present in honey. Honey consists of a concentrated solution of two monosaccharides, D-glucose and D-fructose, which constitute approximately 95% of the honey solids. In addition, honey contains a small amount of higher sugars (oligosaccharides) found in concentrations of up to 5%.

Siddiqui and Furagala (1967, 1968) isolated and identified the minor oligosaccharides found in honey and showed that they mainly consisted of D-glucose–D-glucose and D-glucose–D-fructose disaccharides. Their analysis was accomplished employing charcoal–Celite column chromatography and was extremely laborious and time-consuming.

High-performance liquid chromatography (HPLC) was applied to the concentration of honey oligosaccharides (by the removal of the larger monosaccharide portion). Treatment of this concentrated oligosaccharide fraction to reduction (sodium borohydride) and ^{13}C NMR analysis allowed for the rapid identification and quantitation of the disaccharides present.

MATERIALS AND METHODS

Standard Disaccharides. Cellobiose (*O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose), gentiobiose (*O*-β-D-glucopyranosyl-(1→6)-D-glucopyranose), isomaltose (*O*-α-D-glucopyranosyl-(1→6)-D-glucopyranose), palatinose (*O*-α-D-glucopyranosyl-(1→6)-D-fructose), trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), and turanose (*O*-α-D-glucopyranosyl-(1→3)-D-fructose) were supplied by Sigma Chemical Co. (St. Louis, MO). Maltose (*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose) and sucrose (α-D-glucopyranosyl-β-D-fructofuranoside) were supplied by Anachemia Ltd. (Montreal, Quebec, Canada). Kojibiose (*O*-α-D-glucopyranosyl-(1→2)-D-glucopyranose) was supplied by Koch-Light Laboratories (Colnbrook Berks, England). Neotrehalose (α-D-glucopyranosyl-β-D-glucopyranoside) was a gift from Dr. I. R. Siddiqui, Agriculture Canada. Laminaribiose (*O*-β-D-glucopyranosyl-(1→3)-D-glucopyranose) was a gift from Dr. E. Reese of the U.S. Army Natich Research and Development Laboratories. Maltulose (*O*-α-D-glucopyranosyl-(1→4)-D-fructose) and nigerose (*O*-α-D-glucopyranosyl-(1→3)-D-glucopyranose) were synthesized (Hicks et al., 1983; Excoffier et al., 1976) in our laboratory.

Reagents. Sodium borohydride (BDH Chemicals), glacial acetic acid (Fischer Scientific Co.), methanol (Anachemia Ltd.), and acetyl chloride (Terochem Laboratories Ltd.) were of reagent grade. Acetonitrile was HPLC grade with a UV cutoff of 190 nm (Caledon Laboratories Ltd.).

Purification of Chromium Acetylacetonate. Chromium acetylacetonate (1 g) (Aldrich Chemical Co.) was dissolved in chloroform (10 mL) and the resultant mixture passed through a 25 mm × 8 mm column of Chelex 100 (Bio-Rad Laboratories). The product was recrystallized from this chloroform solution.

Reduction and ^{13}C NMR Preparation of Standard Disaccharides. For each reducing disaccharide, 3–70 mg was dissolved in 5 mL of distilled water. To this solution was added 50 equiv of sodium borohydride. The resulting solution was stirred at room temperature for 1 h. The reaction was neutralized by the dropwise addition of 10% glacial acetic acid. This solution was passed through a column (160 × 8 mm/or larger, depending on the concentration of sodium borohydride used) of Dowex 50W-X8 ion-exchange resin, and well eluted with distilled water. The eluent was then evaporated in vacuo (Buchi rotovapour R) and coevaporated with methanol (5 × 5 mL). The colorless oil was dissolved in 0.5 mL of deuteriated dimethylsulfoxide (DMSO-d_6) and transferred quantitatively to a 5-mm thin-walled NMR tube. To this solution was added 11.33 mg of *O*-methyl β-D-ribofuranoside (internal standard) and 2–3 mg of chromium acetylacetonate (relaxing agent). The nonreducing disaccharides were prepared as above without sodium borohydride reduction and workup.

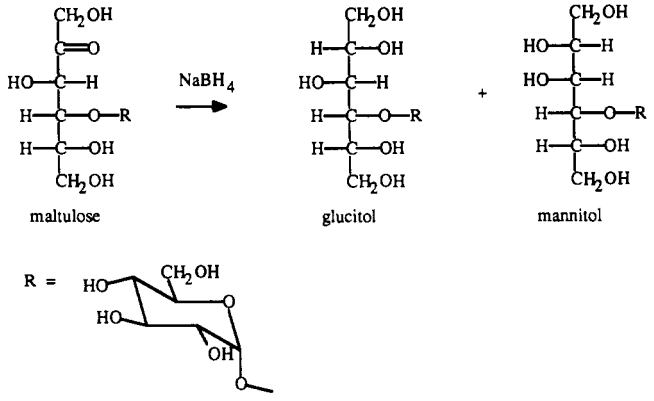
High-Performance Liquid Chromatography: Honey Oligosaccharide Isolation. In order to separate the oligosaccharides from the major monosaccharides in honey, 10 g of honey was dissolved in HPLC-grade water to a total volume of 30 mL. This solution was then passed through a Swinney-25 syringe adapter with a 0.45-μm nylon-66 membrane filter (25-mm diameter). A 6.0-mL aliquot of this solution was injected (Rheodyne Model 7120; 5-mL injector loop) onto a Whatman Partisil M 20/50 preparative HPLC column (50 cm × 18 mm) equipped with a guard column (140 mm × 11 mm) packed with CO:Pell PAC media (30–38 μm). This system was in series with a Whatman differential refractometer (Model R401) maintained at 25.5 °C by a circulating water bath. Elution of the monosaccharide fraction was accomplished with acetonitrile–water (80:20, v/v) as the mobile phase at a flow rate of 7 mL/min (Beckman Model 110A pump). Approximately 850 mL of this mobile phase was required. The mobile phase was then changed to acetonitrile–water (50:50, v/v) to elute the oligosaccharide fraction (450 mL of solution was collected). This procedure was repeated until all of the original honey solution was processed. Evaporation of the combined oligosaccharide fractions in vacuo yielded approximately 350 mg of a slightly colored syrup.

Reduction and Preparation of Honey Oligosaccharides for ^{13}C NMR Analysis. The entire 350 mg of the aforementioned honey oligosaccharide fraction was dissolved in 20 mL of distilled water. The remainder of the conditions for the reduction were the same as those previously described for the standards, except for employing a 300 mm × 25 mm column of Dowex 50W-X8 and coevaporation with 5 × 15 mL of methanol. The resulting slightly colored oil was dissolved in 0.5 mL of warmed (<40 °C) DMSO-d_6 and transferred quantitatively to a 5-mm thin-walled NMR tube.

To this solution were added 11.33 mg of the internal standard and 10–12 mg of the relaxing agent.

Synthesis of *O*-Methyl β-D-Ribofuranoside (Internal Standard). To 2 g (0.0133 mol) of D-ribose in 75 mL of reagent-grade methanol was added 1.0 mL of distilled acetyl chloride (0.0141 mol). The resulting slightly yellow solution was stirred at room temperature for 4 h and was monitored by thin-layer chromatography [Merck, Kieselgel 60 F254 plates; SSE as the solvent (upper phase of ethyl acetate–1-propanol–water, 4:1:2)] to ensure the greatest yield of the desired faster moving product. The reaction was quenched by adding 5 mL of Dowex 1-X2 (OH^-) ion-exchange resin and stirred until neutral by pH paper. The resin was filtered and the solution removed in vacuo (Buchi rotovapour R). The resulting yellowish oil was dissolved in the minimum amount of water and placed on a Dowex 1-X2 (OH^-) ion-exchange resin column (2 × 20 cm), and the minor products were removed by

Scheme I. Products of Maltulose Reduction



elution with water. The major product, *O*-methyl β-D-ribofuranoside, was eluted with 3% methanol–water. Evaporation of the solvent followed by crystallization from ethanol–water yielded 1.57 g (72%) of white needlelike crystals, mp 77–79 °C [lit. mp 79–80 °C (Bishop and Cooper, 1963)]. Anal. Calcd for $C_6H_{12}O_5$: C, 43.90; H, 7.37. Found: C, 43.65; H, 7.42.

^{13}C NMR Analysis. ^{13}C NMR analysis was carried out on a Bruker WH 400 NMR spectrometer operating in the FT mode. Accumulation times varied from 20 min to 10 h to account for variations in the oligosaccharide concentration. The acquisition time and repetition rate was 0.5571 s; the sweep width was 29 411.765; the pulse angle was 90°; the number of data points was 32K; the temperature of the sample was maintained at 297 K; the solvent employed was DMSO-d_6; and the relaxation reagent was chromium acetylacetonate. Zero filling was carried out to ensure that at least five points were employed for each peak. Areas of peaks were determined by instrumental integration and triangulation of the expanded anomeric region.

RESULTS AND DISCUSSION

Complete ^{13}C NMR data on glucobioses and glucotrioses as well as fructose containing di- and trisaccharides are known (Bock and Pedersen, 1983; Bradbury and Jenkins, 1984; Usui et al., 1973). From these data it became obvious that a "fingerprint" region exists for the anomeric carbons of these oligosaccharides in the region 90–110 ppm.

Reducing sugars differ from most other organic compounds in one characteristic property. When a reducing sugar is dissolved in a solvent, the solution can contain up to six compounds. Angyl (1984) noted two pyranoses, two furanoses, and the acyclic carbonyl form and its hydrate.

Sodium borohydride reduction of the hemiacetal form greatly simplifies the analysis by ^{13}C NMR. For example, analysis of maltose by ^{13}C NMR indicates that three peaks appear in the anomeric carbon region (90–110 ppm): C1′ (100.8), C1α (93.1), and C1β (97.1). Reduction of maltose with sodium borohydride yields only one peak, C1′ (100.8), for this compound.

Reduction of fructose-containing reducing disaccharides (such as maltulose, palatinose, and turanose) resulted in two peaks appearing in the anomeric carbon region. Quantitation of these three disaccharides in the presence of the three glucose-containing reducing disaccharides (maltose, nigerose, isomaltose) with which they coincided could pose a problem. In order to alleviate this problem, maltulose, palatinose, and turanose were each reduced at three different concentrations and analyzed. The ratio of *O*-α-D-glucopyranosyl-(1→4)-D-glucitol to *O*-α-D-glucopyranosyl-(1→4)-D-mannitol for maltulose was 1:1 (see Scheme I). For palatinose, the *O*-α-D-glucopyranosyl-(1→6)-D-glucitol to *O*-α-D-glucopyranosyl-(1→6)-D-mannitol ratio was 1:1. For turanose, the *O*-α-D-glucopyranosyl-(1→2)-D-glucitol to *O*-α-D-glucopyranosyl-(1→2)-mannitol ratio was 1:2. With this information, maltulose, maltose, nigerose, turanose, palatinose, and isomaltose could be successfully quantitated.

Table I. Standard Curves for Disaccharide Standards after 1000 Scans

disaccharide	slope[a]	lin correln coeff	reson used, ppm
trehalose	0.94	0.996	92.93
sucrose	1.94	0.999	103.87
maltose (reduced)	1.77	0.984	100.77

[a] Milligrams of oligosaccharide/milliliter of DMSO-d_6 per area units relative to internal standard, *O*-methyl β-D-ribofuranoside (see the Experimental Section).

Sucrose, trehalose, and reduced maltose were used to prepare standard curves in the initial investigation for the applicability of ^{13}C NMR spectroscopy for quantitative analysis. Relaxation delay times from 0 to 6 s were employed, and concentrations ranging from 3 to 70 mg were used. By employing these techniques (with both D_2O and DMSO-d_6 as the solvent), deviations in the standard curves of 10–30% were quite common.

We investigated the use of the paramagnetic relaxation reagent (PARR) chromium acetylacetonate ($Cr(acac)_3$) at various concentrations in our experiments and found that if the amount of relaxing agent added to the sample was greater than 2 mg and less than 6 mg/70 mg of carbohydrate, then deviations in our standard curves could be reduced to 1–2% if less than 2 mg of PARR/70 mg of carbohydrate was used. Insufficient addition of PARR (less than 2 mg/70 mg of carbohydrate) resulted in standard deviation of 10–30% in standard curves due to incomplete relaxation of the anomeric carbons before the next pulses were applied. If the addition of PARR of greater than 6 mg/70 mg of carbohydrate was employed, then peaks broadened and loss of resolution occurred.

The addition of a relaxation reagent resulted in normalization of the NOE, which was important in quantitative measurements. The addition of the relaxation reagent caused spin–lattice relaxation to be more efficient and therefore enabled pulsing more frequently. If no relaxation reagent were used, it would be necessary to wait 5–6T_1's (or to employ the gating technique for quantitation) between pulses. Each pulse required 0.5571 s; therefore, it was possible to acquire at least 11 times the number of scans in an experiment with a PARR. Coxon (1980) indicated that an experiment with a PARR required nine times as many scans as one without to obtain the same signal-to-noise ratio [$(2.98)^2$]. Though this enhancement was lost by addition of the relaxing agent, the signal-to-noise ratio could be enhanced to better than original levels because of the increased number of scans possible (due to T_1 being shorter).

By employing $Cr(Acac)_3$, an internal standard (*O*-methyl β-D-ribofuranoside), and deuteriated dimethyl sulfoxide, standard curves (concentration of oligosaccharide in grams per milliliters of DMSO-d_6 versus area units relative to the internal standard) were obtained for trehalose, sucrose, and reduced maltose (Table I). Note that areas were measured relative to the total area for the internal standard, 11.33 mg of *O*-methyl β-D-ribofuranoside (i.e., the area for the sample was divided by the internal standard area). Linear relationships were found for each of these oligosaccharides for the concentrations studied (3–70 mg). In addition, this linear relationship was maintained when any of these

Table II. Assignments of the ^{13}C Resonance of the Anomeric Carbon(s) of Disaccharides (Reduced Where Applicable)

disaccharide	chem shift, ppm	disaccharide	chem shift, ppm
O-methyl β-D-ribo-furanoside	108.53	maltose	100.77
		turanose	100.32
			99.57
laminaribiose	104.30	nigerose	99.59
sucrose	103.87	kojibiose	99.20
	91.64	palatinose	98.70
gentiobiose	103.61		98.61
cellobiose	103.59	isomaltose	98.62
neotrehalose	103.56	trehalose	92.93
	100.68		
maltulose	100.79		
	99.56		

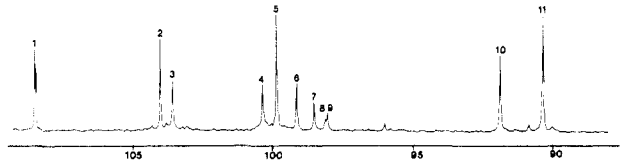


Figure 1. ^{13}C NMR spectrum of the anomeric carbon region for some reduced (where applicable) disaccharides: 1, internal standard (*O*-methyl β-D-ribofuranoside; 108.53 ppm chemical shift); 2, sucrose; 3, gentiobiose; 4, maltose; 5, turanose; 6, turanose/nigerose; 7, kojibiose; 8, palatinose; 9, isomaltose/palatinose; 10, trehalose; 11, sucrose.

standards were mixed. Analysis of five identical samples of reduced cellobiose and reduced maltose under the same conditions produced standard deviations of 0.44% and 0.51%, respectively. Since the concentration of oligosaccharides varies in food products, a check on the sensitivity of this method was carried out by subjecting reduced maltose to analysis from 500 to 60000 scans. In this scanning range there was a linear relationship between the number of scans and the peak area for the anomeric carbon of reduced maltose. Therefore, the sensitivity of this analytical method could be increased by increasing the number of scans.

The results of these experiments lead to the conclusion that ^{13}C NMR analysis employing a relaxation reagent, an internal standard, and DMSO-d_6 would allow for reproducible qualitative and quantitative determination of oligosaccharides. A typical ^{13}C NMR spectrum (anomeric carbon region) of a mixture of disaccharides is shown in Figure 1. Table II contains the chemical shift(s) of the anomeric carbon(s) for each of the 13 disaccharides analyzed. The standard deviations associated with each of the chemical shifts is ±0.02 ppm.

This technique was applied to the identification of the oligosaccharides found in two honeys from our geographical location (Alberta, Canada). In addition, these honey oligosaccharides were analyzed both qualitatively and quantitatively by capillary gas chromatography (Low and Sporns, 1988). Comparisons of the results obtained for each of these honeys by each method are shown in Tables III and IV. The results obtained by these two techniques showed a good correlation.

Limitations of this methodology were realized when we started to examine the ^{13}C NMR spectra of a number of trisaccharides. Since the positions and types of linkages of the monosaccharide units in trisaccharides were the same as in the disaccharides, many of the ^{13}C chemical shifts of the anomeric carbons were very close to the disaccharide values. Therefore, spectra containing comparable amounts of similarly linked di- and trisaccharides would present increasingly difficult problems in resolution. Gas chromatographic analysis of the oligosaccharides present in each of the honeys analyzed indicated that the percentages of trisaccharides present in honey were much smaller than the disaccharide amounts. Although the trisaccharides present in honey would not have had a large influence on the final disaccharide results obtained by ^{13}C NMR, they could have accounted for some of the variation in results obtained when comparing these methods.

Table III. Comparison of Disaccharide Ratios in Alfalfa Honey, Determined by ^{13}C NMR with Comparison to Gas Chromatographic Determination

disaccharide	^{13}C NMR[a]	GC[a]
maltose	1.0	1.0
sucrose	3.2	3.9
kojibioise	0.58	0.25
turanose	0.41	0.46
palatinose	0.36	0.39
gentiobiose	0.13	0.15
neotrehalose	0.11	0.10
nigerose	0.10	0.12
isomaltose	0.10	0.11

[a] All values are given relative to the maltose concentration set at 1.

Table IV. Comparison of Disaccharide Ratios in Sweet Clover Honey, Determined by ^{13}C NMR with Comparison to Gas Chromatographic Determination

disaccharide	^{13} NMR[a]	GC[a]
maltose	1.0	1.0
turanose	0.96	1.23
palatinose	0.68	0.77
kojibiose	0.58	0.64
sucrose	0.29	0.26
neotrehalose	0.19	0.22
gentiobiose	0.19	0.36
nigerose	0.19	0.21
isomaltose	0.16	0.19

[a] All values are given relative to the maltose concentration set at 1.

The ^{13}C NMR method as applied to the identification of minor honey disaccharides had advantages over other techniques in that the method was relatively rapid and allowed for the complete identification and quantitation of the disaccharides present. The analysis of the sugars in honey represented an extremely complicated case for ^{13}C NMR analysis because of the number of oligosaccharides present, their relatively low concentrations, and their very similar structures. It is our opinion that in other food products where the number, concentration, and complexity of the carbohydrates present are less, greater, and lower, respectively, the type of methodology we have reported could be very useful.

ACKNOWLEDGMENT

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Registry No. Chromium acetylacetonate, 21679-31-2; trehalose, 99-20-7; sucrose, 57-50-1; maltose, 69-79-4; kojibiose, 2140-29-6; turanose, 547-25-1; palatinose, 13718-94-0; gentiobiose, 554-91-6; neotrehalose, 585-91-1; nigerose, 497-48-3; isomaltose, 499-40-1; laminaribiose, 34980-39-7; *O*-methyl β-D-ribofuranoside, 7473-45-2; maltulose, 17606-72-3.

LITERATURE CITED

Angyl, S. J. "The Composition of Reducing Sugars in Solutions". *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 15–68.

Berry, J. M.; Hall, L. D.; Wong, K. F. "Concerning Tumbling Motion of Disaccharides in Aqueous Solution". *Carbohydr. Res.* **1977**, *56*, C16–C20.
Bishop, C. T.; Cooper, F. P. "Methanolysis of D-Xylose, D-Arabinose, D-Lyxose and D-Ribose". *Can. J. Chem.* **1963**, *41*, 2743–2758.
Blunt, J. W.; Munro, H. G. "An Automated Procedure for Qualitative and Quantitative Analysis of Mixtures by Means of Carbon Magnetic Resonance Spectroscopy: Applications to Carbohydrate Analysis". *Aust. J. Chem.* **1976**, *29*, 975–986.
Bock, K.; Thoegersen, H. "Nuclear Magnetic Resonance Spectroscopy in the Study of Mono- and Oligosaccharides". *Annu. Rep. NMR Spectrosc.* **1982**, *13*, 1–57.
Bock, K.; Pedersen, C. "Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Monosaccharides". *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.
Bradbury, J. H.; Jenkins, G. A. "Determination of the Structures of Trisaccharides by ^{13}C-N.M.R. Spectroscopy". *Carbohydr. Res.* **1984**, *126*, 125–156.
Cerbulis, J.; Pfeffer, P. E.; Farrell, H. M., Jr. "Reaction of Lactose with Urea". *Carbohydr. Res.* **1978**, *65*, 311–313.
Coxon, B. "Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Food-related Disaccharides and Trisaccharides". *Dev. Food Carbohydr.* **1980**, *2*, 351–390.
Czarniecki, M. F.; Thornton, E. R. "C-13 Nuclear Magnetic Resonance Spin–Lattice Relaxation in *N*-Acylneuraminic Acids: Probes for Internal Dynamics and Conformational Analysis". *J. Am. Chem. Soc.* **1977**, *99*, 8273–8279.
Excoffier, G.; Gagnaire, D. Y.; Vignon, M. R. "Synthesis of Oligosaccharides on Polymer Supports. VIII. Synthesis of Nigerose and Laminaribiose on Polymer Supports". *Carbohydr. Res.* **1976**, *51*, 280–286.
Freeman, R.; Hill, H. D. W.; Kaptein, R. "Proton-decoupled NMR Spectra of Carbon-13 with the Nuclear Overhouser Effect Suppressed". *J. Magn. Reson.* **1972**, *7*, 327–329.
Gorin, P. A. J. "Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Polysaccharides". *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 13–104.
Hicks, K. B.; Symanski, E. V.; Pfeffer, P. E. "Synthesis and High-Performance Liquid Chromatography of Maltulose and Cellobulose". *Carbohydr. Res.* **1983**, *112*, 37–50.
Inch, T. D. "Nuclear Magnetic Resonance Spectroscopy in the Study of Carbohydrates and Related Compounds". *Annu. Rep. NMR Spectrosc.* **1972**, *5A*, 305–352.
Levy, G. C.; Nelson, G. L. *Carbon-13 Nuclear Magnetic Resonance for Chemists*; Wiley-Interscience: New York, 1972.
Low, N. H.; Sporns, P. "Analysis and Quantitation of Minor Di- and Trisaccharides in Honey, Using Capillary Gas Chromatography". *J. Food Sci.* **1988**, *53*, 558–561.
Pfeffer, P. E. "High Resolution Solid ^{13}C NMR and Its Applications in Carbohydrate Chemistry". *J. Carbohydr. Chem.* **1984**, *3*, 613–639.
Rathbone, E. B. *Analysis of Food Carbohydrates*; Elsevier Applied Science: London, 1985.
Siddiqui, I. R.; Furagala, B. "Isolation and Characterization of Oligosaccharides From Honey. Part I. Disaccharides". *J. Apic. Res.* **1967**, *6*, 139–145.
Siddiqui, I. R.; Furagala, B. "Isolation and Characterization of Oligosaccharides From Honey. Part II. Trisaccharides". *J. Apic. Res.* **1968**, *7*, 51–59.
Tamate, J.; Bradbury, J. H. "Determination of Sugars in Tropical Root Crops Using ^{13}C N.M.R. Spectroscopy: Comparison with H.P.L.C. Method". *J. Sci. Food Agric.* **1985**, *36*, 1291–1302.
Usui, T.; Yamaoka, N.; Matsuda, K.; Tuzimura, K.; Sugiyama, H.; Seto, S. "C-13 Nuclear Magnetic-resonance Spectra of Glucobioses, Glucotrioses and Glucans". *J. Chem. Soc., Perkin Trans. 1* **1973**, *20*, 2425–2432.
Vliegenthart, J. F. G.; Dorland, L.; Van Halbeek, H. "High-resolution Proton Nuclear Magnetic Resonance Spectroscopy as a Tool in the Structural Analysis of Carbohydrates Related to Glycoproteins". *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 209–374.
Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 N.M.R. Spectra*; Heyden: London, 1976.

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Rapid Liquid Chromatographic Analysis of Chlorothalonil in Fresh Produce Using Photoconductivity and UV Detectors in Tandem

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Liquid chromatography with a photoconductivity (PC) detector connected in tandem with a UV detector was evaluated for determining chlorothalonil residues in extracts of strawberries, tomatoes, and sweet and sour cherries. Samples were prepared by a rapid, multiresidue screening procedure consisting of acetone extraction, partitioning into petroleum ether plus methylene chloride, and concentrating. Sensitivity and selectivity of the PC detector were found adequate for reliable quantitation of residues at levels both above and well below those of current regulatory concern. UV detection was more prone to interferences at lower levels but served as a useful monitor of the chromatographic system and provided additional data. Recoveries were essentially complete from samples fortified at 0.05–5 ppm. Results for samples with field-incurred residues were in reasonable agreement with those previously determined by gas chromatography.

Chlorothalonil is a broad-spectrum fuungicide used on fruits, vegetables, and other agricultural products. Recovery of chlorothalonil by the rapid, multiresidue method of Luke et al. (1981), using gas chromatography (GC) for determination, has been reported. This widely used method, which eliminates adsorption chromatography cleanup (i.e., Florisil) for relatively polar compounds such as chlorothalonil, results in the injection of considerable coextracted sample material. Selective detectors are therefore required for reliable quantitation of residues in these crude extracts. Variable GC responses due to sample matrix effects on chromatography are commonly encountered, however.

In the present study, liquid chromatography (LC) with photoconductivity (PC) detection was investigated for the

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