Evidence for the Formation of Glucose (Not Sucrose) in the Metabolism of Germinating Sunflower Seeds†

Raimundo Santos do Nascimento‡ and Peter Rudolf Seidl*,§

Instituto Militar de Engenharia, Praça General Tibúrcio 80, 22.290 Rio de Janeiro, Brazil

Robin K. Harris

Department of Chemistry, University of Durham, South Road, Durham, Great Britian DH1 3LE

The application of carbon-13 nuclear magnetic resonance to studies of intact seeds has provided a technique for direct observation and determination of chemical constituents in living matter. A reexamination of products formed during the germination of sunflower, castor bean, and peanut seeds revealed that for the latter two sucrose is the main product from metabolism of germinating seeds, as expected. In the case of sunflower seeds, however, precise assignment of absorptions of individual carbon atoms reveals the presence of glucose and not sucrose, as was assumed from classical studies on mechanisms of germinating seed metabolism (this discrepancy is probably due to processes that occur during the extraction and labeling required for tracer studies). The results emphasize the quality of carbon-13 NMR spectra which can be obtained for mobile components of intact seeds during germination using a standard Fourier transform spectrometer.

INTRODUCTION

Our previous work has shown (Colnago and Seidl, 1983, 1983–1984; Colnago, 1983) that ^{13}C NMR spectroscopy can be used to follow the germination of soybean, castor bean, and peanut seeds. The disappearance of triglycerides and the appearance and subsequent disappearance of sugars could be monitored by following the relative intensity (measured by signal-to-noise ratios) of their respective peaks. These could be correlated with biological changes and were in good agreement with results from other techniques.

We have now taken a closer look at the sugars that are formed during the germination of sunflower, castor bean, and peanut seeds. Although work at higher field (5.87 T instead of the 1.88 T used in the original work) confirms the presence of sucrose as the main sugar in the seed during germination of peanut and castor bean seeds, this is not the case for sunflower seeds. Glucose and not sucrose, as was originally proposed (Bradbeer and Stumpf, 1959) and recently confirmed (Rutar, 1989), was found in germinating sunflower seeds.

EXPERIMENTAL PROCEDURES

Commercial sunflower, castor bean, and peanut seeds were treated with a 10% sodium hypochlorite solution and placed in a germinator with automatic temperature control (25 °C), in the absence of light. Conversion of triglycerides to sugars was followed by placing the germinating seeds in 10-mm NMR tubes and adjusting their position for best resolution. Detailed spectral analyses were run on samples in which sugar concentration reaches a maximum. These are 4.5–5.5 days for sunflower, 6.5–7.5 days for peanut, and 6 days for castor bean.

The NMR spectra were run on a Bruker AC 250 spectrometer operating in the FT mode. All ^{13}C chemical shifts quoted are given in parts per million with respect to the signal of tetramethylsilane (TMS) by referencing the instrument to deuterated chloroform at 77 ppm and replacing the sample tube.

Operating parameters were chosen to optimize sensitivity for seed spectra, which resulted in the choice of a 0.135168-s acquisition time, a 0.5-s pulse delay, and a pulse duration of 4 μs. The number of transients varies according to the sample and is given for Figures 1–3.

RESULTS AND DISCUSSION

The analysis of sugars in aqueous solution is complicated by the fact that in this medium sugars may assume different structures. Glucose, one of the most frequently encountered sugars, for example, can be found in its α-D or β-D forms. Fructose, the other ring in sucrose, can cyclize as α- or β-D-furanoses or pyranoses, forming four different structures (Figure 4).

There seems to have been some discussion as to the assignments of chemical shifts of these sugars (Morris and Hall, 1981; Breitmayer and Voelter, 1978; Pfeffer *et al.*, 1979). We assumed the most recent publication (Morris and Hall, 1981) to be the correct one.

A large number of peaks fall in the region of interest between 105 and 60 ppm. Chemical shifts vary according to conditions, and it would be difficult to introduce a standard during germination, so differences between shifts of a given sugar were used to locate and assign the corresponding peaks. This approach is exemplified for the interpretation of the spectrum of aqueous glucose. Table 1 compares the assignments used in the present work with those found in the literature. Figure 5 shows the differences in chemical shifts that should be observed when these structures are present. Thus, α-glucose should show an absorption that is 19.3 ppm to low frequency of the peak at 92.9 ppm, another one at 1.3 ppm from that one, and so on. A similar approach was used for the identification of the other structures of interest (Tables

† This work was reported at the 203rd National Meeting of the American Chemical Society, San Francisco, CA, April 1992 (AGFD 090).

‡ Present address: Departamento de Química, Universidade do Amazonas, Estrada do Contorno, Campus Universitário, 69.000 Manaus, Amazonas, Brazil.

§ Present address: Centro de Tecnologia Mineral-CETEM/CNPq, Cidade Universitária, 21.941/590 Rio de Janeiro, Brazil.

0021-8561/94/1442-0882$04.50/0

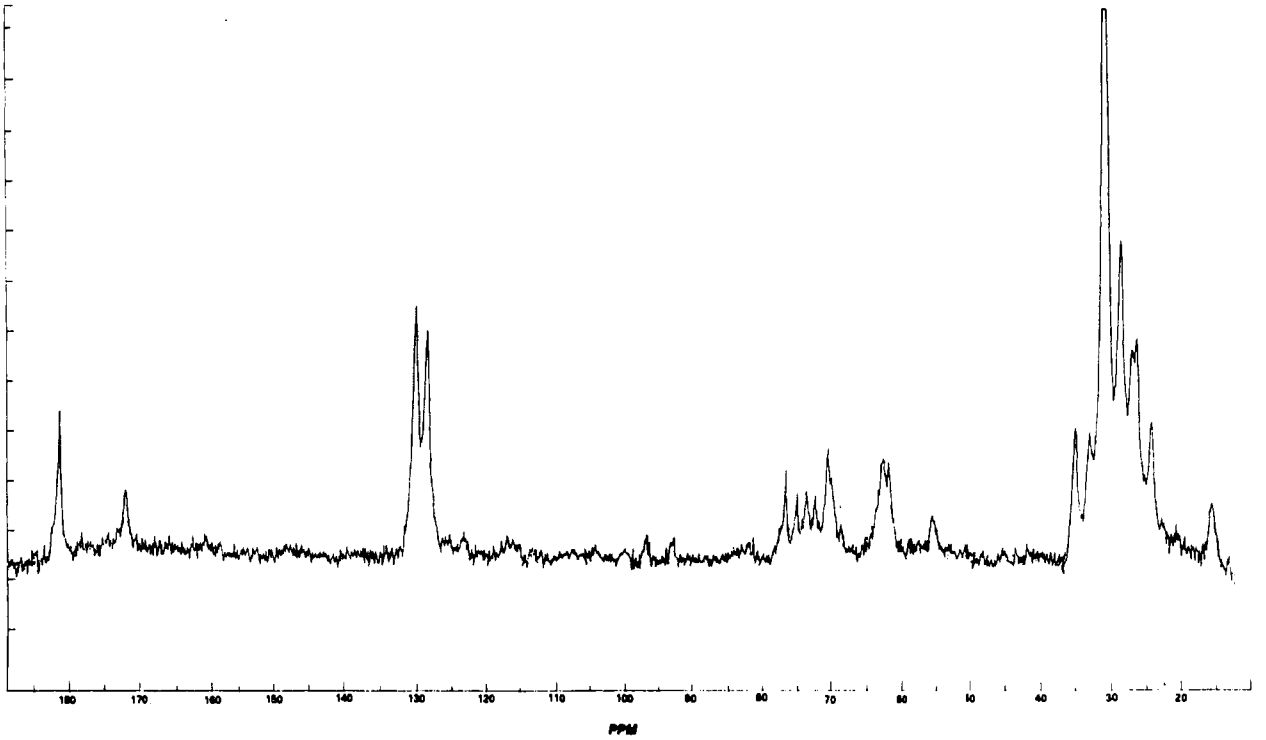

Figure 1. ^{13}C NMR spectrum of germinating sunflower seeds obtained after 50 000 transients.

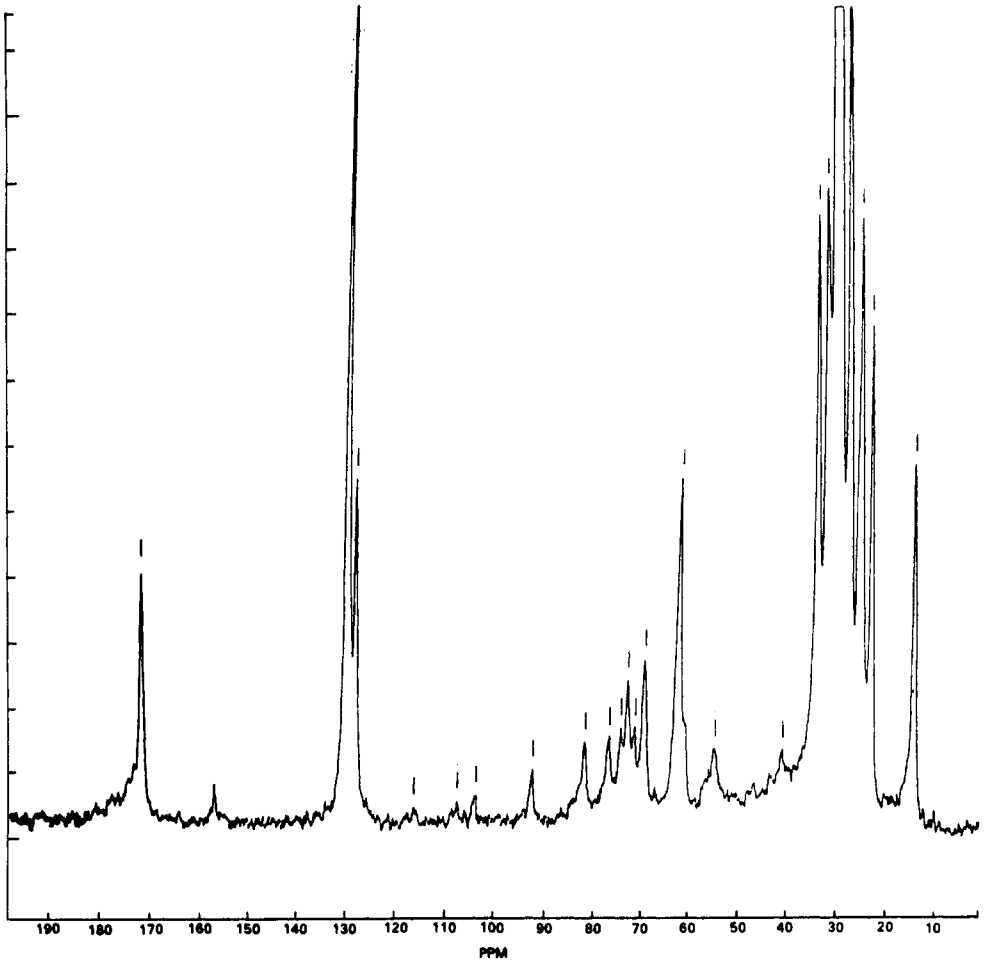

Figure 2. ^{13}C NMR spectrum of germinating peanut seeds obtained after 1964 transients.

2 and 3). This procedure can be applied to shifts reported in the literature (Morris and Hall, 1981; Breitmayer and Voelter, 1978; Pfeffer *et al.*, 1979) to verify that most variations in these differences are under 0.5 ppm. When more than one sugar of interest is present, differences between their respective shifts can be used for the same purpose (see, for example, $\Delta\delta$ between α-D- and β-D-glucose in Figure 5).

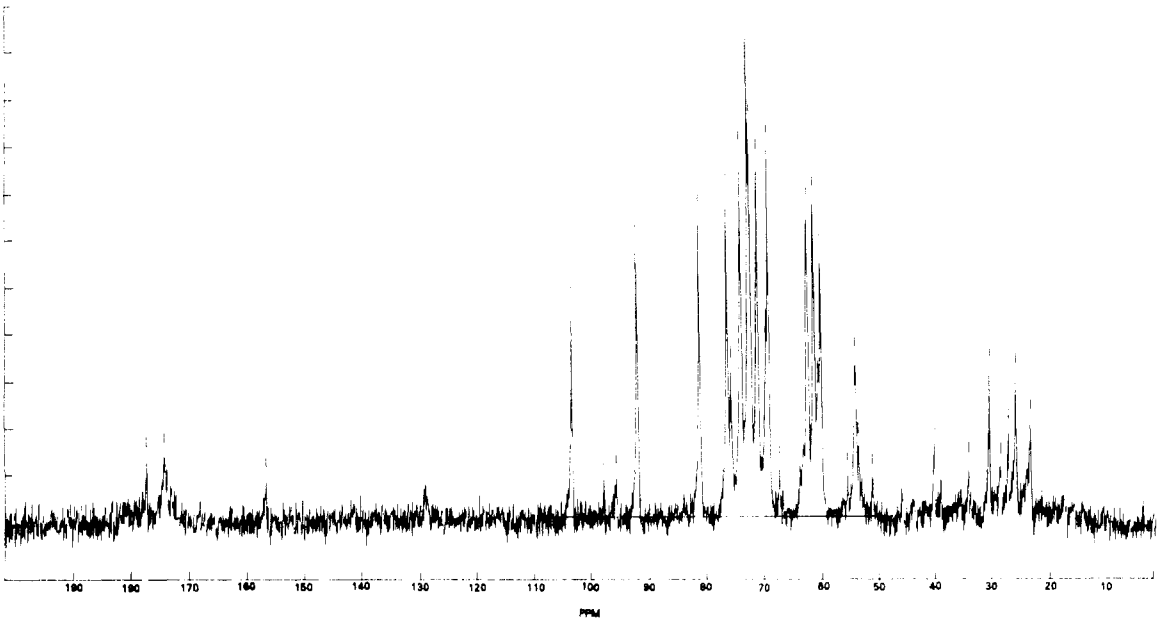

Figure 3. ^{13}C NMR spectrum of sliced sunflower seedling extracts after 7 h in water obtained after 2620 transients.

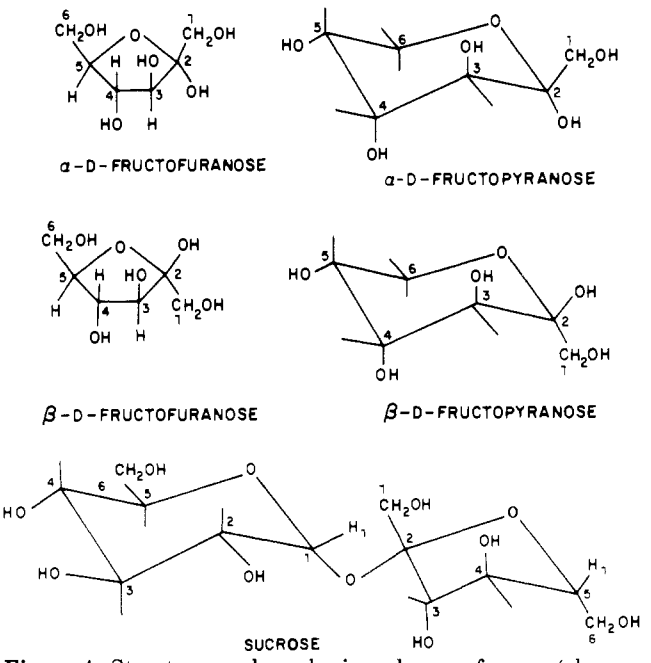

Figure 4. Structures and numbering schemes of sugars (glucose is the same as the corresponding subunit of sucrose).

Table 1. Carbon-13 Chemical Shifts of α-D- and β-D-Glucoses

	C1	C2	C3	C4	C5	C6
α						
Morris	92.89	72.28	73.58	70.44	72.24	61.40
Breitmayer	91.85	71.25	72.55	69.40	71.25	60.40
Pfeffer	92.94	72.47	73.75	70.56	72.28	61.59
this work	91.30	70.65	71.98	68.79	70.65	59.90
β						
Morris	96.71	74.94	76.74	70.40	76.56	61.57
Breitmayer	95.70	73.90	75.55	69.40	75.55	60.60
Pfeffer	96.74	75.14	76.71	70.60	76.58	61.74
this work	95.08	73.34	74.96	68.79	74.96	59.90

Analysis of dormant species revealed the expected peaks for the triglycerides in sunflower (oleic and linoleic acid esters), castor bean (mostly ricinoleic), and peanut (oleic and linoleic) seeds. The corresponding interpretation is given in Table 4.

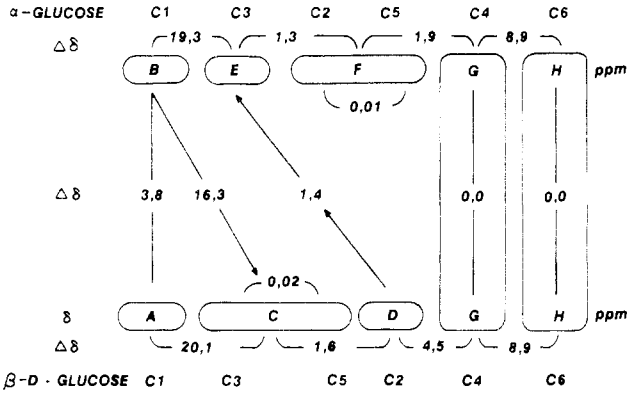

Figure 5. ^{13}C chemical shifts for α and β forms of glucose and their respective differences.

Table 2. Experimental Values for Carbon-13 Chemical Shifts of Fructoses

carbon	α-furanose	β-furanose	β-pyranose
C1	64.99	62.49	63.67
C2	104.12	101.18	97.76
C3	81.73	75.15	67.33
C4	75.77	74.18	69.45
C5	80.93	80.30	68.94
C6	60.87	62.14	63.01

Table 3. Chemical Shift Differences for the More Commonly Found Sugars in Germination of Seeds

α-glucose		β-glucose		β-fructopyranose	
carbons	Δδ	carbons	Δδ	carbons	Δδ
C1–C3	19.3	C1–C3	20.3	C2–C4	28.3
C3–C2; C5	1.3	C3; C5–C2	1.6	C4–C5	0.5
C5–C4	1.9	C2–C4	4.5	C5–C3	1.6
C4–C6	9.0	C4–C6	8.9	C3–C1	3.7

When conversion to sugars reaches its maximum, the different sugars present can be identified. Germinating sunflower seeds reveal peaks at 96.6, 92.6, 76.4, 74.8, 73.4, 72.0, 70.2, and 61.3 ppm (Figure 1). These can be assigned to α- and β-glucose and checked by recourse to Table 3 and Figure 1. There are other less intense peaks that can be ascribed to other metabolites that are formed in the biological processes.

The possibility of distinguishing between α- and β-glucose and sucrose by NMR can be verified by comparison

Table 4. Assignment of ^{13}C Spectra for Sunflower, Castor Bean, and Peanut Seeds

group[a]	sunflower	peanut	castor bean
RCOOR'	172.38	171.63	172.73
C9H(r)			131.47
C9H,C10H(o) and	130.37	129.68	129.95
C9H,C13H(l)	130.37	129.68	129.95
C10H C12H(l)	128.64	128.08	128.00
C10H(r)			126.52
C12HOH(r)			71.34
CHOH(g)	69.77	69.08	69.48
CH2OH(g)	62.50	61.94	62.30
CH2(t)	34 to 23	33 to 22	37 to 23
CH3(m)	14.67	14.09	14.36

[a] Obs. (o), oleic acid; (l), linoleic acid; (r), ricinoleic acid; (g) glycerol; (t), remaining methylenes of the fatty acid chains; (m), terminal methyl of the fatty acid chains.

of spectra of germinating sunflower and peanut seeds. Only sucrose is found in germinating peanut seeds, as can be verified by interpretation of its spectrum (Figure 2) which shows peaks at 104.2, 92.8, 82.1, 77.1, 74.7, 73.3, 71.8, 69.7, and 62.4 ppm.

The transformation of triglycerides into sugars was studied by carbon-14 tracers around 30 years ago (Bradbeer and Stumpf, 1959; Beevers, 1961). Acetate labeled at C-1 and C-2 was fed to excised seedlings, and its incorporation into products resulting from enzyme activity was followed. Sucrose was identified as the main product in experiments run on sunflower, castor bean, and peanut seeds.

A possible source of this discrepancy would be the fact that for NMR observation the seed remains undisturbed, whereas it is necessary to interrupt the process and extract the soluble enzyme systems for tracer studies. To verify the influence of procedures employed in the original work (Bradbeer and Stumpf, 1959) on final sugar composition, 5-day-old sunflower seedlings were finely sliced and left in distilled water for 7 h, the approximate time it takes for incorporation of labeled compounds, before extraction.

Absorptions at 103.8, 92.4, 81.5, 76.7, 74.3, 72.9, 72.6, 71.4, 69.5, 62.7, 61.7, 61.7, and 60.4 ppm (Figure 3) confirm the presence of sucrose in samples treated this way. It is interesting to point out that less intense absorptions are found at 98.5, 96.2, and 76.1 ppm, which would correspond, respectively, to C2 of β-D-fructopyranose and C1 and C3/C5 of β-D-glucose, potential metabolites in the formation of sucrose (the remaining absorptions of these sugars would coincide with those of sucrose).

It is thus apparent that NMR was able to distinguish between sugars that are taking part in biological processes and those that were isolated for other studies. It is possible that closer scrutiny of older work by nondestructive techniques will lead to a more precise description of other metabolic pathways as well.

ACKNOWLEDGMENT

A doctoral fellowship for R.S.N. was provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (work in Brazil) and the Cordenação de Aperfeiçoamento de Pessoal de Nível Superior—CAPES (work in the U.K.).

LITERATURE CITED

Beevers, H. Metabolic Production of Sucrose from Fat. *Nature* **1961**, *191*, 433.

Bradbeer, C.; Stumpf, P. K. Fat Metabolism in Higher Plants XI. The Conversion of Fat into Carbohydrate in Peanut and Sunflower Seedlings. *J. Biol. Chem.* **1959**, *234*, 498.

Breitmayer, E.; Voelter, W. *^{13}C NMR Spectroscopy: Methods and Applications in Organic Chemistry*; Verlag Chemie: New York, 1978; p 261.

Colnago, L. A. Analysis of Seeds by ^{13}C NMR. D.C. Thesis, Instituto Militar de Engenharia, Brazil, 1983.

Colnago, L. A.; Seidl, P. R. Application of Carbon-13 Nuclear Magnetic Resonance to the Germination of Soybean Seeds in Vivo. *J. Agric. Food Chem.* **1983**, *31*, 459.

Colnago, L. A.; Seidl, P. R. Analysis of Germinating Castor Bean and Peanut Seeds by ^{13}C NMR. *An. Assoc. Bras. Quim.* **1983–1984**, *34/35 (1–4)*, 133.

Morris, G. A.; Hall, L. D. Experimental Chemical Shift Correlation Maps from Heteronuclear Two-Dimensional NMR Spectroscopy. 1. Carbon-13 and Proton Chemical Shifts of Raffinose and Its Subunits. *J. Am. Chem. Soc.* **1981**, *103*, 4703.

Pfeffer, P. E.; Valentine, M. K.; Parrish, W. F. Deuterium-Induced Differential Isotope Shift ^{13}C NMR. 1. Resonance Reassignments of Mono- and Disaccharides. *J. Am. Chem. Soc.* **1979**, *101*, 1265.

Rutar, V. J. Magic Angle Sample Spinning NMR Spectroscopy of Liquids as a Nondestructive Method for Studies of Plant Seeds. *J. Agric. Food Chem.* **1989**, *37*, 67.

Received for review June 7, 1993. Revised manuscript received November 29, 1993. Accepted December 9, 1993.[⊛]

[⊛] Abstract published in *Advance ACS Abstracts*, January 15, 1994.