ELSEVIER

Carbohydrate Research 270 (1995) 71–75

CARBOHYDRATE RESEARCH

Note

A new synthesis of sucrose 6′-phosphate

K.B. Kim, E.J. Behrman *

Department of Biochemistry, The Ohio State University, Columbus, OH 43210, USA

Received 20 September 1994; accepted 30 November 1994

Keywords: Sucrose 6′-phosphate; 2,1′ : 4,6-Di-*O*-isopropylidenesucrose

Sucrose 6′-phosphate (**3**) is the key intermediate for sucrose (**1**) synthesis in plants [1]. It has recently become commercially available at ca. $1500/g (Sigma). The only chemical synthesis is that of Buchanan et al. [2]. This six-step procedure, while unambiguous, gives an overall yield of only ca. 6%. We describe here a simplified route (2 steps) with an unoptimized yield of ca. 15%. Our strategy was to use a phosphorylation reagent selective for primary hydroxyl groups and thus to avoid the necessity for blocking all of the secondary ones. Sowa and Ouchi [3] described a suitable system which they used very effectively for the synthesis of 5′-nucleotides from unprotected nucleosides. We applied this reagent to 2,1′ : 4,6-di-*O*-isopropylidenesucrose (**2**) [4] in which the only unprotected primary hydroxyl group is that at the 6′-position (Scheme 1).

The identity of the product was established by comparison of its rotation with the literature value and by the correspondence of its ^{1}H and ^{13}C NMR spectra with those of an authentic sample synthesized by Buchanan's method (Sigma). ^{1}H assignments were made with the help of the assignments of du Penhoat et al. [5] for sucrose and the results of a one-bond H–C COSY experiment (Fig. 1). The ^{13}C spectrum showed that all of the resonances were shifted downfield by ca. 0.5 ppm as compared with sucrose [6] except for the C-5′ doublet which was shifted upfield by 0.5 ppm and the C-6′ doublet which was shifted downfield by 2.2 ppm (Table 1).

An abstract of this work has appeared [7].

* Corresponding author.

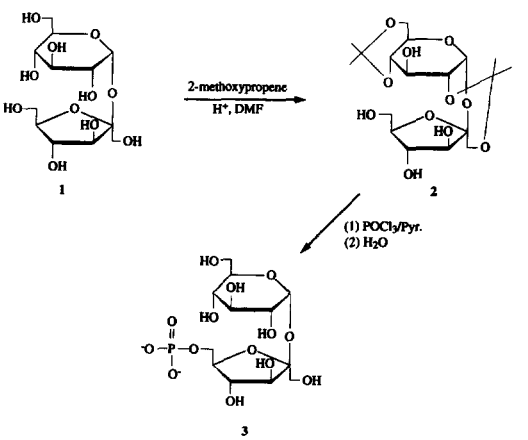
Elsevier Science Ltd.

SSDI 0008-6215(94)00003-4

Experimental

TLC was performed on Silica Gel 60 T6895 glass plates (Sigma) with detection by charring with 5% H_2SO_4 in EtOH. Column chromatography was performed on Aldrich Silica Gel (Merck, grade 60) using a 27 × 2.3 cm column. Evaporations were carried out by using a Rotavapor R110 (Büchi) under reduced pressure. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. ^1H NMR spectra were recorded at 500.1 MHz (Bruker AM-500). The ^{13}C NMR spectra were recorded at 125.8 MHz (Bruker AM-500) and ^{31}P spectra at 121.5 MHz (Bruker MSL-300). Paper electrophoresis was performed on Schleicher & Schuell Paper No. 589 (White Ribbon) with 0.05 M NH_4OAc buffer (pH 5) at 1.3 kV cm^{-1} for 2.5 h. The detection of phosphates on paper was carried out using Ames' improved ammonium molybdate procedure [8]. 2-Methoxypropene (Aldrich) and $POCl_3$ were freshly distilled before use. All other reagents were reagent grade and were used without further purification.

2,1′ : 4,6-Di-O-isopropylidenesucrose (**2**). — This was made by a slight modification of the method of Fanton et al. [4]. A solution of sucrose (5 g, 14.6 mmol) in DMF (150 mL, anhydrous 99 + %, Aldrich) containing Drierite (2 g), toluene-*p*-sulfonic acid (~ 10 mg) was stirred with 2-methoxypropene (7.2 mL, 73 mmol) for 45 min at 70°C. The mixture was cooled to room temperature and neutralized with anhydrous Na_2CO_3 (6 g). The mixture was filtered and the filtrate was concentrated at 60–65°C. TLC showed two



Scheme 1.

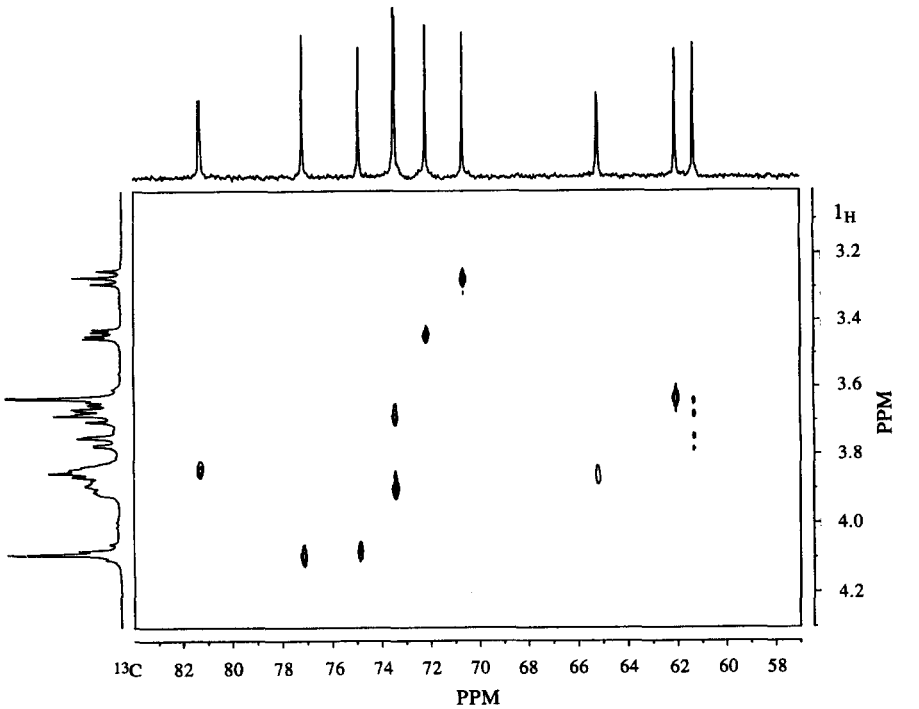


Fig. 1. One-bond H–C COSY spectrum of sucrose 6′-phosphate (pD 11.5, D_2O).

Table 1
NMR data

		1	2	3	4	5	6a	6b
2	^1H, δ [a]	6.65	4.10	4.47	3.8–3.9	4.20	3.96	3.8–3.9
	^1H, J, Hz	3.8	8.7	9.0	10.3	5.3	10.4	
	^{13}C, δ [b]	91.9	75.1	70.2	74.6	64.1	62.7	
3	^1H, δ [c]	5.3	3.4	3.6–3.7	3.24	3.9	3.76	3.6–3.7
	^1H, J, Hz	3.85	9.9	9.4	9.6	2.3, 4.8	12.6	
	^{13}C, δ [c]	93.5	72.5	73.9	70.8	73.4	61.4	

		1′a	1′b	2′	3′	4′	5′	6′a	6′b
2	^1H, δ [a]	4.5–4.6	3.78	–	4.4	5.05	4.5–4.6	4.27	4.27
	^1H, J, Hz	12.3			7.2	7.65			
	^{13}C, δ [b]	67.1		99.6	80.3	75.4	84.8	64.0	
3	^1H, δ [c]	3.62	3.62		4.01——4.06		3.8——3.87		
	^1H, J, Hz	62.3		104.9	77.8	75.4	81.6 [d,e]	65.4 [e]	
	^{13}C, δ [c]								

[a] Solvent, pyridine-d_5 at δ 8.71 7.57, 7.20. Other protons: OH at δ 7.50, 7.00, 6.85, 5.46; methyl groups at δ 1.53, 1.47, 1.38, 1.31.

[b] Solvent, pyridine-d_5 at δ 149.9, 135.6, 123.5. Other carbons: acetal carbons at δ 104.5, 101.8; methyl groups at δ 29.4, 25.5, 24.5, 19.3.

[c] Solvent D_2O, HDO at δ 4.63; external dioxane at δ 67.4; K_2 salt, pD 11.5.

[d] $J_{5',P}$ 7.7 Hz.

[e] At pD 7, Ba salt, C-5′ (d, $^3J_{5',P}$ 7.6 Hz), C-6′ (d, $^2J_{6',P}$ 3.0 Hz); ^{31}P NMR (H_3PO_4 external) δ 4.15.

major spots, R_f 0.5 and 0.15, using 1:1 EtOAc–acetone as eluent. The crude product (R_f 0.5) was purified by column chromatography with 1:1 EtOAc–acetone to give **2** (2.8 g, 43% yield); mp 116–120°C (MeOH–ether); $[\alpha]_D^{25}$ + 26.5° (*c* 0.1, MeOH); lit. [4] $[\alpha]_D^{25}$ + 25.5° (MeOH). Anal. Calcd for $C_{18}H_{30}O_{11} \cdot H_2O$: C, 49.08; H, 7.32. Found: C, 49.43; H, 7.44. The crystalline anhydrous form (mp 164–166°C) has also been reported [9].

Sucrose 6′-phosphate (**3**). — Phosphorus oxychloride (0.41 mL, 5.2 mmol), water (0.72 mL, 4 mmol), and pyridine (0.38 mL, 5.6 mmol) were added to anhydrous MeCN (5 mL) and stirred for 5 min at 0–2°C (ice–water bath). An acetonitrile solution (5 mL) containing 2,1′:4,6-di-*O*-isopropylidenesucrose · H_2O (0.42 g, 1 mmol) was then added. The mixture was stirred for 3 h at 0–2°C. The reaction was monitored with TLC which showed a major product at R_f 0.4 using 80:20 MeCN–water. This is, presumably, the di-*O*-isopropylidene derivative of **3**. The starting material has $R_f \sim 1$. The reaction mixture was poured into 20 mL of ice–water and then stirred for 1 h at 0–2°C. The isopropylidene groups were removed in this step. A saturated aqueous solution of $Ba(OH)_2$ (150 mL) was added to the solution, and the resulting white precipitate was filtered off and washed with water. The filtrate was concentrated to 50 mL under reduced pressure. Methanol (150 mL) was added to the concentrated solution, and the mixture was kept overnight at 0–5°C. The precipitate was filtered off and washed with MeOH, acetone, and ethyl ether to give a crude product (white solid, 200 mg). The crude product was dissolved in water (7 mL), and some insoluble material removed by centrifugation. Methanol (20 mL) was added to the supernatant solution, and the resulting precipitate was filtered off and washed with acetone and ethyl ether. This purification was repeated one more time to yield a white powder (150 mg, 35%). Paper electrophoresis of the product showed the same R_f value as an authentic sample of the potassium salt (Sigma); $[\alpha]_D^{25}$ + 32°, $[\alpha]_{578}^{25}$ + 33°, $[\alpha]_{546}^{25}$ + 36.4°, $[\alpha]_{436}^{25}$ + 60.4°, $[\alpha]_{365}^{25}$ + 91.6° (*c*0.55, water); lit. [2] $[\alpha]_D^{25}$ + 35.4° (water). Anal. Calcd for $C_{12}H_{21}BaO_{14}P \cdot 3H_2O$: C, 23.57; H, 4.45. Found: C, 23.20; H, 4.36.

Acknowledgments

We thank G. Barber and M. Baldoni for preliminary work. The FT-NMR spectra were run at The OSU Chemical Instrument Center by Dr C.E. Cottrell using equipment funded in part by NIH Grant No. 1S10RR01458-01A1.

References

[1] G. Avigad, in P.M. Dey and J.B. Harborne (Eds.), *Methods in Plant Biochemistry*, Vol. 2, Chap. 4, Academic Press, London, 1990.
[2] J.G. Buchanan, D.A. Cummerson, and D.M. Turner, *Carbohydr. Res.*, 21 (1972) 283–292.
[3] T. Sowa and S. Ouchi, *Bull. Chem. Soc. Jpn.*, 48 (1975) 2084–2090.
[4] E. Fanton, J. Gelas, D. Horton, H. Karl, R. Khan, C.-K. Lee, and G. Patel, *J. Org. Chem.*, 46 (1981) 4057–4060.

[5] C.H. du Penhoat, A. Imberty, N. Roques, V. Michon, J. Mentech, G. Descotes, and S. Pérez, *J. Am. Chem. Soc.*, 113 (1991) 3720–3727.
[6] K. Bock and R.U. Lemieux, *Carbohydr. Res.*, 100 (1982) 63–74.
[7] K.B. Kim and E.J. Behrman, Great Lakes-Central ACS Meeting, Ann Arbor, MI, June 1994, Abstract #370.
[8] B.N. Ames, B.R. Bochner, and D.M. Maron, *Anal. Biochem.*, 117 (1981) 81–83.
[9] R. Khan, M.R. Jenner, and H. Lindseth, *Carbohydr. Res.*, 65 (1978) 99–108.