ELSEVIER

Carbohydrate Research 289 (1996) 137–142

CARBOHYDRATE
RESEARCH

Note

NMR structural analysis of a tri-*O*-isopropylidene derivative of D-*glycero*-D-*ido*-2-octulose, the major sugar found in the resurrection plant *Craterostigma plantagineum*

Oliver W. Howarth [a,*], Nicoletta Pozzi [b], Giovanna Vlahov [b], Dorothea Bartels [c]

[a] *Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK*
[b] *Istituto Sperimentale per la Elaiotecnica, Contrada Fonte Umano, 65013 Città S. Angelo (PE), Italy*
[c] *Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Cologne, Germany*

Received 27 October 1995; accepted in revised form 22 March 1996

Keywords: D-*glycero*-D-*ido*-2-Octulose; Resurrection plant; *Craterostigma plantagineum*

Resurrection plants are a small group of higher plants that can survive severe dehydration [1]. We have directed our research towards the molecular basis for this unique property, and in particular at sugars in selected resurrection plants, because of the known role of sucrose and larger oligosaccharides in promoting tolerance to desiccation. The carbohydrate composition and its chemical conversions in the leaves of the resurrection plant *Craterostigma plantagineum* [2] are remarkable. The leaves of *C. plantagineum* in its fully hydrated state contain about 50% of their dried matter in the unusual form of a 2-octulose. Upon dehydration of the plant, this is converted into sucrose, with the reverse process occurring upon rehydration.

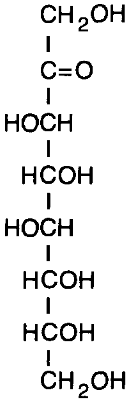
The 2-octulose represents almost 90% of the carbohydrates in the fully hydrated leaves. It was identified as a C_8 sugar by MS and NMR spectroscopy, and further as a 2-octulose from ^1H and ^{13}C NMR data on the underivatised sugar and the acetyl derivatives [2]. It was also believed to have a furanose structure on the basis of the vicinal couplings of the ring protons. Although its structure was not directly determined in our previous work, a tentative proposal of D-*glycero*-D-*ido*-2-octulose (**1**) was put

---

[*] Corresponding author.

PII S0008-6215(96)00106-1

forward on the basis of a biosynthetic hypothesis. This assumed that the interconversion of 2-octulose and sucrose preserves both the glucose and the fructose configurations in the octulose.



$$\begin{array}{c} CH_2OH \\ | \\ C{=}O \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HOCH \\ | \\ HCOH \\ | \\ HCOH \\ | \\ CH_2OH \end{array}$$

1

To evaluate its general significance and to understand the biochemical pathways leading to the formation of octulose, the structure of the 2-octulose is required. NMR data on octuloses are sparse, but Williams and co-workers have reported the formation of the mono- and bis-phosphates of D-*glycero*-D-*altro*-2-octulose and D-*glycero*-D-*ido*-2-octulose, as intermediates of carbon fixation reactions in photosynthesis [3], along with NMR data for the same compounds prepared enzymatically [4]. Because phosphorylation has relatively little effect on the ^1H shifts, these data (included in Table 1) are sufficient to support our earlier proposal of a D-*glycero*-D-*ido*-2-octulose structure. However, the shift fit was insufficiently precise for certainty, especially in view of the multiple forms of the octulose present in aqueous solution, the unavailability of ^{13}C enrichment in the present case, and the perturbations of shift arising from phosphorylation. We have therefore prepared a tri-*O*-isopropylidene derivative of the natural 2-octulose, and determined its structure independently using shift-correlation and NOE-difference NMR spectroscopy. The results, given below, confirm the proposed structure.

1. Experimental

Conversion of 2-octulose into tri-O-isopropylidene-2-octulose [5,6].—2-Octulose, 90% pure by GC analysis, was extracted from fresh *C. plantagineum* leaves as previously reported [2]. A solution of it (450 mg, 1.89 mmol) in 2,2-dimethoxypropane (10 mL) and acetone (10 mL) was stirred for 60 min at 60 °C in the presence of a few crystals of *p*-toluenesulfonic acid. Aliquots of the neutralised reaction mixture were examined by TLC (6:1 ether–hexane) and GLC in a 25-m Nordion column NB54,

Table 1
^{1}H and $^{13}C\{^{1}H\}$ NMR and modelling data for the underivatised octulose, tri-*O*-isopropylidene-octulose, octulose 8-monophosphate, and a comparable di-*O*-isopropylidene-sorbose

Atom (n)	Tri-*O*-isopropylidene-octulose ($CDCl_3$)				Major form of octulose (D_2O)		Octulose 8-P (D_2O) [4]		Di-*O*-isopropylidene-sorbose ($CDCl_3$)		
	δ_H [a]	$^{3}J_{n,n+1}$		δ_C [a]	δ_H [a]	$^{3}J_{n,n+1}$	δ_H	$^{3}J_{HH}$	δ_H [a]	$^{3}J_{HH}$	δ_C [a]
		Obsd	Calcd							Obsd	
1	3.88			63.80	3.63				3.91		63.6
	(2 H)				3.68				3.96		
2	–			98.59	–				–		98.1
3	4.13	1.0	1.7	78.03	4.20	5.8	4.18	6.2	4.19	1.1	78.3
4	4.32	2.6	3.2	75.13	4.47	7.2	4.46	6.6	4.33	2.4	74.7
5	4.22	2.2	2.2	73.02	4.46	1.8	4.47	2.2	4.20	2.1	73.7
6	3.91	6.7	9.7 [b]	69.92	3.82		3.89	8.3	4.11		61.2
7	4.38	6.2	5.2 or 6.3 [c]	74.52	3.75		3.80				
8	3.99	5.2	11.3 or 1.5 [c]	66.20	3.69		3.94				
	4.06				3.85						
Me-a [d]	1.48			19.29							
Me-b	1.44			18.89							
Me-c	1.41			26.65							
Me-d	1.40			29.42							
Me-e	1.38			27.73							
Me-f	1.36			25.12							
C(Me-b, Me-d)				98.22							
C(Me-a, Me-e)				98.02							
C(Me-c, Me-f)				108.92							

[a] Present paper.

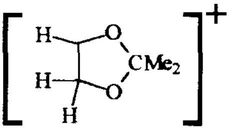
[b] Ignoring rotation about the C-6–C-7 bond.

[c] Alternative, almost isoenergetic conformations.

[d] Isopropylidene methyl labelling is in reverse order of H shifts.

programmed for a rise from 100 to 170 °C over 20 min. Two major TLC spots and GLC peaks were obtained, with the major (70%) migrating more rapidly than the minor in the TLC, but less rapidly in the GLC.

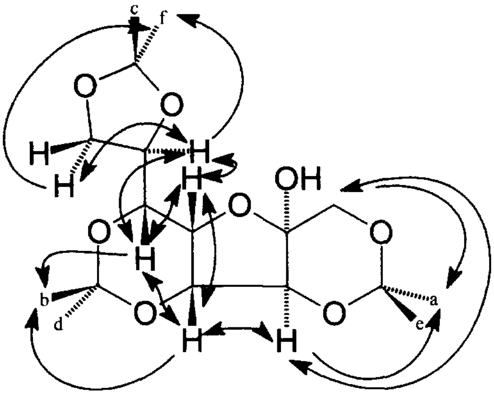
Complete derivatisation was ensured by evaporative removal of the methanol produced, plus a twofold repetition of the derivatisation (68% yield). The oily residue was dissolved in $CHCl_3$ (5 mL) and resolved into its two components by column chromatography on silica gel (Merck 60H), with eluent as above plus an added trace of triethylamine to prevent decomposition. Major product: 90 mg; $[\alpha]_D$ +14.3° (*c* 1.8, $CHCl_3$); EIMS: *m/z* 345 $[M - Me]^+$, 101 for



NMR spectroscopy.—Spectra were obtained for solutions in $CDCl_3$ or in D_2O at 295 K, by standard means, using a Bruker ACP400 spectrometer, and referenced to Me_4Si or to added CH_3OD (3.35 ppm). Two-dimensional (2D) ^1H–^{13}C (short- and long-range) and ^1H–^1H (COSY) shift-correlation experiments [7] were performed for assignment, and then the averaged solution structure was determined by 1D NOE-difference spectra, using a 2-s pre-irradiation interval at each ^1H resonance in turn, followed by molecular modelling (PCMODEL, Serena Software, Bloomington, IN, USA, v.3.0). In combination with the interproton couplings, this enabled all alternative structures to be rejected.

2. Results

NMR data for both the major component of the natural octulose and for the derivatised 2-octulose are given in Table 1, together with vicinal proton coupling constants calculated by PCMODEL. The octulose ^1H resonances were readily ordered via the COSY spectrum. The positions of the three isopropylidene groups were identified as follows. The long-range CH shift-correlation identified two isopropylidene carbons with shifts below 100 ppm, plus a third at ca. 110 ppm. These shifts are as expected for one 5-membered and two 6-membered isopropylidene rings [8,9]. One of the less-shifted isopropylidene carbons also showed a long-range shift-correlation with H-1, thus proving that O-1 is substituted. Also, each isopropylidene carbon could be shift-correlated with its specific methyl pair. NOEs were also observed between the following hydrogens: Me-a from H-1b and H-3, Me-b from H-4 and H-6, and Me-f from H-7 and H-8a, although the methyl resonances were too similar in shift to permit their selective irradiation. Thus the 5-membered ring is (7,8) and the 6-membered rings are (1,3) and (4,6). In combination with the interproton couplings in the main furanose ring, these fix all the conformations, apart from rotation about the C-6–C-7 bond, and possible twist equilibria within the otherwise unconstrained 7,8-ring. In particular, the *β*



Scheme 1. 1,3:4,6:7,8-Tri-*O*-isopropylidene-D-*glycero*-α-D-*ido*-2-octulofuranose, with arrows to indicate observed interproton NOEs.

anomer is excluded, because the β linkage would necessitate a distortion of the main ring which would greatly alter these couplings.

Mutual NOEs were also observed for the pairs H-1b:H-3, H-3:H-4, H-4:H-5 and H-6, H-5:H-6 and H-7(weaker), H-6:H-7(weaker), H-7:H-8a, and H-8a:H-8b. Also, no H-5:H-8a,b NOEs were detected. The relative intensities and absences of these NOEs were consistent with the modelled structure, except that the H-5 and H-6:H-7 NOEs were a little larger than expected for the modelled distances. This indicates that there is significant conformational flexibility about the C-6–C-7 bond, also reflected in an H-6:H-7 coupling somewhat smaller than that calculated for a rigid model. Table 1 presents two sets of calculated couplings in the C-7:C-8 ring, for different envelope conformations having similar calculated energies. The observed couplings support an approximately 1:2 mixture of these conformations. Thus the 1,3:4,6:7,8-tri-*O*-isopropylidene-D-*glycero*-α-D-ido-2-octulofuranose structure (Scheme 1) is confirmed. The corresponding structure of the original octulose is given in the Fischer projection **1**.

Further confirmation of the stereochemistry for C-1 to C-5 came from ^{1}H and ^{13}C NMR spectra [1] of 1,3:4,6-di-*O*-isopropylidene-β-L-sorbofuranose [10], which closely resembles a major portion of the octulose triacetal. Table 1 shows that its shifts and couplings, where comparable, are very similar to those of the octulose derivative.

The definition of structure of the octulose will enable further study of its biosynthesis. It suggests either glucose or fructose as likely precursors. Two transketolase enzymes have recently been identified which are induced during rehydration, the process during which the octulose is being synthesised [11]. Transketolases catalyse the reversible transfer of a two-carbon glyco-aldehyde and could thus be involved in the synthesis of octulose, perhaps transferring a C_2 unit to a C_6 sugar. This will have to be confirmed by in vivo labelling studies and by biochemical analysis.

---

[1] Kindly supplied by a referee.

References

[1] D. Bartels, R. Alexander, K. Schneider, R. Elster, R. Velasco, J. Alamillo, G. Bianchi, D. Nelson, and F. Salamini, in T.J. Close and E.A. Bray (Eds.), *Plant Responses to Cellular Dehydration During Environmental Stresses*, The American Society of Plant Physiologists, 1993, pp 119–127.

[2] G. Bianchi, A. Gamba, C. Murelli, F. Salamini, and D. Bartels, *The Plant Journal*, 1 (1991) 355–359.

[3] J.F. Williams, K.K. Arora, and J.F. Longenecker, *Int. J. Biochem.*, 19 (1987) 749–817.

[4] I.F. Flanigan, J.G. Collins, K.L. Graham, J.K. MacLeod, and J.F. Williams, *Magn. Reson. Chem.*, 33 (1995) 231–232.

[5] E. Fanton, J. Gelas, and D. Horton, *J. Org. Chem.*, 46 (1981) 4057–4060.

[6] H. Regeling, E. De Rouville, and G.J.F. Chittenden, *Recl. Trav. Chim. Pays-Bas*, 106 (1987) 461–464.

[7] J.K.M. Sanders and B.K. Hunter, *Modern NMR Spectroscopy*, Oxford University Press, Oxford, 1993.

[8] J.G. Buchanan, A.R. Edgar, D.I. Rawson, P. Shahidi, and R.H. Wightman, *Carbohydr. Res.*, 100 (1982) 75–86.

[9] P. Dais and A. S. Perlin, *Carbohydr. Res.*, 146 (1986) 177–191.

[10] T. Maeda, *Bull. Chem. Soc. Jpn.*, 40 (1967) 2122–2128.

[11] G. Bernacchia, G. Schwall, F. Lottspeich, F. Salamini, and D. Bartels, *The EMBO Journal*, 14 (1995) 610–618.