Carbohydrate Research, 102 (1982) 294–297
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Solid-state ^{13}C-n.m.r. spectroscopy of D-*threo*-2,5-hexodiulose (5-keto-D-fructose)

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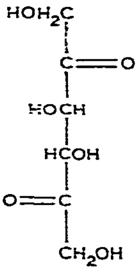
(Received October 19th, 1981; accepted for publication, November 16th, 1981)

D-*threo*-2,5-Hexodiulose (**1**; "5-keto-D-fructose", 5KF) is a symmetrical hexodiulose isolated from cultures of several strains of Acetobacter growing on D-fructose as the sole source of carbon[1–3]. Our previous ^{13}C-n.m.r. spectral studies established the structure of 5KF in solution[4]; in aqueous solution at 25°, it exists mainly (>95%) in the β-pyranose form, **2**, in which the 5-ketone group is hydrated to form a *gem*-diol. In anhydrous Me_2SO-d_6, on the other hand, the sugar appears to exist as a tricyclic, spiran dimer, **3**, of fused β-pyranose and β-furanose rings. X-Ray crystallographic analysis of 5KF indicated[5] that, in the crystal state, it exists as the dimer **3**.

Recent instrumental advances have allowed high-resolution ^{13}C-n.m.r. spectra



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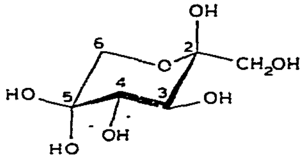

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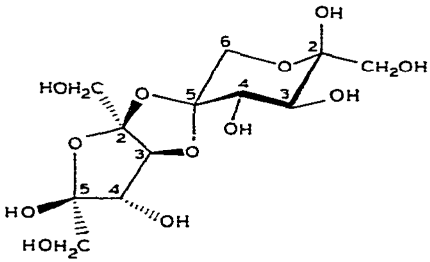

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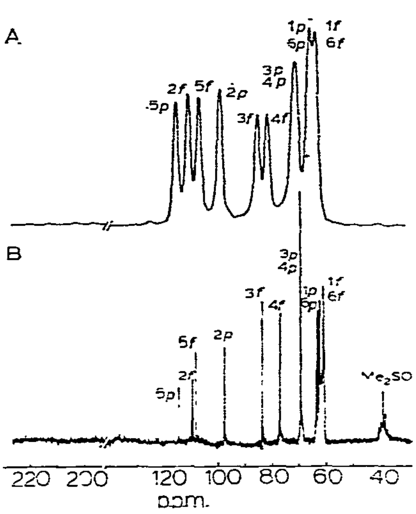

Fig. 1. (A) Magic-angle spinning, cross-polarization ^{13}C-n.m.r. spectrum, recorded at 15.1 MHz, of 5-keto-D-fructose, 1.8 ms contact time and 50,000 scans. (B) 25.2-MHz, proton-decoupled, ^{13}C-n.m.r. spectrum of 0.8M 5-keto-D-fructose in Me$_2$SO-d_6 at 30°, 18,200 scans [reprinted, with permission, from *Biochemistry*, 21 (1982) 75–81, copyright (198X) by the American Chemical Society].

of solid samples to be measured with magic-angle spinning (m.a.s.) and cross polarization (c.p.) under high-power, proton-decoupling conditions[6,7]. Several m.a.s.-c.p. ^{13}C-n.m.r. spectra have recently been reported for high-molecular-weight polysaccharides of limited solubility[8,9] and high solution-viscosity[10], where natural-abundance, ^{13}C-n.m.r. spectroscopy of polysaccharides in solution is of limited value. Because of the similarity in structure of 5KF in crystal form and Me$_2$SO-d_6, we have examined the m.a.s.-c.p., ^{13}C-n.m.r. spectra of 5KF under various conditions.

The m.a.s.-c.p. ^{13}C-n.m.r. spectrum of solid 5KF is shown in Fig. 1A. Nine resonances are observed between 60 and 120 p.p.m., relative to tetramethylsilane, with a chemical-shift pattern very similar to that observed for 5KF in Me$_2$SO-d_6 (see Fig. 1B). The resonance at 72 p.p.m., upon resolution enhancement, is resolved into two overlapping resonances (not shown). No resonances are observed in the carbonyl region, as would be expected for structure **3**. Tentative assignments of the solid-state resonances were made by comparison with the previously assigned resonances of 5KF in Me$_2$SO-d_6 (see Fig. 1B; *p* and *f* represent pyranose and furanose, respectively). The line widths are ~2.3 p.p.m. (35 Hz) under these conditions, and compare favorably with other reported m.a.s.-c.p., ^{13}C-n.m.r. linewidths. Under the most favorable circumstances, linewidths of 7 Hz have been reported for sucrose[11]. The chemical-shift pattern for solid 5KF differs somewhat from that of 5KF in Me$_2$SO-d_6; for instance, the larger splitting between 2*f* and 5*f* may be noted. Chemical-shift differences between solution and the solid state have been observed for amino acids, peptides, and proteins[12,13].

The four low-field resonances assigned to C-2 and C-5 of 5KF have no directly bonded protons, and thus, the cross-polarization time, T_{is}, should be longer for these

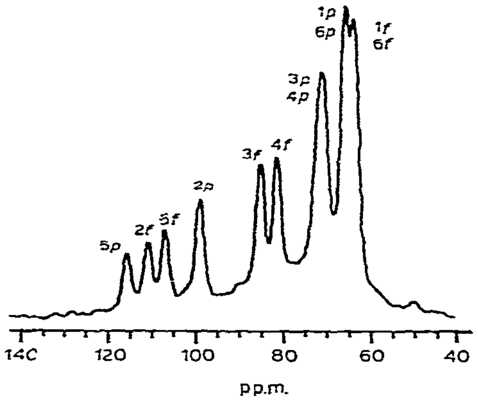

Fig. 2. Magic-angle spinning, cross-polarization, ^{13}C-n.m.r. spectrum, recorded at 15.1 MHz, of 5-keto-D-fructose, 75 μs contact time and 12,000 scans.

atoms than for other sugar carbon atoms possessing directly bonded hydrogen atoms. When the contact time was shortened from 1.8 ms (used to obtain the spectrum in Fig. 1A) to 75 μs, the intensities of the signals of these four carbon atoms are lessened relative to those of the other resonances (see Fig. 2), confirming their assignments as quaternary carbon atoms.

In summary, by comparing the high-resolution ^{13}C-n.m.r. spectrum of 5KF in Me_2SO-d_6 with the m.a.s.-c.p. ^{13}C-n.m.r. spectrum of the crystalline sugar, additional evidence has been obtained for the existence of structure 3 for 5KF in Me_2SO-d_6.

EXPERIMENTAL

D-*threo*-2,5-Hexodiulose (5-keto-D-fructose) was isolated from *Gluconobacter cerinus* cultures as described previously[1], and was a pure product (m.p. 172–174°, uncorr.) as analyzed by thin-layer chromatography on cellulose.

The natural-abundance, proton-decoupled ^{13}C-n.m.r. spectrum of 5KF in anhydrous Me_2SO-d_6 was recorded at 25.15 MHz with a JEOL p.F.t.-n.m.r. spectrometer operating in the pulsed Fourier-transform mode and interfaced with a Nicolet 1080 series computer. Spectra were recorded by using an internal deuterium lock, and ^{13}C-chemical shifts are expressed as p.p.m. downfield from tetramethylsilane, with internal Me_2SO-d_6 (40.5 p.p.m.) as the reference standard.

The solid-state, m.a.s.-c.p. ^{13}C-n.m.r. spectra of 5KF were recorded at 15.1 MHz with a home-built n.m.r. spectrometer. The sample (0.7 g) was packed in a Beams–Andrew, 700-μL, hollow rotor, and spun at 1.667 kHz, with a 3-s repetition time, 40-ms proton-decoupling time, using 10-gauss ^{1}H-decoupling power. Solid-state, ^{13}C-chemical shifts are expressed as p.p.m. downfield from Me_4Si.

ACKNOWLEDGMENTS

We acknowledge the help of Dr. W. Thomas Dixon of Washington University in recording the solid-state, m.a.s.-c.p., ^{13}C-n.m.r. spectra at Monsanto Co., Physical Sciences Center, St. Louis, Mo. This work was supported by National Institutes of Health Postdoctoral Fellowship to J.S.B. (GM-07452), and grant GB-12670 from the National Science Foundation to S.E.

REFERENCES

1 G. Avigad and S. Englard, *J. Biol. Chem.*, 240 (1965) 2290–2296.
2 O. Terada, K. Tomizawa, and S. Kinoshita, *Nippon Nogei-kagaku Kaishi*, 35 (1961) 127–130.
3 M. Ameyama, E. Shinagawa, K. Matsushita, and O. Adachi, *J. Bacteriol.*, 145 (1981) 814–823.
4 J. S. Blanchard, C. F. Brewer, S. Englard, and G. Avigad, *Biochemistry*, 21 (1982) 75–81.
5 L. Hassen, A. Hordvik, and R. Hove, *J. Chem. Soc., Chem. Commun.*, (1976) 572–573.
6 J. Schaefer and E. O. Stejskal, *J. Am. Chem. Soc.*, 98 (1976) 1031–1032.
7 J. Schaefer and E. O. Stejskal, in G. C. Levy (Ed.), *Topics in Carbon-13 NMR Spectroscopy*, Vol. 3, Wiley–Interscience, New York, 1979, pp. 283–324.
8 R. H. Atalla, J. C. Gast, D. W. Sindorf, V. J. Bartuska, and G. E. Maciel, *J. Am. Chem. Soc.*, 102 (1980) 3249–3251.
9 W. E. Earl and D. L. Vander Hart, *J. Am. Chem. Soc.*, 102 (1980) 3251–3252.
10 L. D. Hall and M. Yalpani, *Carbohydr. Res.*, 91 (1981) c1–c4.
11 D. L. Vander Hart, W. E. Earl, and A. N. Garroway, *J. Magn. Reson.*, 44 (1981) 361–401.
12 S. J. Opella, M. H. Frey, and T. A. Cross, *J. Am. Chem. Soc.*, 101 (1979) 5856–5857.
13 M. H. Frey and S. J. Opella, *J. Chem. Soc., Chem. Commun.*, (1980) 474–475.