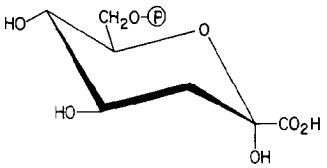
Carbohydrate Research, 132 (1984) 317–322
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Structural analysis of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate by ^{1}H- and natural-abundance ^{13}C-n.m.r. spectroscopy

CRAIG C. GARNER AND KLAUS M. HERRMANN
Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907 (U.S.A.)
(Received December 5th, 1983; accepted for publication in revised form, February 24th, 1984)

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate (**1**) is the first intermediate of the shikimate pathway, the common metabolic route to the aromatic amino acids in bacteria and plants[1]. *In vivo*, compound **1** is synthesized by aldol condensation between C-3 of enolpyruvate phosphate and C-1 of D-erythrose 4-phosphate[2]. The reaction is catalyzed by **1**-synthase[3–6]. The principal tautomer is a pyranose resulting from hemiacetal formation between C-2 and HO-6 of the primary condensation-product[3]. The structure has been confirmed by chemical syntheses[7,8]. This paper confirms by ^{1}H- and ^{1}H-decoupled ^{13}C-n.m.r. analysis the $^5C_2$(D) conformation for **1**; long-range coupling-constants obtained from ^{1}H-coupled ^{13}C-n.m.r. establish **1** as the pure α anomer.



1

RESULTS AND DISCUSSION

Coupled and decoupled ^{1}H- and ^{13}C-n.m.r. spectra of **1** (Fig. 1) yield the chemical shifts and coupling constants shown in Table I. The resonance assignments, are based on selective ^{1}H homo- and hetero-nuclear-decoupling experiments, and on comparison with chemical shifts of similar compounds[9–11].

The ^{1}H-n.m.r. spectrum of **1** (Fig. 1A) is similar to that of methyl (methyl 3-deoxy-D-*arabino*-2-heptulopyranosid)onate[10], except for the H-7 resonances, which are shifted downfield because of deshielding by the 7-phosphate group. In addition, heteronuclear coupling between phosphorus and the H-7 nuclei is observed. The other chemical shifts and the three-bond coupling constants for vicinal protons, which give results in good agreement with X-ray, c.d., and n.m.r. data for

0008-6215/84/$ 03.00

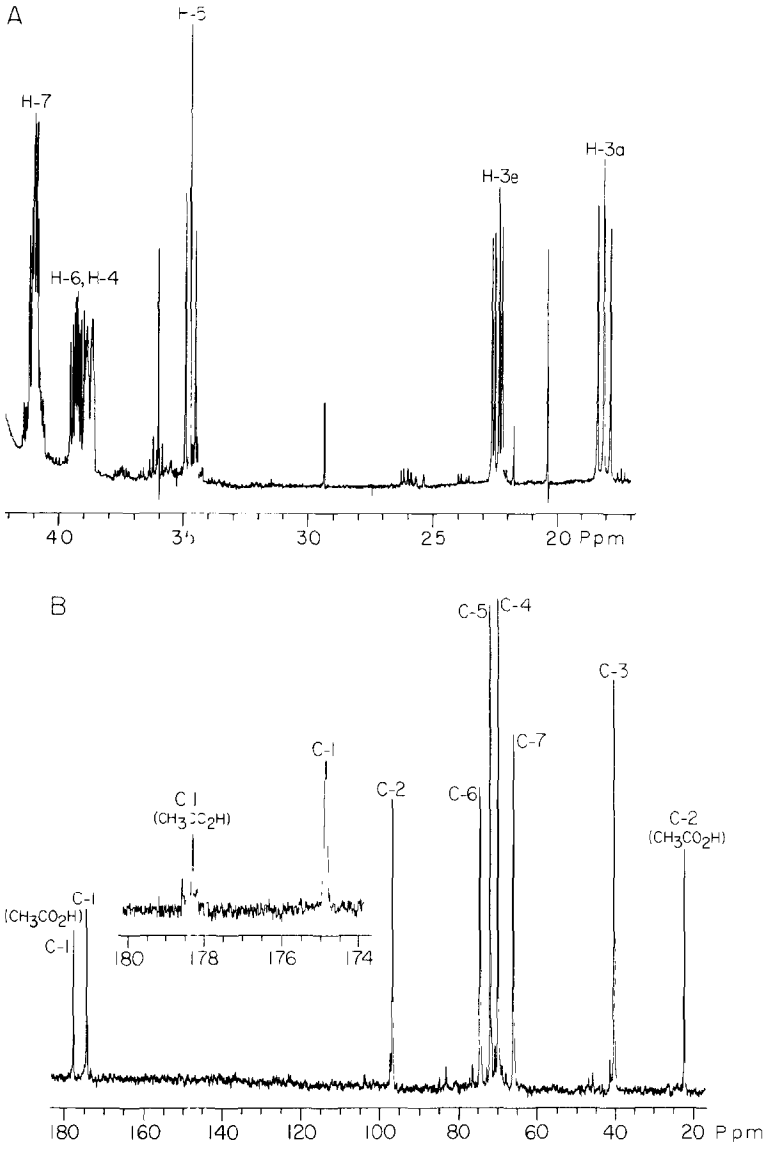


Fig. 1. A, ^{1}H (470 MHz) and B, ^{1}H-decoupled, natural-abundance ^{13}C- (118 MHz) n.m.r. spectra of 3-deoxy-α-D-*arabino*-heptulopyranosonic acid 7-phosphate (**1**) in acetic acid at 20°. The insert in B shows the low-field portion of a ^{1}H-coupled ^{13}C-n.m.r. spectrum of **1** in acetic acid at 20°. Chemical shifts are given in p.p.m. from tetramethylsilane.

methyl glycosides of 3-deoxy-D-*arabino*-2-heptulosonates[10], confirm that **1** is a pyranose chair [$^{5}C_{2}$(D)] having all the bulky substituents in equatorial orientations.

The ^{1}H-decoupled ^{13}C-n.m.r. spectrum of 1 (Fig. 1B) confirms the basic deoxypyranose structure of the molecule. The chemical shift of C-3 (39.4 p.p.m.)

TABLE I

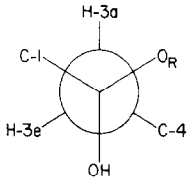
^{1}H- AND ^{13}C-N.M.R. DATA FOR **1** IN ACETIC ACID

Proton	*(P.p.m.)*	*Coupling*	*(Hz)*	*Carbon atom*	*(P.p.m.)*	*Coupling*	*(Hz)*
H-3a	1.80	$^2J_{H\text{-}3a,H\text{-}3e}$	13.2	C-1	174.4	$^3J_{C\text{-}1,H\text{-}3a}$	0.8
		$^3J_{H\text{-}3a,H\text{-}4}$	11.5			$^3J_{C\text{-}1,H\text{-}3e}$	0.8
H-3e	2.23	$^3J_{H\text{-}3e,H\text{-}4}$	5.2	C-2	96.4		
H-4	3.93	$^3J_{H\text{-}4,H\text{-}5}$	9.6	C-3	39.4	$^1J_{C\text{-}3,H\text{-}3}$	132
H-5	3.47	$^3J_{H\text{-}5,H\text{-}6}$	9.6	C-4	69.3	$^1J_{C\text{-}4,H\text{-}4}$	146
H-6	3.88			C-5	71.3	$^1J_{C\text{-}5,H\text{-}5}$	143
H-7	4.10			C-6	73.9	$^1J_{C\text{-}6,H\text{-}6}$	143
				C-7	65.4	$^1J_{C\text{-}7,H\text{-}7}$	146

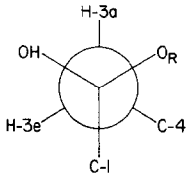
is characteristic for 2-deoxyhexoses in which the replacement of OH by H at C-2 causes an upfield shift[9] for the C-2 resonances of ~30 p.p.m. The C-7, C-4, C-5, and C-6 resonances of **1** between 60 and 80 p.p.m. are similar to the C-6, C-3, C-4, and C-5 chemical shifts, respectively, of aldohexoses. The C-2 resonance of **1** is a singlet at 96.4 p.p.m. indicating **1** to be a single anomer.

The numerical value of the C-2 chemical shift is not sufficient to distinguish between the α and β configuration, as there appears to be no general relationship between anomeric configuration of monosaccharides and the chemical shifts of their anomeric carbon atoms[9]. Whereas the anomeric configuration of ordinary hexoses may be deduced from the $^1J_{C\text{-}1,H\text{-}1}$ coupling constants[9], this cannot be accomplished for **1**, because there is no proton bound to the anomeric carbon atom.

In consequence, long-range ^{13}C–^{1}H coupling was used to deduce the anomeric configuration of **1**. The two-bond coupling constants $^2J_{C\text{-}2,H\text{-}3}$ are small (<2 Hz), consistent with the α configuration of hexose chair forms[12]. Determination of the three-bond coupling constants, $^3J_{C\text{-}1,H\text{-}3a}$ and $^3J_{C\text{-}1,H\text{-}3e}$, proves **1** to be



2



3

the α anomer. This method has previously been used to assign the anomeric configuration of methyl glycosides of 3-deoxy-D-*manno*-octulosonate[13]. The numerical value of $^3J_{C,H}$ is dependent upon the magnitude of the dihedral angle[14] in a similar way as that deduced by Karplus[15] for $^3J_{H,H}$. The Newman projections, looking down the C-2–C-3 bond of **1**, show torsion angles between C-1 and the H-3a and H-3e protons of 60° for the α configuration (**2**) and of 180° and 60°, respectively, for the β configuration (**3**). Predicted values[16] for $^3J_{C,H}$ having *gauche* protons (60° torsion angle) are ~1 Hz, for protons in *trans* (180° torsion angles) disposition, 7.8 Hz.

The low-field portion of a ^{1}H-coupled ^{13}C-n.m.r. spectrum of **1** in acetic acid (Fig. 1B, insert) shows a multiplet for the carboxyl carbon atom of acetic acid, from which a $^2J_{C\text{-}2,H\text{-}1}$ value of 6 Hz is measured. However in this spectrum, the hyperfine structure of the C-1 of **1** resonance is not resolved, because the three-bond coupling-constants are much smaller than 6 Hz. The two $^3J_{C\text{-}1,H\text{-}3}$ values for **1**, obtained from measurements of line broadening after selective decoupling of H-3a and H-3e, are both ~0.8 Hz. Thus, the anomeric configuration of **1** is α.

Compound **1** used in these studies was synthesized enzymically. While the final product of the synthesis is the pure α anomer, it cannot be inferred that the primary product released from the enzyme is α-**1**. Chemically synthesized **1** is quantitatively converted into dihydroquinate by dihydroquinate synthase[8]. If dihydroquinate synthase is stereospecific with respect to the configuration at C-2 of its substrate, the chemical synthesis of **1** also yields only the α anomer. Thus, biosynthesis of α-**1** may not be so much the result of stereospecific enzyme catalysis, but rather of greater stability of the α over the β anomer.

MATERIALS AND METHODS

Chemicals. — D-Erythrose 4-phosphate[17] and enolpyruvate phosphate[18] were synthesized as described. The free acid of enolpyruvate phosphate was obtained by passing the cyclohexylammonium salt over Dowex 50-1. Compound **1** synthase was purified to homogeneity as described before[4]. All other chemicals were obtained commercially at the highest purity available and were used without further purification.

Enzymic synthesis of **1**. — Enolpyruvate phosphate (875 μmol), D-erythrose 4-phosphate (350 μmol), ovalbumin (10 mg), and potassium phosphate (1 mmol) in a total volume of 20 mL were adjusted to pH 6.5. The mixture was warmed to 37° and 700 units of pure 1-synthase[4] were added in several portions during 60 min. The mixture was kept for an additional 60 min at 37° until D-erythrose 4-phosphate had been quantitatively converted into **1**, as judged by the thiobarbiturate assay[8]. The protein was precipitated with concentrated HCl and the precipitate removed by centrifugation. The supernatant solution was adjusted with NaOH to pH 4, concentrated to ~4 mL by rotary evaporation, and clarified by filtration. The remaining salt and excess enolpyruvate phosphate was removed by gel filtration on Sephadex G-10. Solutions containing **1** were pooled, concen-

trated, and further purified by paper chromatography on Whatman 3MM with 3:3:1 (v/v/v) ethyl acetate–acetic acid–water as eluant. Compound **1** was eluted from the paper with water. The overall yield was 70%.

^{1}H-N.m.r. spectra. — Compound **1** (70 mg) was dissolved in 0.5 mL of 0.1μM ethylenedinitrilo(tetraacetate) in ^2H_2O; ^2H_2O served as internal lock, and the sample contained acetic acid, a carryover from the purification of **1**. The solution was placed in a 5-mm n.m.r. tube of a NTC-470 n.m.r. spectrometer. Spectra were recorded at 20° at 470 MHz; 32 acquisitions were taken with 16,384 time-domain points per 4000 Hz, and a recycle time of 5 s. Chemical shifts were measured relative to ^{2}HOH (4.80 p.p.m.) and cross-referenced and reported relative to tetramethylsilane.

^{13}C-N.m.r. spectra. — Compound **1** (70 mg) was dissolved in 0.7 mL of 0.1μM ethylenedinitrilo(tetraacetate) in ^2H_2O; the sample contained acetic acid, a carryover from the purification of **1**. The solution was placed in an 8-mm n.m.r. tube of a NTC-470 spectrometer. Both ^{1}H-decoupled and coupled ^{13}C spectra were recorded at 20° at 118 MHz; 4000 acquisitions were taken with 32,768 time-domain points per 8402.68 Hz, a recycle time of 3.5 s, and a 45° flip angle. Pulse ^{1}H-decoupled ^{13}C spectra were recorded using broadband ^{1}H noise-decoupling with an internal ^{2}H-field–frequency lock. Chemical shifts were measured relative to 1,4-dioxane (67.4 p.p.m.) and cross-referenced and reported relative to tetramethylsilane.

Three-bond coupling constants $^3J_{C\text{-}1,H\text{-}3a}$ and $^3J_{C\text{-}1,H\text{-}3e}$ were calculated from line-width measurements of the C-1 resonance under four sets of conditions: *1*, coupled; *2*, broadband decoupled; *3*, with selective decoupling of H-3a; and *4*, with selective decoupling of H-3e. In each of three separate experiments, conditions *2*, *3*, or *4* were operated simultaneously with condition *1* by an alternate pulse technique. Time-domain points (32,768 per 4402 Hz) were taken with a recycle time of 4.36 s.

ACKNOWLEDGMENTS

We thank Dr. Eldon Ulrich for help with spectral data-acquisition and for critical reading of the manuscript. The n.m.r. spectra were recorded at the Purdue University Biochemical Magnetic Resonance Laboratory, which is supported by U.S. Public Health Service Research Resources Grant RR-01077. These studies were supported by U.S. Public Health Service Training Grant GM-07211 to C.C.G. and Research Grant GM-17678 to K.M.H. This is paper no. 9700 of the Purdue University Agricultural Experiment Station.

REFERENCES

1 K. M. HERRMANN, in K. M. HERRMANN AND R. L. SOMERVILLE (Eds.), *Amino Acids: Biosynthesis and Genetic Regulation*, Addison–Wesley, Reading, MA, 1983, pp. 301–322.

2 P. R. SRINIVASAN, M. KATAGIRI, AND D. B. SPRINSON, *J. Am. Chem. Soc.*, 77 (1955) 4943–4944.

3 P. R. SRINIVASAN AND D. B. SPRINSON, *J. Biol. Chem.*, 234 (1959) 716–722.
4 R. SCHONER AND K. M. HERRMANN, *J. Biol. Chem.*, 251 (1976) 5440–5447.
5 R. J. MCCANDLISS, M. D. POLING, AND K. M. HERRMANN, *J. Biol. Chem.*, 253 (1978) 4259–4265.
6 T. OGINO, C. GARNER, J. L. MARKLEY, AND K. M. HERRMANN, *Proc. Natl. Acad. Sci. U.S.A.*, 79 (1982) 5828–5832
7 D. B. SPRINSON, J. ROTHSCHILD, AND M. SPRECHER, *J. Biol. Chem.*, 238 (1963) 3170–3175.
8 K. M. HERRMANN AND M. D. POLING, *J. Biol. Chem.*, 250 (1975) 6817–6821.
9 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
10 D. CHARON, L. SZABO, M. CESARIO, AND J. GUILHEM, *J. Chem. Soc., Perkin Trans. 1*, (1982) 3055–3063.
11 T. C. CRAWFORD, G. C. ANDREWS, H. FAUBL, AND G. N. CHMURNY, *J. Am. Chem. Soc.*, 102 (1980) 2220–2225.
12 A. S. PERLIN, N. CYR, P. G. S. RITCHIE, AND A. PARFONDRY, *Carbohydr. Res.*, 37 (1974) c1–c4.
13 F. M. UNGER, D. STIX, AND G. SCHULTZ, *Carbohydr. Res.*, 80 (1980) 191–195.
14 R. U. LEMIEUX, *Ann. N.Y. Acad. Sci.*, 222 (1973) 915–934.
15 M. KARPLUS, *J. Am. Chem. Soc.*, 85 (1963) 2870–2871.
16 M. F. CZARNIECKI AND E. R. THORNTON, *J. Am. Chem. Soc.*, 99 (1977) 8273–8279.
17 F. J. SIMPSON, A. S. PERLIN, AND A. S. SIEBEN, *Methods Enzymol.*, 9 (1966) 35–38.
18 V. M. CLARK AND A. J. KIRBY, *Biochim. Biophys. Acta*, 78 (1963) 732.