Carbohydrate Research, 203 (1990) 173–182
Elsevier Science Publishers B.V., Amsterdam

Two-dimensional ^{1}H-, ^{13}C-, and ^{31}P-nuclear magnetic resonance and molecular-mechanics investigation of D-fructose 2,6-bisphosphate

Ronald J. Voll, Subbaraya Ramaprasad, David Vargas, Ezzat S. Younathan*,
Department of Biochemistry, Louisiana State University, Baton Rouge, Louisiana 70803 (U.S.A.)

Saad Laban, and Theodore A. W. Koerner
Department of Pathology, The University of Iowa, Iowa City, Iowa 52242 (U.S.A.)

(Received July 10th, 1989; accepted for publication in revised form November 13th, 1989)

ABSTRACT

Two-dimensional nuclear magnetic resonance studies have been carried out to assign unequivocally all the proton, carbon, and phosphorus resonances of D-fructofuranose 2,6-bisphosphate (**1**) and to verify its structure using a 400-MHz spectrometer. Several unexpected chemical-shift values and coupling constants were obtained. Molecular mechanics calculations (Sybyl) carried out to minimize the conformational energy of **1** yield $\varphi_{C\text{-}1,P\text{-}2} = +84$, $\varphi_{C\text{-}3,P\text{-}2} = -155$, and $\varphi_{C\text{-}5,P\text{-}6} = +175°$. Thus the unusual near-gauche orientations of C-1 and C-3 to P-2 in **1** can explain their small vicinal coupling constants ($^{3}J_{C\text{-}1,P\text{-}2} = 1.2$, and $^{3}J_{C\text{-}3,P\text{-}2} = 3.8$ Hz), in contrast to the expected larger value seen for $^{3}J_{C\text{-}5,P\text{-}6}$ namely, 6.9 Hz. Treatment of a sample of this compound with sodium borohydride did not affect its nuclear magnetic resonance spectrum, substantiating that O-2 is phosphorylated. Oxidation with sodium periodate yielded an intermediate which decomposed by a β-elimination mechanism involving the 6-phosphate group. These data establish unequivocally the ^{1}H, ^{13}C, and ^{31}P assignments and explain the observed anomalous shifts. Moreover they indicate that the activator of fructose 6-phosphate 1-kinase is the β anomer of the $^{4}T_{3}$ conformer of D-fructose 2,6-bisphosphate.

INTRODUCTION

In 1980, the isolation of a low-molecular-weight compound from rat liver which stimulated the activity of D-fructose 6-phosphate 1-kinase was reported[1]. The activator was identified as D-fructose 2,6-bisphosphate (**1**), based on its ^{13}C-n.m.r. spectra and our assignments for carbon signals of D-fructose 6-phosphate (**2**) and D-fructose 1,6-bisphosphate[2,3] (**3**). Independently and simultaneously, the same structure (**1**) of the activator was reported by two other groups[4–7]. A synthetic compound showing the same enzymic effects as the natural modulator was synthesized by these three groups, starting with **3** and *N,N'*-dicyclohexylcarboiimide. This new modulator turned out to be one of the most important discoveries in the area of carbohydrate metabolism in Eukaryotes during the past decade and may have a role in the pathogenesis of diabetes mellitus[8]. Its metabolic role has been reviewed[9–11] and its enzymic stereospecificity has been reported[12].

We have observed certain anomalies in the ^{13}C spectra of this compound as

* To whom correspondence should be addressed.

reported in the literature[5,7,13]. Moreover, because of the sterically nonspecific method of its synthesis, it was deemed appropriate to carry out further work to ascertain its structure. This paper reports detailed one-dimensional (1-D) and two-dimensional (2-D) n.m.r. studies, and molecular-mechanics calculations, as well as chemical studies on **1**. Complete and verified assignments of the ^{1}H, ^{13}C, and ^{31}P chemical shifts are given.

EXPERIMENTAL

Materials. — D-Fructose 2,6-bisphosphate (**1**), sodium salt, was purchased from Sigma Chemical Co., St. Louis. This material was found by 1-D proton-n.m.r. analysis to have some volatile impurities, which were decreased considerably after repeated lyophilization of its D_2O solution.

Methods. — N.m.r.-spectral studies were performed using a 0.6M solution of **1** in D_2O at pH 8.3. The sample was stored at 5° when not in use. Data were acquired on a Bruker AM400 instrument at ambient probe temperature [^{1}H (400.1 MHz), ^{13}C (100.6 MHz), and ^{31}P (162.0 MHz)]. Three types of 2-D n.m.r. experiments were carried out. The homonuclear scalar correlation spectrum (COSY) was obtained according to the procedure of Aue *et al.*[14]. The distortionless enhancement by polarization transfer (DEPT) spectrum was prepared according to Doddrell *et al.*[15]. The heteronuclear COSY experiments were carried out according to Maudsley and Ernst[16] and Bax and Morris[17]. Representative spectral parameters are as follows: For the spectrum of Fig. 2, the 256 t_1 experiments were performed with 32 scans and 2K data points in t_2. Data were zero-filled once in the ω_1 dimension and were matrix-processed in the magnitude mode using sine-bell window functions in both dimensions. Digital resolution along ω_1 and ω_2 was 1.34 and 10.4 Hz, respectively. For the spectrum of Fig. 3, the 256 x 2K data matrix (NS = 16) was processed in the magnitude mode, using sine-bell functions in both directions. Digital resolution along ω_1 and ω_2 was 3.32 and 1.59 Hz, respectively.

Chemical studies. — These were performed using a 0.1M solution of **1** in a 10% D_2O–H_2O mixture. The reactions were monitored at the ^{13}C frequency [50.3 MHz] on a Bruker WP200 instrument operating in the proton-decoupled mode at ambient probe temperature. The sodium borohydride treatment consisted of the addition of the solid reagent (7 equivalents of hydride) to the solution at 0°, with reaction allowed to proceed for 3 h at 5° before the spectrum was acquired. This is an adequate time for at least some of any free carbonyl group to be reduced. The sodium periodate treatment consisted of the addition of a solution of the reagent (5 equivalents) to the solution of **1** at 25° and the reaction allowed to proceed for 30 min before the spectrum was acquired. The sample was then stored for one month at 5° for later acquisition of spectra in order to study the final product composition. Control experiments with 2,5-anhydro-D-mannitol and its 1,6-bisphosphate (**4**) showed that these conditions were adequate to oxidize a *trans*-vicinal glycol unit and to cause a β-elimination of the phosphate group.

Molecular mechanics. — Studies of the conformation of **1** were done by using a structure built-up from tetrahydrofuran which was obtained from the Cambridge database. The absolute stereochemistry of the resulting structure was checked at each

chiral carbon atom. The conformational analysis was conducted while fixing the furanose ring in the $^4T_3$ (D) conformation, as this ring conformation has been observed for all 2-hexuloses in their X-ray crystal structures, and was shown to be the enzymically active conformer in certain cases[3,18,19]. Both molecular building and conformational analyses were carried out using commercial software (Tripos Sybyl 3.5 and 5.1) for force-field calculations. The conformational search was done in two stages: the first involving the simultaneous rotation about bonds O-2–C-2, C-1–C-2, and C-3–O-3. Each bond was rotated in 5° increments from 0 to 360°, while fixing the geometry of the rest of the molecule. Initially, the phosphate groups were assumed not to be protonated, and the lowest conformational energy without charges was calculated. Subsequently, a charge of −0.66 was added to each phosphate non-ester oxygen, the Pullman electrostatic charges calculated[20], and the conformational search reinitiated. After the lowest energy was found for the three bonds around the P-2 phosphate, a similar conformational analysis about O-6–C-6 and C-6–C-5 was carried out.

RESULTS AND DISCUSSION

Two-dimensional n.m.r. studies. — Proton J-connectivities and subspectra. Shown in Fig. 1 are the 1-D highdigital resolution and the 2-D COSY spectra of **1**. Integration of the 1-D spectrum reveals a total of seven non-exchangeable protons. The proton *J*-connectivities shown in the COSY spectrum reveal two subspectra. These are a downfield five-proton AHM(XZ) spin-system and an upfield two-proton A'B' spin-system. As has been previously pointed out[21], such a 5 + 2 pair of subspectra is characteristic of 2-hexuloses. The tightly-coupled M(XZ) part of the five-spin subspectrum is also very characteristic of 2-hexuloses and their derivatives[22]. Both the J_{AH} and J_{HM} vicinal coupling-constants of the AHM(XY) system are seen te be large (> 5 Hz). Thus, the orientation of both A to H and H to M must be closely *trans*-periplanar and the stereochemistry of the 2-hexulose must be either D-*arabino* or L-*arabino*[23]. Given the structure of the starting material (**3**), **1** must be a D-*arabino*-2-hexulose. Thus, the constitution and configuration of the monosaccharide core of **1** have remained intact during synthesis from D-fructofuranose 1,6-bisphosphate (**3**).

Carbon protonation, connectivities, and subspectra. The 1-D ^{13}C spectrum of **1**, which indicates that it contains six carbons, is partially shown in Fig. 2. Determination of the protonation of these six carbons through a DEPT experiment (not shown) reveals that the two upfield carbons are methylenes, the most downfield carbon is unprotonated, and the remaining three carbons are methines. In order to establish the proton–carbon *J*-connectivities of the protonated carbons of **1**, a 2-D heteronuclear COSY experiment was carried out (Fig. 2). This study unambiguously connected the carbon resonances at 78.48, 76.54, 81.07, and 66.55 p.p.m. to the AHM(XY) proton subspectrum and the carbon resonance at 63.79 p.p.m. to the A'B' proton subspectrum. In all cases, the protonation of each carbon was correctly accounted for by the number of ^1H–^{13}C connectivities. Thus, the AHM(XY) proton subspectrum must arise from a –CH–CH–CH–CH_2– fragment in **1**, which for a 2-hexulose can only be the structure

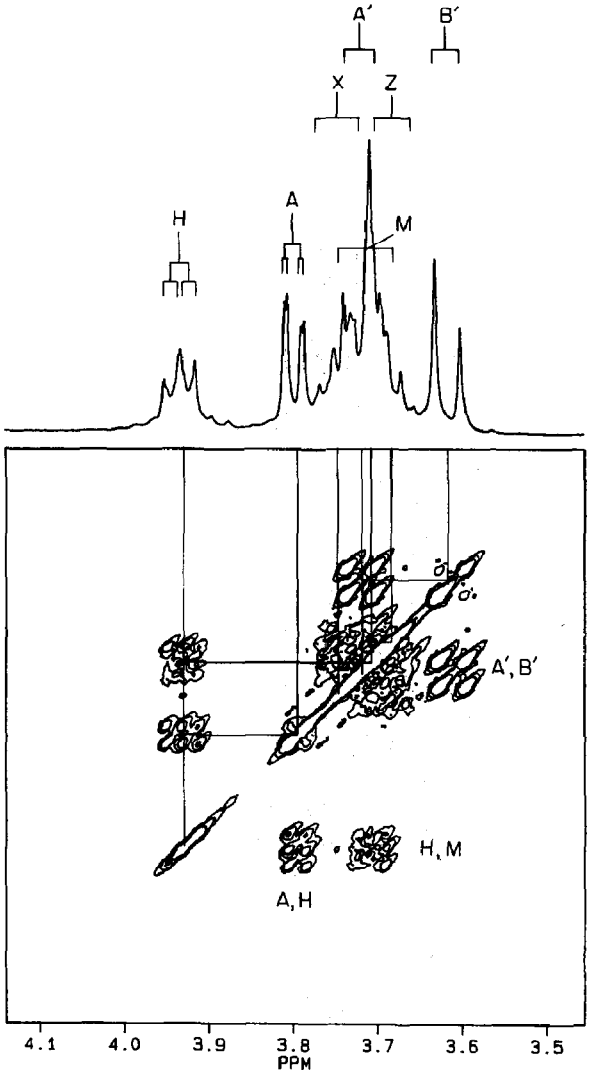
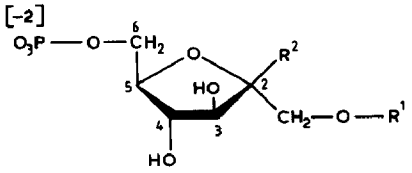


Fig. 1. 1-D ^1H and 2-D scalar ^1H–^1H shift-correlated n.m.r. spectrum of D-fructose 2,6-bisphosphate.

associated with the last four carbons of the molecule (C-3 through C-6). Likewise, the A'B' subspectrum must arise from the C-1 $-CH_2-$ fragment and the unprotonated carbon (104.84 p.p.m.) must be the C-2 anomeric carbon. The extreme downfield shift of this anomeric carbon indicates that **1** has a β-fructofuranose ring[3]. Having thus allocated all seven protons and six carbons to these three structural fragments, complete proton and carbon resonance assignments can be made (Table I). These assignments are confirmed by their similarity with the analogous carbon and proton resonances in other 2-hexuloses (ketohexoses) and related 2,5-anhydrohexitols, especially[3,24–26] **4**.

Phosphorus resonances and couplings. Having established that the carbon and proton skeleton of **1** are unchanged from its precursor **3**, it is clear that the novelty of **1**



1 $R^1 = H, R^2 = OPO_3[-2]$
2 $R^1 = H, R^2 = OH$
3 $R^1 = PO_3[-2], R^2 = OH$
4 $R^1 = PO_3[-2], R^2 = H$

lies in the redisposition of its phosphate groups. Integration of the 1-D ^{31}P spectrum of **1** (Fig. 3, ^{31}P-axis) shows two resonances at 0.35 and 4.77 p.p.m. In order to establish the proton–phosphorus connectivity of these two phosphorus nuclei, a 2-D heteronuclear COSY experiment was carried out (Fig. 3). This study showed that the ^{31}P nucleus at 4.77 p.p.m. coupled strongly ($J > 5$ Hz) to two protons, H-6a and H-6b, and the ^{31}P nucleus at 0.35 p.p.m. coupled strongly to no protons. Also seen were weak couplings ($J < 2$ Hz) for the downfield ^{31}P nucleus to H-5 and for the upfield nucleus to H-3. The finding that the downfield ^{31}P is strongly coupled to the H-6 protons indicates that the resonance at 4.77 p.p.m. arises from a C-6 phosphorylated hydroxymethyl group. The failure to find any proton–phosphorus coupling to H-4 rules out any 4,6-cyclic phosphate diester.

The finding that the upfield ^{31}P nucleus fails to manifest any strong coupling is

TABLE I

N.m.r. chemical-shift data and assignments of **1**

$^1H^a$		$^{13}C^b$		$^{31}P^c$	
3.95	H-4	104.84	C-2	4.77	P-6
3.80	H-3	81.07	C-5	0.35	P-2
3.73	H-1b	78.48	C-3		
3.72	H-6b	76.54	C-4		
3.71	H-5	66.55	C-6		
3.69	H-6a	63.79	C-1		
3.62	H-1a				

The observable ^1H–^1H spin-couplings (in Hz) are:
$^2J_{H\text{-}1a,H\text{-}1b} = 12.7$, $^3J_{H\text{-}3,H\text{-}4} = 7.9$, $^3J_{H\text{-}4,H\text{-}5} = 6.7$.
The observable ^1H–^{31}P spin-couplings (in Hz) are:
$^3J_{H\text{-}6a,P\text{-}6}$ and $^3J_{H\text{-}6b,P\text{-}6} = 5.5$,
$^4J_{H\text{-}3,P\text{-}2} = 1.1$, $^4J_{H\text{-}1a,P\text{-}2}$ and $^4J_{H\text{-}1b,P\text{-}2} < 0.1$.
The ^{13}C–^{31}P spin-couplings (in Hz) are:
$^2J_{C\text{-}2,P\text{-}2} = 6.4$, $^2J_{C\text{-}6,P\text{-}6} = 4.2$,
$^3J_{C\text{-}1,P\text{-}2} = 1.2$, $^3J_{C\text{-}3,P\text{-}2} = 3.8$, and $^3J_{C\text{-}5,P\text{-}6} = 6.9$.

[a] In p.p.m. with internal 1,4-dioxane at 3.53 p.p.m. [b] In p.p.m. with internal 1,4-dioxane at 67.40 p.p.m. [c] In p.p.m. with respect to phosphoric acid as external standard. P-2 and P-6 are the phosphates attached to C-2 and C-6 respectively.

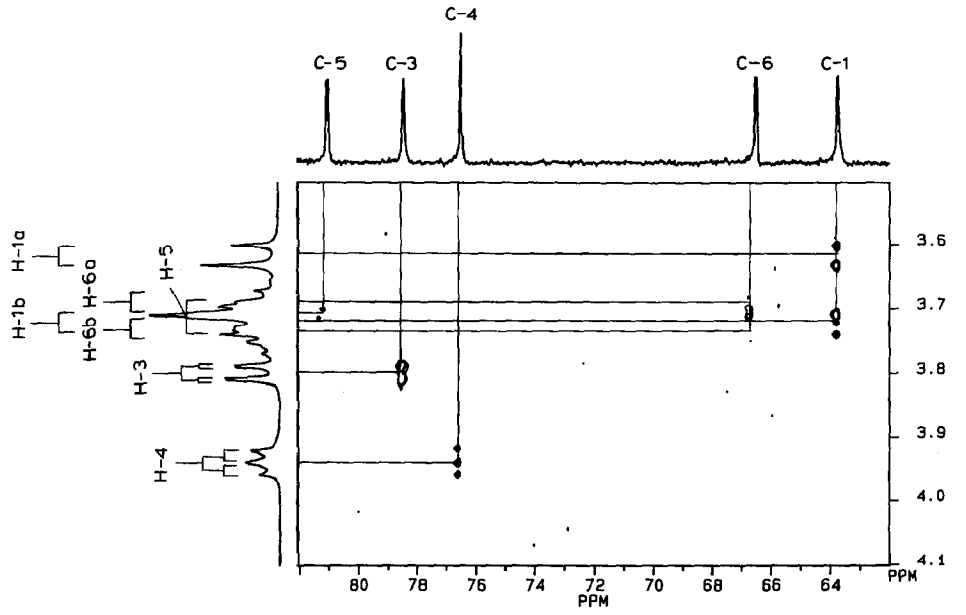


Fig. 2. ^{1}H–^{13}C shift-correlated n.m.r. spectrum of D-fructose 2,6-bisphosphate. The proton–carbon correlation spectrum and the corresponding 1-D spectra along the ω_1 and ω_2 dimensions.

very significant. Since vicinal ($^{3}J_{H,P}$) coupling should always be greater than 2 Hz, its absence indicates that the upfield ^{31}P nucleus is linked to a carbon that fails to present a vicinal proton for ^{31}P coupling. This circumstance can only arise if the linkage carbon is unprotonated. Only one carbon is unprotonated, namely the anomeric carbon C-2. Thus, the upfield ^{31}P resonance must be ester linked to C-2. The failure to find any other proton–phosphorus couplings in Fig. 3 rules out any 1,2- or 2,3-cyclic phosphate diesters. Thus, it may be concluded that all 2-D n.m.r. data are consistent with the assigned structure of the activator, namely β-D-fructofuranose 2,6-bisphosphate.

Chemical studies. — Borohydride reduction. The first chemical study involved the addition of one equivalent of sodium borohydride to the solution of **1** in an attempt to reduce any free anomeric carbon present in the molecule. The initial ^{13}C spectra before the addition contained only the six signals of the six carbons in the molecule, with splittings caused by the phosphate ester groups. After the treatment, the resulting spectra showed no change in any of the original signals and no new signals were observed. These results indicate that the hemiacetal group of the molecule is blocked by a base-stable group. This information is consistent with the O-2 phosphorylation of **1**.

Periodate oxidation. The second chemical study involved the addition of one equivalent of sodium periodate to a fresh solution of **1** in an attempt to oxidize any free vicinal glycol unit or equivalent group present in the molecule. The ^{13}C spectrum before the addition was the same as already described. The resulting spectra showed several new peaks in the methylene region and in the anomeric region. In particular a new peak appeared at ~ 110 p.p.m., indicating the creation of a new hemiacetal carbon. Another

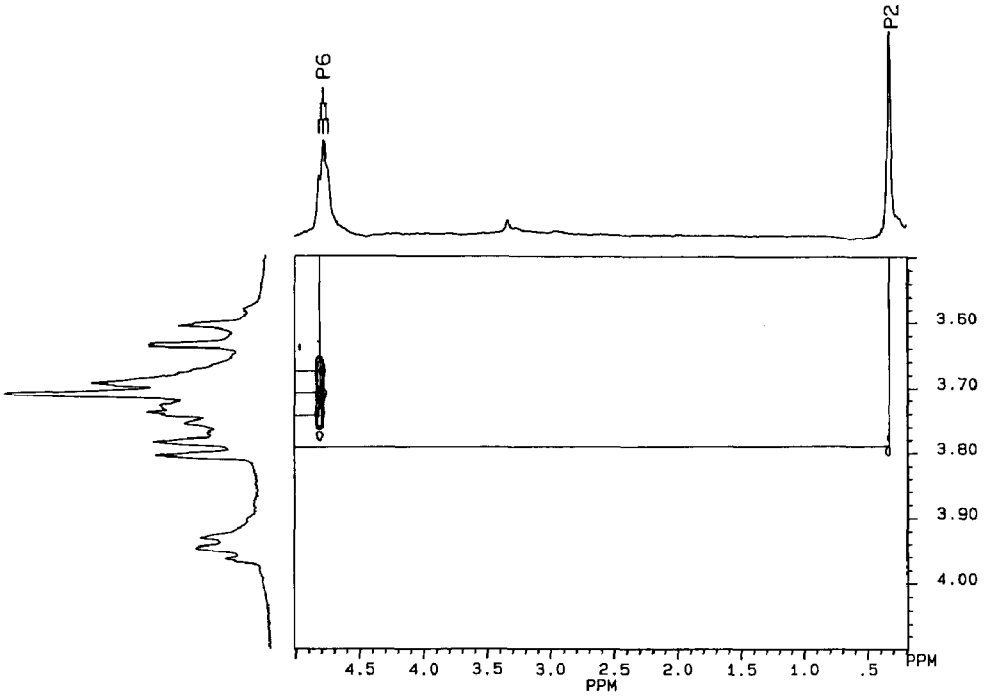


Fig. 3. ^{1}H–^{31}P shift-correlated n.m.r. spectrum of D-fructose 2,6-bisphosphate. The proton–phosphorus correlation spectrum and the corresponding 1-D spectra along the ω_1 and ω_2 dimensions.

spectrum was acquired after an extended period (see Experimental) which showed signals for vinyl carbons, indicating that a β-elimination of the phosphate group on C-6 had occurred. These results show that a vicinal glycol unit is present in **1** and that the molecule also contains a leaving group beta to one of the carbonyl groups of the cleavage product. Such a structural fragment is present in the D-fructofuranose 2,6-bisphosphate structure, but not in other possible structures such as the 3,6-bisphosphate. In summary, the chemical studies corroborate the findings obtained from the 2-D n.m.r. studies.

Unexpected couplings. — The fact that **1** is phosphorylated at C-6 is confirmed by the observed ^{13}C–^{31}P couplings. Both $^{2}J_{C\text{-}6,P\text{-}6} = 4.2$ and $^{3}J_{C\text{-}5,P\text{-}6} = 6.9$ Hz are equal to the values expected from studies of many ketohexose phosphates[3]. However, the conclusion that **1** is phosphorylated at C-2 yields ^{13}C–^{31}P couplings that are anomalous. Thus the observed $^{2}J_{C\text{-}2,P\text{-}2} = 6.4$ Hz is 2 Hz larger than the value reported for other ketohexose phosphates. Moreover, the vicinal couplings $^{3}J_{C\text{-}1,P\text{-}2} = 1.2$ and $^{3}J_{C\text{-}3,P\text{-}2} = 3.8$ Hz are at least 5 and 2 Hz smaller, respectively, than expected. The finding that $^{2}J_{C\text{-}2,P\text{-}2}$ is larger than expected can be explained as due to the effect of the C-2 carbon lying within the fructofuranose ring, adjacent to the ring oxygen. No $^{2}J_{C,P}$ has previously been reported in such an environment. The unexpected $^{3}J_{C,P}$ values are more problematic and may only be explained if the P-2–C-1 and P-2–C-3 dihedral angles are unusual. This turned out to be the case (see later).

Another coupling problem that results from the assignment of a 2,6-bisphosphate structure (**1**) to the activator is that it means the ^{1}H–^{31}P coupling at H-3 and H-5 must result from a four-bond or long-range coupling. Such long-range couplings are certainly possible; however, it is not clear why the two protons at H-1 do not also participate in such coupling. In order to explain the unexpected P-2 vicinal couplings, as well as the weak ^{4}J proton–phosphorus couplings observed at H-3 and H-5 but not at the H-1 protons, we undertook a molecular-mechanics study of the *β*-D-fructofuranose 2,6-bisphosphate structure.

Molecular-mechanics studies. — Shown in Fig. 4 is the lowest energy conformation of *β*-D-fructofuranose 2,6-bisphosphate (**1**), as determined by molecular-mechanics calculations. The calculated dihedral angles of pertinent fragments of **1** are presented in Table II for comparison with n.m.r. coupling constants. These angles have been sequentially numbered in Table II for convenience in reference.

Considering the problem of the unexpected ^{13}C–^{31}P vicinal coupling-constants, it is seen that the pertinent angles 3 and 6 are in fact different from the $\sim 180°$ angle expected for POCC dihedral angles[3] and actually observed for angle 1 of **1**. This difference is seen even more clearly for the angles computed after introducing the Pullman charges in the calculation. Angles 1, 3 and 6 are then seen to be $+175$, $+84$, and $-155°$, respectively, and therefore closely follow a Karplus relation with vicinal ^{13}C–^{31}P couplings of 6.9, 1.2, and 3.8 Hz, respectively. Thus, the unexpected ^{13}C–^{31}P vicinal couplings may be rationalized as resulting from deviations from *trans*-periplanarity in POCC dihedral angles, a situation unavoidable with 2-phosphorylation, but previously not encountered in ketohexose phosphates[3].

Concerning the problem of selective ^{1}H–^{31}P long-range coupling, it is important to recall the established requirement for such coupling[27], namely that it is confined to a

TABLE II

Dihedral angles present in the lowest energy conformation of *β*-D-fructofuranose 2,6-bisphosphate obtained from molecular-mechanics calculations, and comparison with observed ^{31}P couplings of **1**

Angle No.	*Four atom fragment*	*Dihedral angle*[a]	*Observed coupling constants (Hz)*	
			^{13}C–$^{31}P(^{3}J)$	^{1}H–$^{31}P(^{4}J)$
1	P-6, O-6, C-6, C-5	$+175°$	6.9	
2	O-6, C-6, C-5, H-5	$-174°$		<2[b]
3	P-2, O-2, C-2, C-1	$+69°$ ($+84°$)	1.2	
4	O-2, C-2, C-1, H-1a	$-80°$		<0.1
5	O-2, C-2, C-1, H-1b	$+160°$		<0.1
6	P-2, O-2, C-2, C-3	$-170°$ ($-155°$)	3.8	
7	O-2, C-2, C-3, H-3	$-153°$		1.1

[a] Angles calculated using force-field equations (Sybyl 3.5). Angles in parentheses were calculated using Pullman charges (Sybyl 5.1). Angles are accurate to $+/-3°$, as calculations were carried out at 5° increments. [b] Coupling is clearly present in the ^{1}H–^{31}P COSY experiment (Fig. 3); however, accurate measure of ^{4}J is not possible due to overlapping resonances in the high-resolution proton spectrum.

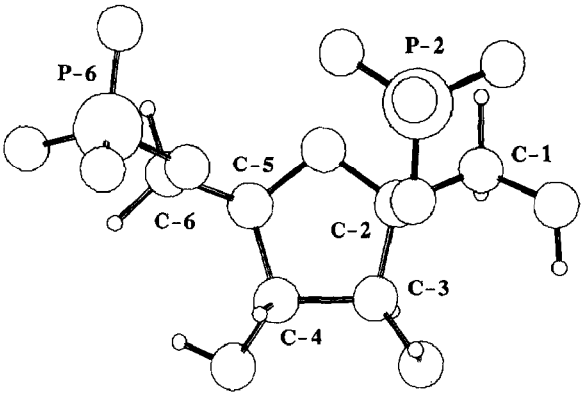


Fig. 4. The lowest-energy conformation of β-D-fructose 2,6-bisphosphate obtained through Sybyl force-field calculations.

near-planar zig-zag configuration for the five atoms involved. Such a "W-configuration" is in fact calculated for the P-2, O-2, C-2, C-3, H-3 fragment, since both angles 6 and 7 are within 30° or less of *trans*-periplanarity (−170 and −153°, respectively). On the other hand, no "W-configuration" is calculated for P-2, O-2, C-1, H-1a, or H-1b with at least angle 3 for both being very non-planar (+84°). Thus, the longe-range ^{1}H–^{31}P coupling seen for H-3, but not H-1a and H-1b, can be explained by the unique orientation between P-2 and H-3 that is found in the lowest energy conformation of β-D-fructofuranose 2,6-bisphosphate. In fact, this "W-configuration" is so difficult to create that the observation of $^{4}J_{H\text{-}3,P\text{-}2}$ coupling can be used as a confirmation of the β-anomeric configuration of **1**. It is impossible to create a "W-configuration" between P-2 and H-3 in the α-anomer ($^{4}J_{H\text{-}1,P\text{-}2}$ would be observed in this case). Finally, it should be noted in Table II that both angles 1 and 2 are calculated to be within 10° or less of *trans*-periplanarity (+175 and −174°, respectively). Thus, the long-range coupling observed between H-5 and P-6 may also be rationalized in terms of the "W-configuration".

In conclusion, this investigation of the structure of **1** using 2-D n.m.r. techniques confirms the previously proposed β-D-fructofuranose 2,6-bisphosphate structure. The anomalous ^{13}C–^{31}P couplings have been rationalized through molecular-mechanics studies that show these couplings to be expected for the β-D-fructofuranose $^{4}T_{3}$ 2,6-bisphosphate structure.

ACKNOWLEDGMENT

This investigation was supported in part by grants No. AM31676 (to ESY) and No. DK25295 (to TAWK) from the National Institutes of Health and financial support from The College of Basic Sciences at LSU. The purchase of the 400-MHz n.m.r. spectrometer was made possible through an NIH shared instrumentation grant No. RR02459.

REFERENCES

1 E. van Schaftingen, L. Hue, and H. G. Hers, *Biochem. J.*, 192 (1980) 897–901.
2 T. A. W. Koerner, Jr., L. W. Cary, N. S. Bhacca, and E. S. Younathan, *Biochem. Biophys. Res. Commun.*, 51 (1973) 543–550.
3 T. A. W. Koerner, Jr., R. J. Voll, L. W. Cary, and E. S. Younathan, *Biochemistry*, 19 (1980) 2795–2801.
4 T. H. Claus, J. R. Schlumpt, M. R. El-Maghrabi, J. Pilkis, and S. J. Pilkis, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 6501–6505.
5 S. J. Pilkis, M. R. El-Maghrabi, J. Pilkis, T. H. Claus, and D. A. Cumming, *J. Biol. Chem.*, 256 (1981) 3171–3174.
6 E. Furuya and K. Uyeda, *J. Biol. Chem.*, 255 (1980) 11656–11659.
7 K. Uyeda, E. Furuya, and A. D. Sherry, *J. Biol. Chem.*, 256 (1981) 8679–8684.
8 M. Kuwajima and K. Uyeda, *Biochem. Biophys. Res. Commun.*, 104 (1982) 84–88.
9 H. G. Hers, J. Francois, and E. Schaftingen, *Curr. Top. Cell. Reg.*, 27 (1985) 399–410.
10 M. R. El-Maghrabi and S. J. Pilkis, *J. Cell. Biochem.*, 26 (1984) 1–17.
11 R. L. Heath and J. Preiss (Eds.), *Regulation of Carbon Partitioning in Photosynthetic Tissue*, Am. Soc. Plant Physiologists, Rockville, Maryland, 1985.
12 E. L. Kelley, R. J. Voll, V. A. Voll, and E. S. Younathan, *Biochemistry*, 25 (1986) 1245–1248.
13 A. M. Hesbian-Frisque, E. van Schaftingen, and H. G. Hers, *Eur. J. Biochem.*, 117 (1981) 325–327.
14 W. P. Aue, E. Bartholdi, and R. R. Ernst, *J. Chem. Phys.*, 64 (1976) 2229–2246.
15 D. M. Doddrell, D. T. Pegg, and M. R. Bendall, *J. Magn. Reson.*, 48 (1982) 323–327.
16 A. A. Maudsley and R. R. Ernst, *Chem. Phys. Lett.*, 50 (1977) 368–372.
17 A. Bax and G. A. Morris, *J. Magn. Reson.*, 42 (1981) 501–505.
18 P. Swaminathan, L. Anderson, and M. Sundaralingam, *Carbohydr. Res.*, 75 (1979) 1–10.
19 E. S. Younathan, R. J. Voll, and T. A. W. Koerner, Jr., in C. M. Veneziale (Ed.), *The Regulation of Carbohydrate Formation and Utilization in Mammals*, University Park Press, Baltimore, Maryland, 1981, pp. 69–98.
20 H. Berthod and A. Pullman, *J. Chim. Phys.*, 62 (1965) 942–946.
21 T. A. W. Koerner, Jr., J. H. Prestegard, and R. K. Yu, *Methods Enzymol.*, 138 (1987) 38–59.
22 T. A. W. Koerner, Jr. (1975), Ph.D. Dissertation, Louisiana State University, Baton Rouge.
23 C. Altona and C. A. G. Haasnoot, *Org. Magn. Res.*, 13 (1980) 417–429.
24 T. A. W. Koerner, Jr., R. J. Voll, and E. S. Younathan, *Carbohydr. Res.*, 59 (1977) 403–416.
25 T. A. W. Koerner, Jr., R. J. Voll, and E. S. Younathan, *Carbohydr. Res.*, 70 (1979) 165–168.
26 R. J. Voll, T. A. W. Koerner, Jr., P. A. Bartlett, N. S. Bhacca, D. C. Lankin, and E. S. Younathan, *Carbohydr. Res.*, 95 (1981) 145–154.
27 L. M. Jackman and S. S. Sternhell, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, 2nd edn., Pergamon Press, New York, 1969, pp. 334–341.