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NUCLEAR MAGNETIC RESONANCE STUDIES OF D-ERYTHROSE 4-PHOSPHATE IN AQUEOUS SOLUTION. STRUCTURES OF THE MAJOR CONTRIBUTING MONOMERIC AND DIMERIC FORMS

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ABSTRACT

N.m.r. studies show that a concentrated (~1.0M) aqueous solution of D-erythrose 4-phosphate is composed of an equilibrium mixture of the monomeric aldehyde and hydrated aldehyde forms, which interconvert rapidly, together with a major contribution from three dimeric forms. In dilute solutions (~0.04M), the hydrated monomer is predominant and dimeric forms are not detectable at this concentration. The chemical structures and stereochemistry of the three dimers have been elucidated by ^{1}H- and ^{13}C-n.m.r. spectroscopy of D-erythrose 4-phosphate and its (4,4-$^{2}H_2$) and (3,4,4′-$^{2}H_3$) derivatives, aided by the use of the model compounds, glycolaldehyde, D-glyceraldehyde, and DL-glyceraldehyde 3-phosphate, which also form dimers in concentrated aqueous solution. In some cases, the *tert*-butyldimethylsilyl derivatives of the model dimeric compounds were prepared and isolated. The two major dimers of D-erythrose 4-phosphate, I and II, are asymmetrically substituted 1,3-dioxane and 1,3-dioxolane structures, respectively, and dimer III is the α anomer of dimer I.

INTRODUCTION

D-Erythrose 4-phosphate has been inferred to play a key role in the "classical" non-oxidative pentose pathway[1], in the reaction sequence of the new pentose pathway[2], and in the path of carbon in photosynthesis[3], despite the fact that it has never been convincingly detected and characterized in any tissue. The studies of Kornberg and Racker[4], in which synthetic D-erythrose 4-phosphate was added to transaldolase and D-fructose 6-phosphate to produce heptulose 7-phosphate and triose 3-phosphate, enabled the hypothetical biogenetic "four-carbon fragment" to be confidently assumed to be erythrose 4-phosphate.

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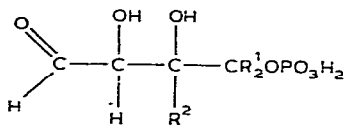
Tentative chromatographic evidence for the presence of erythrose 4-phosphate in *Chlorella* has been presented. Moses and Calvin[5], after examining the ^{14}C metabolites formed in *Chlorella* after 3 min of $^{14}CO_2$ fixation, observed the presence of two chromatographically different ^{14}C-phosphorylated products, both of which, after dephosphorylation, cochromatographed with authentic erythrose.

The phenomenon of erythrose 4-phosphate behaving as more than one compound is also observed when erythrose 4-phosphate is purified by ion-exchange chromatography following its synthesis from D-glucose 6-phosphate by oxidation with lead tetraacetate[6]. Paper chromatography of erythrose 4-phosphate preparations gives two spots of widely differing R_f value, consistent with the proposed presence of monomeric and dimeric forms of this compound. Conclusive proof of the existence of dimeric forms of erythrose 4-phosphate was afforded by the mass spectrum of a trimethylsilylated, freeze-dried preparation of the compound, which showed ions corresponding to an octa-Me_3Si derivative of an erythrose 4-phosphate dimer (or dimers)[7]. Since this study was commenced, dimeric forms of D-erythrose itself have been isolated as acetates. The three major, dimeric compounds were assigned 1,3-dioxane and 1,3-dioxolane structures from a study of their ^1H-n.m.r. spectra[8]. A recent ^1H-n.m.r. study (ref. 9) of a concentrated solution of syrupy D-erythrose in Me_2SOD_2O (4:1) confirms the presence of dimeric forms of this compound, and the ^{13}C-n.m.r. spectrum of a concentrated, neutral solution of [1-^{13}C]-enriched D-erythrose has been reported to contain C-1 signals corresponding to dimeric or oligomeric forms[10].

The present study was undertaken to elucidate by n.m.r. spectroscopy the structures of the different monomeric and dimeric forms of D-erythrose 4-phosphate present in aqueous solution.

RESULTS AND DISCUSSION



1 , $R^1 = R^2 = H$
1a, $R^1 = D$; $R^2 = H$
1b, $R^1 = R^2 = D$

^1H- And ^{13}C-n.m.r. studies showed that dilute aqueous solutions of D-erythrose 4-phosphate at equilibrium consisted largely of monomer aldehyde **1** and its hydrated form **2**. As the concentration of D-erythrose 4-phosphate was increased from ~0.04 to 1.0M, the proportion of monomers **1** and **2** decreased relative to other forms of D-erythrose 4-phosphate (Fig. 1), which were found to convert back into monomers **1** and **2** on dilution (to ~0.04M) over a period of several hours at 20°. Because of earlier studies on D-erythrose 4-phosphate[6,7], glycolaldehyde[11–15], and other similar systems[14–16], these other forms were suspected of being dimeric. This was established by analyzing the ^1H- and ^{13}C-n.m.r. spectra of aqueous solutions of D-erythrose 4-phosphate recorded at various concentrations and pH. Preparation of D-(4,4'-$^2H_2$)-

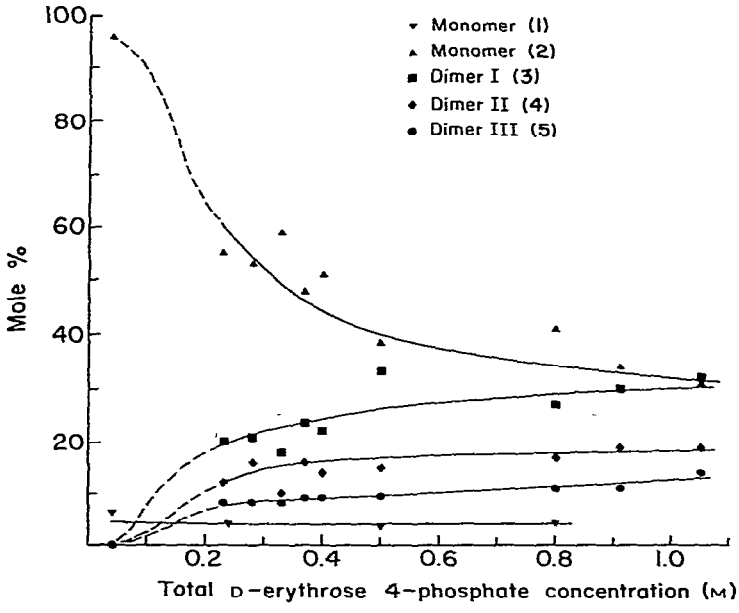


Fig. 1. Relative contributions of the major monomeric and dimeric forms of D-erythrose 4-phosphate present at equilibrium in aqueous solution (D_2O, pD 1.4) at varying dilutions, as measured by ^1H- and ^{13}C-n.m.r.

and (3,4,4′-$^2H_3$)-erythrose 4-phosphate (**1a** and **1b**, respectively) provided greatly simplified ^1H- and ^{13}C-n.m.r. spectra, and made the presence of the monomeric and dimeric forms more obvious.

Detection and assignment of structures to the dimers were complicated by the fact that they could not be isolated pure because of the interconversion between monomers and dimers and possibly between different dimers. Therefore, the system was studied as an equilibrium mixture. A further limitation was the stability of D-erythrose 4-phosphate, which decomposes rapidly in aqueous solution above pH 6 and is unstable at higher temperatures (>40°). Assignment of the signals to each of the major dimers present was initially made on the basis of intensities in the ^{13}C- (ref. 16) and ^1H-n.m.r. spectra of the respective, low-field anomeric carbon atoms (C-1 and C-1′) and attached protons (H-1 and H-1′). For D-erythrose 4-phosphate, three classes could be compared internally; anomeric, secondary hydroxyl, and methylene carbon atoms. In the ^1H-n.m.r. and to some extent in the ^{13}C-n.m.r. spectra (Fig. 2), assignment of the signals to the protons and carbon atoms of all major, contributing forms was complicated by the overlap of signals because of the narrow chemical-shift ranges (δ 3.9–6.2 for protons, δ 64–103 for carbon atoms), and because of coupling. This problem could not be resolved by high-field n.m.r., but was simplified by the use of the deuterated analogues **1a** and **1b**. Their n.m.r. spectra (Fig. 3) enabled assignment of the remaining protons and carbon atoms (without deuterium attached) to the major dimers in the equilibrium mixture. The fully proton-coupled ^{13}C-n.m.r. spectrum of D-(3,4,4′-$^2H_3$) erythrose 4-phosphate

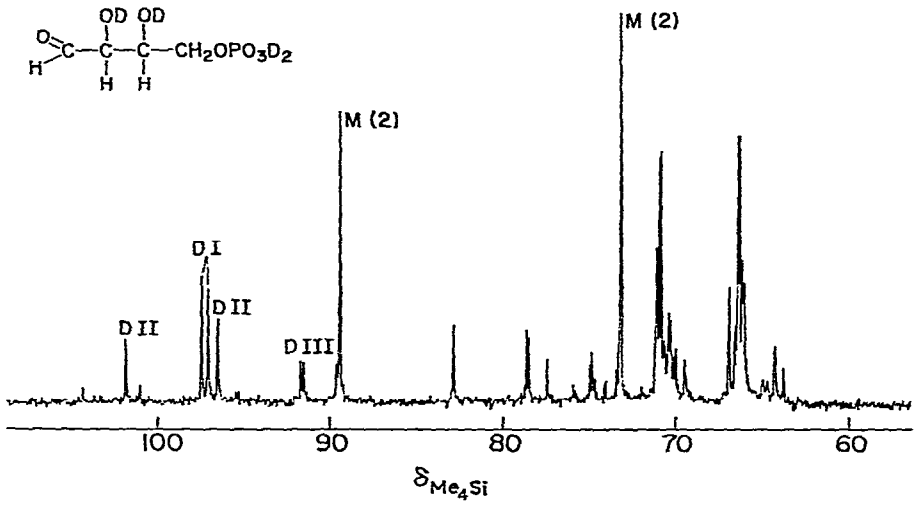


Fig. 2. ^{13}C-n.m.r. (67.89 MHz, proton decoupled) spectrum of D-erythrose 4-phosphate in D_2O ($c \sim 0.3$M, pD 5.4).

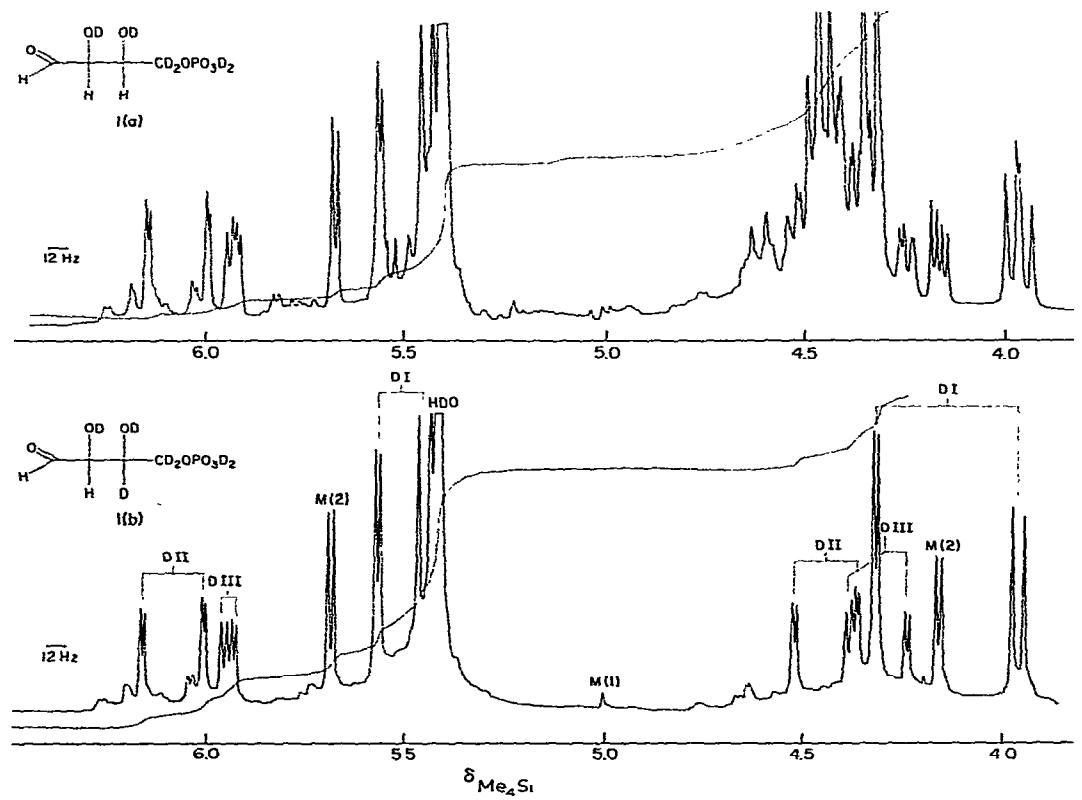


Fig. 3. ^{1}H-n.m.r. (270 MHz) spectra of D_2O solutions ($c \sim 0.5$M, pD 5.4) of the deuterated D-erythrose 4-phosphate derivatives **1a** and **1b**.

TABLE I

^{1}H-N.M.R. CHEMICAL-SHIFT VALUES[a] AND COUPLING CONSTANTS OF THE MAJOR CONTRIBUTING FORMS OF D-ERYTHROSE 4-PHOSPHATE IN D_2O SOLUTION[b,c]

Compound	*pD*	*H-1* *H-1'*	$J_{1,2}$ *(Hz)* $J_{1',2'}$ *(Hz)*	*H-2* *H-2'*	$J_{2,3}$ *(Hz)* $J_{2',3'}$ *(Hz)*	*H-3* *H-3'*
monomer **1**	1.4	10.3				
	5.4	10.3	~0	5.00	5.0	
monomer **2**	1.4	5.66	3.7	4.13	7.7	4.43
	5.4	5.683	3.7	4.138	7.2	4.399
dimer I **3**	1.4	5.42	6.8	3.92	9.4	4.34
		5.54	2.8	4.32	6.8	4.49
	5.4	5.445	8.0	3.935	9.1	4.309
		5.555	2.9	4.300	7.9	4.426
dimer II **4**	1.4	6.13	2.2	4.50		
		5.98	1.8	4.34		
	5.4	6.156	2.9	4.500	8.1	
		5.995	2.5	4.335	8.0	
dimer III **5**[d]	1.4	5.92	3.3	4.21	8.5	
		5.92	4.1	4.35		
	5.4	5.918	3.0	4.219	6.2	
		5.952	3.8	4.361	6.2	

[a]Measured in p.p.m. relative to an external standard of Me_4Si. [b]In discussions in text, δ and J values at pD 5.4 are used, as these are more accurate. [c]Only in the case of monomer **2** could assignments be made to the methylene protons. [d]All pairs of assignments could be reversed.

was simplified to such an extent that, for the monomers, only one-bond and two-bond proton coupling was observed. In the dimers, three-bond couplings through oxygen were also present.

For carbohydrates in general, it has been found that five- and six-membered rings are favored in cases where seven- or eight-membered rings are also possible[17,18a]. Linear dimers were not considered to contribute, as they would be expected to have only a transient existence[12]. With D-erythrose, it has been found by g.l.c. of the acetates and by n.m.r. analysis that major contributing forms in concentrated solution are the monomeric α and β furanoses and dimeric 1,3-dioxane and 1,3-dioxolane forms[8,9]. No cyclic, monomeric, stable forms of D-erythrose 4-phosphate are possible.

Other small monosaccharides having an α-hydroxyaldehydic function and which cannot form cyclic monomers, for example, glycolaldehyde[9,11-13], have been shown to dimerize in concentrated aqueous solution. As part of this study, we have looked at the dimeric forms of D-glyceraldehyde and DL-glyceraldehyde 3-phosphate and extended the work on glycolaldehyde as an aid in making n.m.r. structural assignments to the D-erythrose 4-phosphate dimers. Interestingly, D-erythrose 4-phosphate shows a greater propensity to dimerize than do any of the two- and three-carbon sugars studied.

Structures of the major contributing forms of D-*erythrose 4-phosphate in aqueous*

solution. — *Monomer* (**1**). This simplest monomeric form was readily detected by ^{1}H-n.m.r. as a singlet at δ 10.3 for the aldehydic proton at pD 5.4 and, less readily, at pD 1.4 as a broad singlet. Spin-saturation experiments[19] showed that this form (**1**) was in equilibrium with the hydrated monomer **2** in aqueous solution. A tentative assignment only could be made for H-2 (Table I), because of its low concentration (Fig. 1) and broadening[20] through rapid interconversion with monomer **2**. The kinetics of this interconversion are discussed later.

In the ^{13}C-n.m.r. spectra, the signals arising from monomer **1** were very weak, making assignments of C-2, C-3, and C-4 difficult (Table II), whereas C-1 was not observed in many spectra as it was outside the recorded spectral width. Exchange broadening for C-1 was small[21a], as the frequency separation between it and C-1 of **2** was large (1838 Hz at 15.1 MHz) relative to that between H-1 of **1** and H-1 of **2** (276 Hz at 60 MHz). For C-2 and C-3 of **1**, where the frequency separations from C-2 and C-3 of **2** were small (61 and 56 Hz at 15.1 MHz, respectively), line broadening was observed for the carbon atoms of monomeric **1** at pD 1.4.

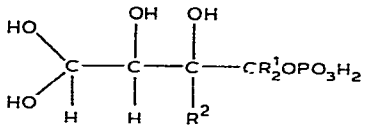
Monomer (**2**). ^{1}H- And ^{13}C-n.m.r. studies showed that this hydrated form of monomer **1** was the most abundant monomeric form present in aqueous solution, varying from ~93% of the total in dilute solution to ~30% in concentrated solution (Fig. 1), thus permitting ready assignment of its n.m.r. signals.

TABLE II

^{13}C-N.M.R. CHEMICAL-SHIFT VALUES[a] AND CARBON–PHOSPHORUS COUPLING CONSTANTS OF THE MAJOR CONTRIBUTING FORMS OF D-ERYTHROSE 4-PHOSPHATE IN D_2O SOLUTION[b]

Compound	*pD*	*C-1* *C-1′*	*C-2* *C-2′*	*C-3* *C-3′*	J_{CCOP} *(Hz)*	*C-4* *C-4′*	J_{COP} *(Hz)*
monomer **1**	1.4	211.8	77.51[d]	74.69[d]	7.3		
	5.4	[c]	77.88[d]	75.18[d]	7.3		
monomer **2**	1.4	89.95	73.50	70.97	7.3	67.97	5.9
	5.4	90.00	73.63	71.33	7.3	66.80	5.1
dimer I **3**	1.4	98.00	67.26	78.52	6.6	65.75	4.4
		97.56	71.48	70.42	8.1	67.77	5.1
	5.4	98.04	67.37	79.02	8.1	64.69	5.9
		97.68	71.54	70.79	7.3	66.57	4.4
dimer II **4**	1.4	97.24	83.03	70.06	8.8	~67.7	
		102.48	69.99	74.88	6.7	~67.7	
	5.4	97.11	83.35	70.46	7.4	67.01	5.2
		102.46	69.93	75.34	8.8	66.87	
dimer III **5**	1.4	92.10	64.12	~73.5			
		91.92	71.48	70.69	7.4		
	5.4	92.23	64.20	73.77	7.3	64.46	5.2
		91.88	71.54	71.08	8.1	66.41	5.1

[a]In p.p.m., relative to Me_4Si. [b]In discussions in text, δ and J values at pD 5.4 are quoted, as these are more accurate. [c]Outside measured spectral width. [d]Tentative only.

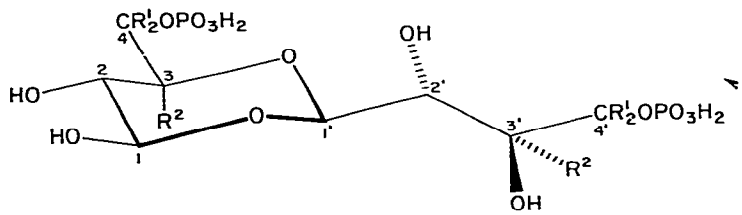


2 , $R^1 = R^2 = H$
2a, $R^1 = D; R^2 = H$
2b, $R^1 = R^2 = D$

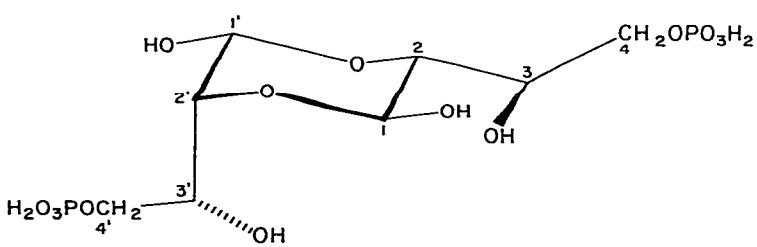
The H-1 signal of **2** showed a chemical shift (δ 5.68) characteristic of a *gem*-diol formed by the hydration of an aldehyde[22,23], which was consistent with that observed for model systems (for example, hydrated D-glyceraldehyde, δ 5.48, see Experimental section). Inspection of the spectra of **2**, **2a**, and **2b** (Fig. 3) and decoupling experiments enabled the assignments of H-2 and H-3 and the measurement of $^3J_{1,2}$ and $^3J_{2,3}$ (Table I) to be made.

The ^{13}C-n.m.r. chemical shift of C-1 (δ 90.0) was similar to that reported for the corresponding carbon atom in hydrated erythrose[10] and for the hydrated monomers of model compounds, for example, hydrated DL-glyceraldehyde 3-phosphate, δ 90.2 (see Experimental section). The C-2 signal could be assigned on the basis of the similarity of its chemical shift (δ 73.6) to that of secondary carbon atoms of sugars bearing a hydroxyl group[16]. The C-4 and C-3 signals were readily distinguished by their characteristic two-bond and three-bond couplings[24] with phosphorus (Table II), and are successively "washed out" in the spectra of the deuterated D-erythrose 4-phosphates **2a** and **2b**.

Dimer I (**3**). The molar concentration of the most abundant dimeric form,



3 $R^1 = R^2 = H$
3a $R^1 = D; R^2 = H$
3b $R^1 = R^2 = D$



6

dimer I, varies from 0% in dilute solution to ~30% of the total in concentrated solution (Fig. 1). Pairs of equal intensity signals were observed for each carbon atom and proton in the ^{13}C (Fig. 2) and ^{1}H-n.m.r. spectra (Fig. 3), showing that dimer I was asymmetric. The chemical shifts and couplings in both spectra were consistent with the 1,3-dioxane structure **3**, but initially the 1,4-dioxane structure **6** could not be excluded. Because optically pure D-erythrose 4-phosphate was used in all experiments, the chair forms of any 1,4-dioxane dimers, for example, **6**, must have the opposite stereochemistry at C-2 and C-2', namely, one of the two-carbon substituents must be equatorial and the other axially disposed. The presence of this axial substituent in **6** therefore makes it energetically less favored than the all-equatorial, 1,3-dioxane form **3** for dimer I.

The chemical shift of H-1 of dimer I (δ 5.45, Table I) is similar to that observed for β-D-glucopyranose 6-phosphate (δ 5.15, see Experimental section), which has an equatorial hydroxyl group attached to the anomeric carbon atom. The strong coupling (8.0 Hz) between H-1 and H-2 is comparable to the coupling (7.8 Hz) for

TABLE III

CARBON-PROTON COUPLING-CONSTANTS AND PREDICTED ^{2}J AND ^{3}J VALUES FOR THE CONTRIBUTING MONOMERIC AND DIMERIC FORMS OF D-(3,4,4'-$^{2}H_3$)-ERYTHROSE 4-PHOSPHATE (**1b**) IN D_2O SOLUTION[a]

Compound	*Carbon atom*	δ_{Me_4Si}	^{1}J *(Hz)*	*Observed*	*Predicted*		*Predicted*
				^{2}J *and/or* ^{3}J *(Hz)*	^{2}J *(Hz)*[28]	^{2}J *(Hz)*[27]	$^{3}J^{b}$ *(φ)*[16,21b]
monomer **2**	1	89.95	163.8	2.9			
	2	73.50	144.2	2.0			
dimer I **3**	1	98.00	162.4	5.9, 2.9	−5.7	−5.5	small (60°)
	1'	97.56	164.5	2.6	see rotamers		small (60°)
	2	67.26	147.8	<1.0	<1.0	0	[c]
	2'	71.48	145.2	1.2	see rotamers		[c]
dimer II **4**	1	97.24	177.1	3.3	−3.0	−2.7	very small (~90°)
	1'	102.48	171.3	5.6, mult.	see rotamers		very small (~90°) large (~150°)
	2	83.03	151.5	3.1	+5.0	+5.0	very small (~90°)
	2'	69.99	144.6	7.1	see rotamers		[c]
dimer III **5**	1	92.10	173.4	1.8	<1.0	+2.5	small (60°)
	1'	91.92	167.2	5.5, 2.2	see rotamers		very large (180°)
	2	64.12	147.3	<1.0	<1.0	+2.5	[c]
	2'	71.48	144.0	6.4	see rotamers		[c]
rotamers[d] A	1'				−5.7	−5.5	
B	1'				<1.0	+2.5	
C	1'				<1.0	+2.5	
A	2'				<1.0	0	
B	2'				+7.5	+7.5	
C	2'				<1.0	0	

[a]Measured at pD 1.4, 67.89 MHz. [b]The range of three-bond couplings is reported to be 0 to +7 Hz[16,21b]. [c]No $^{3}J_{CH}$ possible because of deuterium substitution. [d]See Fig. 4.

the *trans* diaxial H-1 and H-2 of β-D-glucopyranose 6-phosphate and to the $J_{1,2}$ value (7.5 Hz) observed for the all-equatorial 1,3-dioxane dimer of acetylated D-erythrose[8].

The shift of H-1′ (δ 5.56) is comparable to that (δ 5.68) of H-1 observed for the hydrated monomer **2** and is consistent with the chemical shift of a proton attached to an acetal carbon (see Table IV). The $J_{1',2'}$ coupling of 2.9 Hz is similar to the $J_{1,2}$ coupling (3.7 Hz) observed for monomer **2**, which is consistent with H-2′ belonging to an acyclic moiety as in **3**. Supporting this, H-2′ (δ 4.30) and H-3′ (δ 4.43) have chemical shifts and coupling ($J_{2',3'}$ 7.9 Hz) comparable with values observed for H-2 (δ 4.14) and H-3 (δ 4.40, $J_{2,3}$ 7.2 Hz) of monomer **2**.

The H-2 signal (δ 3.94) has the highest-field chemical shift in the ^{1}H-n.m.r. spectrum of the D-erythrose 4-phosphate equilibrium mixture. The same situation is observed for H-2 in β-D-glucopyranose[25] and β-D-glucopyranose 6-phosphate (see Experimental section). The strong coupling (9.1 Hz) of H-2 to H-3 in dimer I indicates a *trans*-periplanar arrangement for these two protons, as in **3**.

In the ^{13}C-n.m.r. spectra, because of their similar chemical-shift values, anomeric carbon atoms were assigned on the basis of the carbon–proton couplings (Table III). The chemical shift of C-1 (Table II) is comparable with that observed for C-1 of β-D-glucopyranose 6-phosphate (δ 96.8), supporting the assignment of an equatorial hydroxyl group to C-1.

It has been reported[26] that the one-bond carbon–proton coupling for the anomeric carbon atom and proton in hexopyranoses is ~160 Hz for the β anomer, whereas for the α compound the coupling is ~170 Hz. The one-bond carbon–proton coupling for C-1 (162.4 Hz) indicates that its anomeric hydrogen atom is axial (Table III).

From the two- and three-bond carbon–proton couplings for C-1 and C-2 in D-(3,4,4′-$^2H_3$) erythrose 4-phosphate (**1b**), it may be seen that the predicted two-bond couplings[27,28] fit well with the proposed structure **3** for dimer I if it is assumed that the measured, two-bond couplings have the same sign as the corresponding predicted values (Table III). Much of the merit of two-bond couplings for predicting stereochemistry is lost where the sign of the coupling is unknown, as positive and negative couplings of similar magnitude are possible, depending on the stereochemistry of the substituents on the C–C–H coupling path[28]. Interpretation of the two- and three-bond couplings of C-1′ and C-2′ is further complicated by the rotational flexibility of the acyclic moiety in **3**, which can give rise to three possible, favored rotamers about C-1′ and C-2′. The significance of these values for rotamer assignment is discussed in a later section.

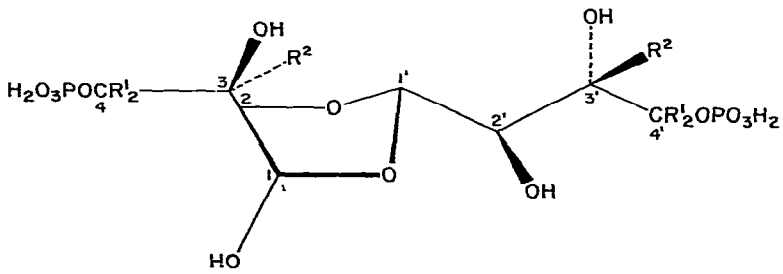
The chemical shift of C-1′ of dimer I (δ 97.68) is within the range observed for acetal carbon atoms in a 1,3-dioxane ring[29]. Furthermore, the similarity of the chemical shifts of C-2′, C-3′, and C-4′ in dimer I and the corresponding carbon atoms of the hydrated monomer of D-erythrose 4-phosphate (**2**) is consistent with these three carbon atoms being present in an acyclic sidechain, as in **3** (Table II).

The C-2 atom in dimer I (δ 67.37) can be considered to bear some similarity to

C-4 in D-glucose 6-phosphate (δ 69.7) (see Experimental section). Incorporation of the oxygen atom on C-3 in the 1,3-dioxane ring-structure **3** is shown by the expected deshielding[16] of C-3 by 8 p.p.m. compared with C-3 of monomer **2**.

The foregoing assignments confirmed that dimer I had the 1,3-dioxane structure **3** rather than the 1,4-dioxane structure **6**.

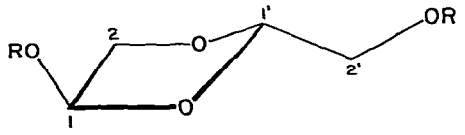
Dimer II (**4**). The concentration of this second-most abundant dimeric form (dimer II) varied from 0% in dilute solution to ~18% of the total in concentrated solution (Fig. 1). As with dimer I, ^{1}H- and ^{13}C-n.m.r. spectra (Figs. 2 and 3) of dimer II show the asymmetric nature of the compound and strongly support a tri-substituted 1,3-dioxolane structure.



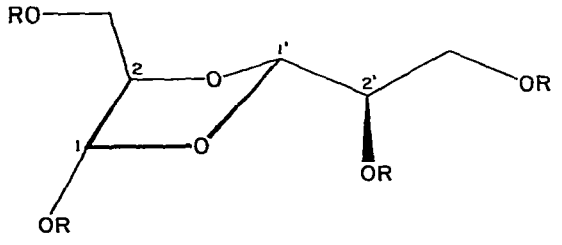
4 $R^1 = R^2 = H$
4a $R^1 = D$; $R^2 = H$
4b $R^1 = R^2 = D$

The stereochemistry of the substituents is assumed to be as shown in **4**, where the two carbon substituents are *anti* and the hydroxyl group attached to the ring is *trans* to the vicinal carbon substituent. The flexibility of the five-membered ring would result in staggering of substituents to partially relieve the *syn*-1,3- and *cis*-1,2-non-bonded interactions. Through pseudorotation, interconversion between conformers can occur without any 1,2-eclipsing interactions, and n.m.r. data can therefore be interpreted as a time average of interconverting conformers[18b].

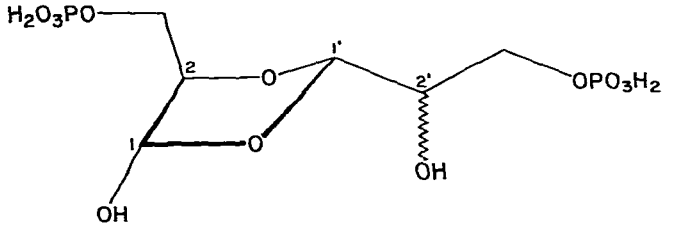
A search of the literature revealed that, apart from a ^{1}H-n.m.r. study of glycolaldehyde[11–13] and the acetylated dimers of D-erythrose[8], little information is available on the n.m.r. properties of substituted dioxolanes having structures similar to that proposed for dimer II. Therefore, to assist in the interpretation of the n.m.r. data, glycolaldehyde, D-glyceraldehyde, and DL-glyceraldehyde 3-phosphate were selected as models in the expectation that the latter two compounds would also form 1,3-dioxolane dimers. The *tert*-butyldimethylsilyl derivatives of the contributing forms of glycolaldehyde and D-glyceraldehyde were also prepared and isolation of individual derivatized dimers attempted. Glycolaldehyde gave a mixture of silylated dimers having ^{1}H- and ^{13}C-n.m.r. signals (other than those arising from the *tert*-butyldimethylsilyl groups) similar to those observed for glycolaldehyde in aqueous solution (Table IV). The derivatized dimers were readily separated from the monomeric



7 R = H
8 R = *tert*-butyldimethylsilyl



9 R = H
10 R = *tert*-butyldimethylsilyl



11

forms, but isolation of individual dimers by preparative g.l.c. was not possible. As well as being able to confirm previous ^{1}H-n.m.r. assignments[11] for the major 1,3-dioxolane form **7** of glycolaldehyde, the less-complex ^{1}H-n.m.r. spectrum of the silylated dimers allowed assignments to be made to dimeric forms not reported previously. These will not be discussed in this paper, but both ^{1}H- and ^{13}C-chemical shifts for these compounds are given (see Experimental section).

The ^{1}H- and ^{13}C-n.m.r. spectra of aqueous solutions of D-glyceraldehyde, which had not previously been investigated, showed a complex of (presumably) dimeric forms, as well as the monomer and the hydrated monomer. In the n.m.r. complex, signals stood out that could be assigned to a 1,3-dioxolane structure **9** by analogy with those observed for the major glycolaldehyde dimer. Derivatization of D-glyceraldehyde gave a mixture of di-*tert*-butyldimethylsilyl-D-glyceraldehyde monomer and tetra-*tert*-butyldimethylsilylated dimers of D-glyceraldehyde. The major dimer was separated by preparative g.l.c. and isolated as a low-melting solid. Its analysis and

TABLE IV

SELECTED ^1H AND ^{13}C N.M.R. CHEMICAL-SHIFT VALUES AND ONE-BOND CARBON–PROTON COUPLING CONSTANTS FOR MODEL COMPOUNDS

Compound	*Solvent*	*H-1*	*H-1′*	*C-1*	^1J *(Hz)*	*C-1′*	^1J *(Hz)*	*C-2*	*C-2′*
7	D_2O	5.70[a]	5.29[a]	95.58	177	102.72	169	72.27	61.75
8	$CDCl_3$	5.57	5.18	95.69	172	103.08	169	73.53	64.44
9	D_2O	5.93	5.79	97.88	176	102.85	167	84.09	71.81
10	$CDCl_3$	5.33	5.18	98.05	173	102.72	167	84.41	73.63
11	D_2O	6.11[b] 6.06[b]	5.88	97.14		102.95		82.56	70.81

[a]Ref. 11. [b]Diastereoisomers.

mass spectrum confirmed its composition and dimeric form, and its ^{13}C- and ^1H-n.m.r. spectra indicated that it was the derivatized form of the major dimer **9** that had been observed in aqueous solution. This compound (**10**) has ring substituents similar to those of **4** and is, therefore, a suitable model for dimer II.

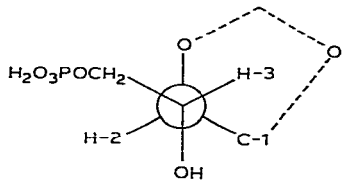
An ^1H- and ^{13}C-n.m.r. study of aqueous solutions of DL-glyceraldehyde 3-phosphate indicated that dimers were much less readily formed in this system. A major dimeric form was present, however, which showed ^1H- and ^{13}C-n.m.r. signals (Table IV) consistent with a 1,3-dioxolane structure **11**.

In the major glycolaldehyde dimer **7**, the hydroxyl group would be expected to be *anti* to the hydroxymethyl group on the 1,3-dioxolane ring, although there is also a minor 1,3-dioxolane dimer present in which the substituents must be *syn*. For dimer II (**4**) and the other model compounds **9**, **10**, and **11**, the large carbon substituents would be *anti*, thus forcing the hydroxyl substituent on C-1 to be *trans* to the carbon substituent on the 2 position and *syn* to the carbon substituent on the 1′ position.

The H-1 and H-1′ signals of **9** and **11** (Table IV) had similar chemical shifts to those of H-1 and H-1′ of dimer II (**4**). In dimer II, however, the ^1H-n.m.r. chemical shifts and couplings for the pairs of protons are similar, making individual assignments difficult (Table I). A basis for distinguishing between these protons could be by comparison with chemical shifts reported[11,12] for H-1 and H-1′ of the glycolaldehyde dimer **7** (Table IV). In this instance, the lower-field signal belongs to H-1, which is 0.4 p.p.m. more deshielded than H-1′, whereas for dimer II the difference is 0.16 p.p.m. The smaller difference between the chemical shifts of H-1 and H-1′ of dimer II (**4**), **9**, **10**, and **11** compared with that observed for **7** and **8** is most probably due to the additional carbon substituent on the 1,3-dioxolane ring in the former compounds.

The $^3J_{1,2}$ ^1H-n.m.r. coupling (2–3 Hz) observed for dimer II, **7**, **8**, and **9** is consistent with that generally observed for five-membered rings where pseudorotation within the ring would result in the observation of time-averaged coupling con-

stants[18b]. The strong $^3J_{2,3}$ coupling (8.1 Hz) for dimer II indicates that rotamer **12**, where H-3 and H-2 are approximately *trans* coplanar, is the most favored.



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In the ^{13}C-n.m.r. spectra of dimer II (**4**), C-1 (δ 97.11) and C-1′ (δ 102.46) showed a distinct chemical-shift difference (Table II). This difference permitted the assignment of C-1 on the basis of its similarity in chemical shift to the C-1 shift of many pentofuranosides[30,31] and pentofuranose 5-phosphates, such as, α- and β-arabinose 5-phosphate[32] (δ 101.84 and 96.03), and α- and β-ribose 5-phosphate (δ 97.23 and 101.94)[32]. The other signal (δ 102.46), which appears at lowest field for the major D-erythrose 4-phosphate dimers, may be assigned to C-1′ and is typical of that observed for acetals[33], for example, D-erythrose 4-phosphate dimethyl acetal sodium salt[32] (δ 105.51) and DL-glyceraldehyde 3-phosphate diethyl acetal sodium salt (δ 105.46)[32]. The major dimer of glycolaldehyde (**7**) shows chemical shifts similar to those of dimer II for C-1 and C-1′, whereas the chemical shifts observed for C-1 and C-1′ of the major dimers (**9** and **11**) of D-glyceraldehyde and DL-glyceraldehyde 3-phosphate are almost identical with those observed for dimer II (Table IV).

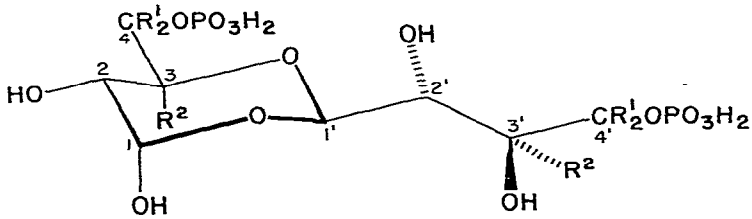
Large carbon–proton, one-bond couplings are observed for C-1 of dimer II (177.1 Hz) as well as for the model compounds **7** (176.5 Hz), **9** (176.0 Hz), and **10** (173.3 Hz, Table III and IV), consistent with the large couplings found for C-1 of some furanoses, such as, 1,2-*O*-isopropylidene-α-D-glucofuranose (186 Hz)[34], β-D-ribofuranose[32] (172.9 Hz), and α-D-ribofuranose[32] (174.9 Hz).

The C-2 signal (δ 83.35) of dimer II (**4**) was the most heavily deshielded C-2 of the major dimers of D-erythrose 4-phosphate. This chemical shift falls within the range of that observed for the C-4 of furanoses[30–32], and is very similar to the chemical shifts observed for C-2 of the major dimers (**9** and **11**) of D-glyceraldehyde and DL-glyceraldehyde 3-phosphate (Table IV). The C-2′ signal (δ 69.93) has a chemical shift comparable to that of C-2 of D-erythrose 4-phosphate dimethyl acetal sodium salt (δ 71.36), with which some structural correlation would be expected.

Because of the conformational mobility of five-membered rings and the steric effects of the large substituents on the ring in dimer II, interpretation of the two- and three-bond carbon–proton couplings was difficult. However, two-bond couplings predicted from rules of Cyr, Hamer, and Perlin[28] and Bock and Pedersen[27] are in broad agreement with the observed couplings (Table III).

Dimer III (**5**). After taking into account the ^{1}H- and ^{13}C-n.m.r. signals for dimers I (**3**) and II (**4**), other signals for a third major dimeric form remained, which

varied from 0% of the total in dilute solution to ~13% of the total in concentrated solution (Fig. 1). The ^{1}H- (Fig. 3) and ^{13}C-n.m.r. spectra of D-(3,4,4′-$^2H_3$) erythrose 4-phosphate showed that these consisted of two sets of equal intensity H-1, H-2, C-1, and C-2 resonances, indicating that these signals arose from a single asymmetric dimer. Of the possible structures that could be written for dimer III, only the 1,3-dioxane **5** could be accommodated by the n.m.r. data. Structure **5** is the α anomer of dimer I, and would therefore be expected to be a contributing form.



5 $R^1 = R^2 = H$
5a $R^1 = D$; $R^2 = H$
5b $R^1 = R^2 = H$

Individual assignment of the two groups of protons in dimer III was not possible because of their similar chemical shifts and coupling constants (Table I). As for dimer II, H-3 could not be detected because of the weakness of its signal and the complexity of the signals of other monomers and dimers in the same region. The H-1 signal (δ 5.92 or 5.95) is shifted ~0.50 p.p.m. to lower field compared with H-1 of **3**, in keeping with the difference of 0.57 p.p.m. observed for H-1 in the β and α anomers of D-glucose 6-phosphate (see Experimental section). Furthermore, H-2 (δ 4.22 or 4.36) is shifted to lower field relative to H-2 of dimer I by 0.28 or 0.42 p.p.m., a $\Delta\delta$ value similar to that (0.30 p.p.m.) observed for H-2 in the β and α anomers of D-glucose 6-phosphate. There is a further similarity between $J_{1,2}$ (3.0 or 3.8 Hz) of dimer III (**5**), and $J_{1,2}$ (3.5 Hz) of α-D-glucose 6-phosphate, consistent with an equatorial H-1 being coupled to an axial proton on C-2, as in **5**. This coupling also compares favorably with the $J_{1,2}$ value of 3.5 Hz reported for an acetylated dimer of D-erythrose[8], having a structural arrangement similar to that of **5**.

Signals of H-1′ (δ 5.92 or 5.95) and H-2′ (δ 4.22 or 4.36) have chemical shifts and couplings ($J_{1',2'}$ 3.0 or 3.8 Hz) consistent with structure **5** having an acetal group at C-1′ and an acyclic sidechain as in dimer I (**3**) and dimer II (**4**) (Table I).

Unlike the ^{1}H-n.m.r. spectrum, significant differences were observed for the chemical shifts and/or couplings in the ^{13}C-n.m.r. spectrum for the two groups of carbon atoms, enabling individual assignments to be made. The C-1 resonance (δ 92.23) is shifted upfield relative to C-1 of **3** (Table II) consistent with dimer III being the α anomer of **3**. A similar shift is observed for C-1 in going from the β to the α anomer of D-glucose 6-phosphate and of D-glucose[25]. The C-1′ resonance (δ 91.88) is also shifted upfield relative to C-1′ (δ 97.68) of **3**, in accordance with the

expected shielding of C-1′ in **5** by an axial hydroxyl group at C-1. This is comparable with the shielding observed for C-5 (4.6 p.p.m.) in going from β- to α-D-glucose[25].

Because of the similarity in their chemical-shift values, assignment of C-1 and C-1′ was made on the basis of their carbon–proton couplings. In general, the one-bond carbon–proton coupling for C-1 in α-pyranosides having H-1 equatorial is ~10 Hz greater than that observed when there is an axial proton on C-1, as in the β anomer[26]. On this basis, the ^{13}C signal at δ 92.10 (92.23) may be assigned to C-1 of **5**, as it has a $^{1}J_{C,H}$ value of 173.4 Hz, which is 11 Hz greater than the $^{1}J_{C,H}$ value for C-1 of **3**. The signal at δ 91.92 (91.88), with a one-bond carbon–proton coupling of 167.2 Hz, must therefore be that of C-1′ (Table III).

Interpretation of the two- and three-bond couplings is complicated by their similarity in magnitude and their lack of sign. The weaker coupling of 1.8 Hz observed for C-1 approaches the value observed[16] (see Experimental section) for the α anomer of D-(5,6,6′-$^{2}H_3$) glucose and D-(5,6,6′-$^{2}H_3$) glucose 6-phosphate (see Experimental section) of 0 Hz, and is in accordance with the predicted values for **5** of <1 (ref. 28) or +2.5 Hz (ref. 27) for $^{2}J_{C\text{-}1,H\text{-}2}$ and ~0 Hz (refs. 16, 21b) for $^{3}J_{C\text{-}1,H\text{-}1'}$.

The C-2 signal (δ 64.20) is shifted upfield by 3.17 p.p.m. relative to C-2 of **3** (Table II). A similar effect is observed for C-2 in D-glucose 6-phosphate and D-glucose[25] on changing from the β to the α anomer. The C-3 signal (δ 73.77) is shifted upfield relative to C-3 of **3** by 5.25 p.p.m. because of the γ-shielding effect[35] of the axial hydroxyl group at C-1 in **5**. The C-2′, C-3′ and C-4′ atoms have chemical shifts almost identical to those of C-2′, C-3′, and C-4′ in **3** and C-2, C-3, and C-4 in monomer **2**, which is in keeping with these carbon atoms belonging to the same acyclic moiety.

No two- or three-bond carbon–proton coupling was observed for C-2 in the deuterated analog **5b**. This is consistent with the weak or zero couplings predicted for $^{2}J_{C\text{-}2,H\text{-}1}$, and no $^{3}J_{C,H}$ couplings are possible (Table III).

Rotamers of the C-1′ sidechain in dimers I, II, and III. — The structures **3**, **4**, and **5** assigned to dimers I, II, and III, respectively, have in common the three-carbon substituent on C-1′. Analysis of the two- and three-bond carbon–proton coupling constants for C-1′ and C-2′ using D-3,4,4′-($^{2}H_3$) erythrose 4-phosphate (**1b**), and their comparison with values predicted[16,21b,27,28] for the favored rotamers A, B, and C (Fig. 4) has been performed (Table III). Whereas no three-bond couplings will be observed for C-2′ in all three dimers because of deuterium atoms at C-3′ and C-4′,

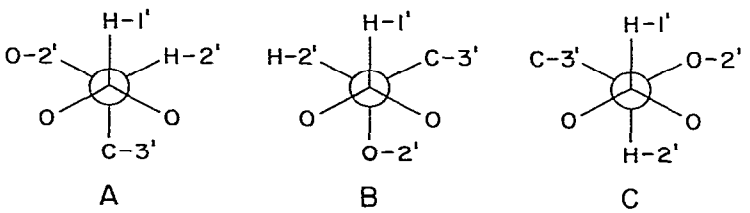


Fig. 4. Rotamers about C-1′ and C-2′ in dimers I, II, and III.

both two- and three-bond couplings to C-1′ are possible, the latter arising from coupling through oxygen with protons on ring carbon atoms.

It is apparent from the large observed $^2J_{C\text{-}2',H\text{-}1'}$ values of 7.1 Hz in dimer II and 6.4 Hz in dimer III that B (predicted $^2J_{C,H}$ +7.5 Hz) must be the preponderant rotamer in these two compounds. The larger of the two couplings (~5.5 Hz) observed for C-1′ in dimers II and III can be assigned to a three-bond coupling with ring protons (predicted large), while the smaller coupling corresponds to the predicted $^2J_{C,H}$ value of 2.5 Hz for rotamer B. Further support for B as the favored rotamer comes from the proton–proton coupling constants between H-1′ and H-2′ in dimers II and III of 2.5 Hz and 3.0 or 3.8 Hz, respectively. These are within the range of values expected for a *gauche* arrangement of protons attached to adjacent carbon atoms possessing electronegative substituents[36], as is the case in both rotamers A and B. Rotamer B, however, is strongly favored on steric grounds over A.

The $J_{H\text{-}1',H\text{-}2'}$ values of 2.9 Hz for dimer I also suggests that either rotamer A or B is favored in this compound, but the observed two-bond carbon–proton value of 1.2 Hz appears to rule out B as a major contributor. A more logical explanation for the low carbon–proton and proton–proton values observed for dimer I is that the three-carbon sidechain in 3 is less constrained about the C-1′–C-2′ bond than in the other two dimers and, therefore, rotates freely. The resultant coupling-constants will be an average of all rotamers and would be expected to be small.

Minor dimers of D-*erythrose 4-phosphate.* — Low-intensity ^{1}H- and ^{13}C-n.m.r. signals were detected that could be due to the presence of at least two more asymmetric dimers. ^{1}H-N.m.r. chemical shifts and couplings, and ^{13}C-n.m.r. signals at δ 104.98 and 101.67, were consistent with these minor contributors being two of the three possible stereoisomers of the 1,3-dioxolane dimer II (**4**). This observation was supported by the model systems glycolaldehyde, D-glyceraldehyde, and D-glyceraldehyde 3-phosphate, which showed comparable signals for possible substituted 1,3-dioxolane stereoisomers (see Experimental section).

^{31}P-N.m.r. spectra. — ^{31}P-N.m.r. spectrometry has found use in enzyme studies because the relatively low abundance and distribution of phosphorus (^{31}P) in biological systems (compared with ^{1}H and ^{13}C) enable individual phosphorus-containing compounds to be measured and identified. Several biological applications of ^{31}P-n.m.r. have been reported[37–39] where rates of substrate binding and product formation have been determined. With this possibility in mind, we recorded the 24.28-MHz ^{31}P-n.m.r. proton-decoupled spectrum of D-erythrose 4-phosphate (Fig. 5). Unfortunately, the phosphate signals for the monomeric and dimeric forms of D-erythrose 4-phosphate were incompletely resolved and not readily assignable. It was also evident that the instrument used was insufficiently sensitive to measure concentrations of phosphates in the order of 1–10mM required for enzyme reactions. This does not preclude the possibility that ^{31}P-n.m.r. spectroscopy could be usefully applied to the study of D-erythrose 4-phosphate in enzyme systems with an n.m.r. spectrometer capable of better resolution, dispersion and sensitivity.

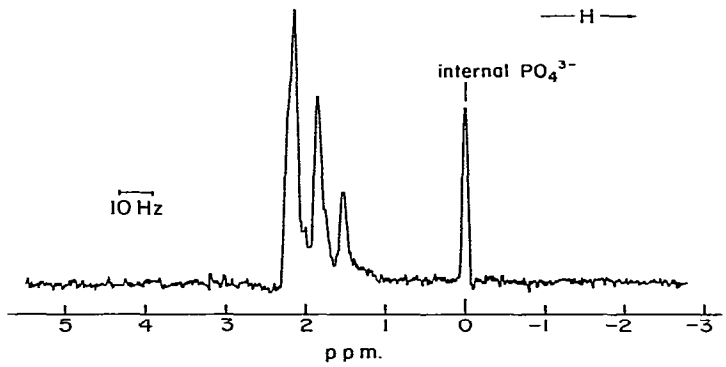


Fig. 5. ^{31}P-n.m.r. spectrum (24.28 MHz, proton decoupled) of an aqueous solution (pH 7.5) of D-erythrose 4-phosphate.

Interconversion of monomers and dimers. — The rate of conversion of dimers into monomers was found to be low. A concentrated solution containing a high proportion of dimers, on dilution to 0.04M, took several hours at 25° to disproportionate completely to monomers, as determined by ^{1}H-n.m.r. spectroscopy. As mentioned earlier, the interconversion of monomers **1** and **2** was readily detected by ^{1}H-n.m.r. through line broadening of the aldehydic proton resonance of **1** and by spin-saturation experiments. Because of the relatively large amount of hydrated monomer **2** and, therefore, its lower rate of conversion into **1**, no broadening of the H-1 signals of **2** could be detected. The spin-saturation experiments[19] and broadening[20] of the H-1 signal of **1** at low pD indicated that the rate of interconversion of the two forms was higher at lower pD. From the change in line width at half-height of H-1 of **1**, approximate rates of conversion of monomer **1** into hydrated monomer **2** were calculated[20] as 46 s^{-1} at pD 1.4 and 9 s^{-1} at pD 6.4. The calculated rates of conversion of **2** into **1** of 6 s^{-1} at pD 1.4 and 1 s^{-1} at pD 6.4 at 27° are comparable with the reported[23] rate of conversion of the hydrated aldehyde form of D-glyceraldehyde 3-phosphate into the free aldehyde form of 8.7×10^{-2} s^{-1} in the pH range 7.3–8.6 at 20°.

CONCLUSIONS

D-Erythrose 4-phosphate readily forms stable dimers in aqueous solution at concentrations >0.2M. Although these dimers are not normally significant at biological concentrations, they could interfere in some of the *in vitro* enzymic studies using D-erythrose 4-phosphate as a substrate, if frozen or freeze-dried preparations of the compound, which would contain a substantial proportion of dimers, are not allowed sufficient time (namely, several hours at 25°) to reach equilibrium before use. The biochemical significance of these results is reported elsewhere[40]. The structures **3**, **4**, and **5** assigned to dimers I, II, and III, respectively, parallel those deduced for the three isolated, acetylated dimers of D-erythrose[8].

EXPERIMENTAL

General methods. — All organic solvents were distilled by using a 100-cm, vacuum-jacketed, glass, helices-packed fractionating column. Water was twice distilled from a glass distillation apparatus. Accurate pH or pD measurements were made by using a Pye Model 78 pH meter adapted to measure small volumes (~1 mL) with a glass electrode. Aqueous solvents were removed on a rotary evaporator at an oil-pump pressure of less than 1 torr and a water-bath temperature of 30°, and the solvents condensed with an ice–water-cooled condenser and a Dry-Ice–acetone condenser. D-Erythrose 4-phosphate could be stored indefinitely at —20° as a frozen aqueous solution, pH ~1. At pH 6 and above, D-erythrose 4-phosphate decomposed rapidly[6] and could only be stored for short periods at this pH. For column and thin-layer chromatography on cellulose, the following solvent mixtures were used: solvent *A*, 8:3:2:1 1-butanol–ethanol–water–acetic acid; and solvent *B* (GW_3)[41a] 8:4:5:5:1 1-butanol–1-propanol–acetone–80% (w/v) formic acid–30% (w/v) trichloroacetic acid, developed twice. In t.l.c., sugar phosphates and inorganic phosphate were made visible by spraying with molybdate–perchloric acid reagent followed by exposure to u.v. light[41b], and reducing sugars were made visible by dipping plates in silver nitrate dissolved in aqueous acetone and spraying them with 0.5M sodium hydroxide in aqueous ethanol[41].

For ^{13}C-n.m.r. spectra of compounds in aqueous solvents, 1,4-dioxane was used as the internal standard and the chemical shifts were calculated relative to tetramethylsilane by the following expression[42] $\delta(Me_4Si) = \delta(1,4\text{-dioxane}) + 67.40$. For ^{1}H-n.m.r. spectra of compounds in aqueous solvents, tetramethylsilane was used as the external standard. ^{1}H-N.m.r. spectra (100 MHz) were recorded with a Varian HA-100 high-resolution spectrometer at the probe temperature of 27°. ^{1}H-N.m.r. (60 MHz) and ^{13}C-n.m.r. spectra (15 MHz) were recorded with a JEOL JNM FX-60 spectrometer at the probe temperature of 25°, in the pulse (Fourier) mode, using 10-mm sample tubes. Phosphorus (^{31}P) decoupling was accomplished by irradiating with an amplified signal generated by a Schlumberger FSD 120 B frequency synthesizer. A Bruker HX-270 superconducting-magnet spectrometer, operating in the pulse (Fourier) mode, was used to record the 270–MHz ^{1}H-n.m.r. spectra (5-mm sample tubes) and 67.89–MHz ^{13}C-n.m.r. spectra (10-mm sample tubes) at the probe temperature of 25°. During recording of proton-decoupled, ^{13}C-n.m.r. spectra at 67.89 MHz, the probe temperature was set at 10° in order that the temperature of the sample did not rise above 30°. A Bruker, pulsed (Fourier) n.m.r. spectrometer B-KR322s equipped with a Varian electromagnet was used to record 24.28-MHz, ^{31}P-n.m.r. proton-decoupled spectra (10-mm sample tubes). The probe temperature was set at 5° such that the sample temperature was ~25°.

Mass spectra were recorded with an AEI MS-902 mass spectrometer, and g.l.c.–m.s. analyses made with a Varian MAT III instrument. Gas chromatography was performed analytically with a Varian Aerograph Series 1400 (nitrogen flowrate

15 mL/min) and preparatively with a Varian Aerograph Series 1700 instrument (helium flowrate 20 mL/min).

*1,2:5,6-Di-*O*-isopropylidene-α-*D*-(6,6'-$^2H_2$)-glucofuranose.* — This compound was prepared by the method of Lemieux and Stevens[43] and isolated as colorless needles: m.p. 108–110° (lit.[43] 108–110°); *m/z* (%) 247 ($M^{+\cdot}$ — CH_3, 68), 246 (1.2), and 103 (100).

*1,2:5,6-Di-*O*-isopropylidene-α-*D*-(5,6,6'-$^2H_3$)-glucofuranose.* — 1,2-*O*-Isopropylidene-α-D-5,6,6'-($^2H_3$)glucofuranose (1.67 g, prepared by the method of Mackie and Perlin[44]) was dissolved in freshly distilled, dry acetone (100 mL), cooled to 0°, and concentrated sulfuric acid (2 mL) was added to the stirred solution. The homogeneous solution was allowed to warm to 20° and then kept for 4.5 h. The solution was cooled to 0° and made neutral with sodium hydroxide (6 g in 10 mL of water). The salts were removed by filtration and washed with acetone. Removal of the solvent gave a white solid that was extracted with toluene and filtered off. Evaporation of the toluene gave a crystalline solid (1.99 g) which, on recrystallization from toluene–light petroleum, gave colorless needles (1.11 g), m.p. 105–110° (lit.[43] 108–110°); *m/z* (%) 248 ($M^{+\cdot}$ — CH_3, 89), 247, and 104 (100).

D-(*6,6'-$^2H_2$*)- *And* (*5,6,6'-$^2H_3$*)-*glucose.* — As required, the appropriate deuterated D-glucose was generated by hydrolysis, catalyzed by Dowex-50W X8 cation-exchange resin (H^+), from one of the following D-glucose derivatives: 1,2:5,6-di-*O*-isopropylidene-α-D-(6,6'-$^2H_2$)-glucofuranose, 1,2-*O*-isopropylidene-α-D-(5,6,6'-$^2H_3$)-glucofuranose, or 1,2:5,6-di-*O*-isopropylidene-α-D-(5,6,6'-$^2H_3$)-glucofuranose. T.l.c. examination (cellulose, solvent *A*) indicated the completion of hydrolysis. Conversion into the deuterated D-glucose was assumed to be quantitative for calculation of the stoichiometry for the subsequent phosphorylation procedure.

D-(*6,6'-$^2H_2$*)- *And* -(*5,6,6'-$^2H_3$*)-*glucose 6-phosphate.* — The phosphorylation procedure was adapted from the large-scale enzymic synthesis of D-glucose 6-phosphate reported by Pollack, Baughn, and Whitesides[45]. Deuterated D-glucose (0.78 g), ATP (38.4 mg), magnesium chloride (132.5 mg), and EDTA (12.7 mg) were dissolved in water (15 mL) by stirring under nitrogen with a magnetic stirrer. 1,4-Dithiothreitol (21.2 mg) was added and washed in with water (5 mL), and the temperature was set to 30° and the pH adjusted to 7 with 4M potassium carbonate. The pH measurements were made by sampling, with a glass capillary, through a small opening against a flow of nitrogen, and then spotting onto a narrow-range pH paper. Slow stirring and a stream of nitrogen over the solution were maintained for the remainder of the procedure. Hexokinase (15 μL, ATP:D-hexose 6-phosphotransferase, 140 U/mg, 10 mg/mL; Boehringer–Mannheim) and acetate kinase (18 μL, ATP:acetate phosphotransferase, 200 U/mg, 5 mg/mL; Boehringer–Mannheim) were added through a septum by means of a syringe. After 45 min, acetyl diammonium phosphate (775 mg, 100 mg/mL solution kept at 0° before addition) was added in 0.5-mL portions with a syringe, over a period of 30 h. More hexokinase (8 μL) and acetate kinase (9 μL) were added at 7.5-h intervals and the pH throughout the procedure was maintained between 6 and 7 with 4M potassium carbonate. The progress of the phosphorylation

was monitored by t.l.c. (cellulose, solvent *A*), using a glass capillary to sample the mixture. After 30 h, examination by t.l.c. indicated that all of the glucose had been converted into glucose 6-phosphate.

The aqueous solution was filtered to remove a small amount of white solid and passed through Dowex-50W X8 cation-exchange resin (H^+, 55 × 80 mm) to give a strongly acidic solution. This was immediately concentrated to ~20 mL and loaded onto a column of Bio-Rad AGl X8 anion exchanger 100–200 mesh (formate form in 0.2M aqueous formic acid) (25 × 450 mm) washed with water (50 mL) and 0.2M aqueous formic acid (400 mL) and eluted with a gradient of aqueous 0.2M formic acid–0.05M ammonium formate and 0.2M formic acid–0.1M ammonium formate, and the eluate was collected in 16-mL fractions. The fractions containing glucose 6-phosphate (by the phenol–sulfuric acid test or by t.l.c. examination) were combined, passed through Dowex-50W X8 (H^+), and concentrated. Ammonium formate and formic acid were removed completely by passing the solution three times through Dowex-50W X8 (H^+) and concentrating the eluate to ~2 mL. After purification by ion-exchange chromatography and deionization, glucose 6-phosphate was isolated in 63% yield (as determined by the phenol–sulfuric acid method) and stored as the barium salt or as a frozen aqueous solution at −20°.

D-(*5,6,6'-*$^2H_3$)-*Glucose 6-phosphate* (H^+). — ^{1}H-N.m.r. (100 MHz, D_2O, external Me_4Si): δ 5.72, d, J 3.5 Hz, H-1α, 5.15, J 7.8 Hz, d, H-1β, and 4.33–3.60, m, H-2, H-3, and H-4. Irradiating at δ 5.72 collapsed the multiplet at δ 4.07 to a simpler multiplet and irradiating at δ 5.15 caused the multiplet at δ 3.77 to collapse to a complex sextet. The ^{13}C-n.m.r. (15 MHz, D_2O, internal 1,4-dioxane) data are given next.

Carbon atom	δ_{Me_4Si}	^{1}J (*Hz*)	^{2}J (*Hz*)	^{3}J (*Hz*)
αC-1	93.0	169.9	C-1–H-2,0	C-1–H-3,0
βC-1	96.8	156.2	C-1–H-2,br	C-1–H-3,br
αC-2	72.2	144.8	C-2–H-3,4.6	C-2–H-4,0
βC-2	74.8	144.5	C-2–H-3,br	C-2–H-4,br
αC-3	73.4	146.0	C-3–H-2,5.4	C-3–H-1,5.4
			C-3–H-4,5.4	
βC-3	76.3	143.1	C-3–H-2,5.4	C-3–H-1,0
			C-3–H-4,5.4	
C-4	69.8	~146	—	—
C-4	69.7	~146	—	—

D-*Erythrose 4-phosphate*. — D-Erythrose 4-phosphate was prepared by oxidation of D-glucose 6-phosphate with lead tetraacetate by a method adapted from that of Sieben, Perlin, and Simpson[6]. The most significant modifications were in the method of removing acetic and formic acid, the ion-exchange chromatography,

and deionization procedures. These enabled D-erythrose 4-phosphate to be obtained in sufficient purity for n.m.r. studies and, because of the relatively large amount of material required for ^{13}C-n.m.r. studies, the method was scaled up from 1 to 3 mmol of D-glucose 6-phosphate starting material.

D-Glucose 6-phosphate (3mM, 912 mg of the disodium salt; Boehringer–Mannheim) was dissolved in water (6 mL), and then distilled acetic acid (700 mL) was added to give a homogeneous solution. Lead tetraacetate in acetic acid (5.1mM, ~20 mg/mL, concentration determined by iodometric titration) mixed with 3M aqueous sulfuric acid (1.8 mL), was then added dropwise to the stirred solution of D-glucose 6-phosphate during 3 h. After stirring for a further 30 min, moistened starch–iodide paper indicated no excess of oxidant. The solution was then filtered through a sintered-glass funnel, the lead sulfate cake was washed with water (200 mL), and the aqueous and acetic acid filtrates were combined and concentrated (~50 mL) under diminished pressure (water bath 30°). Water (100 mL) was added and the solution concentrated (30 mL) and this process was repeated twice, and then the solution was filtered to remove precipitated lead sulfate. The filtrate and washings (50 mL) were passed through cation-exchange resin (H^+ form, Bio-Rad AG-50W X8, 200–400 mesh, 15 mL). The solution was then concentrated (20 mL), water was added (50 mL), and the solution was again concentrated (20 mL) and loaded onto an anion-exchange column (formate form 0.2M formic acid, Bio-Rad AG-1 X8, 100–200 mesh, 25 × 450 mm) and washed with water (50 mL) and 0.2M formic acid (100 mL). Elution was performed with the following gradients: (*a*) 0.2M formic acid–0.05M ammonium formate (1000 mL) and 0.2M formic acid–0.10M ammonium formate (1000 mL); (*b*) 0.2M formic acid–0.10M ammonium formate (500 mL) and 0.2M formic acid–2.0M ammonium formate (500 mL). The eluate was collected in test tubes as 15-mL fractions and tested by the phenol–sulfuric acid method[6]. After the excess of D-glucose 6-phosphate had been eluted with gradient (*a*), gradient (*b*) was applied. A plot of concentration *vs.* tube number showed a chromatographic separation similar to that of Sieben, Perlin, and Simpson[6], except that the "dimeric" D-erythrose 4-phosphate band in our second gradient consisted of two bands. The test-tube fractions containing D-erythrose 4-phosphate were combined into three fractions corresponding to the "monomeric" band and two "dimeric" bands. Each fraction was treated as follows to remove formic acid and ammonium formate. Without concentration, the solution was passed through Bio-Rad AG-50W X8 resin (H^+ form, 55 × 80 mm), and the acidic eluate collected and then concentrated to 20 mL. This solution was passed through the regenerated Bio-Rad AG-50W X8 resin (H^+ form), the acidic eluate concentrated (from 5 to 1 mL), and the process repeated twice. Each fraction, after the removal of formic acid and ammonium formate, was stored as a frozen aqueous solution (~20 mL) at −20°. The amount of D-erythrose 4-phosphate was determined by taking an aliquot (1 mL) from a known volume of an aqueous solution and diluting this to 25 mL. Various amounts of this solution (5–100 μL) were subjected to the phenol–sulfuric acid test and the straight-line plot of absorbance *vs.* amount of D-erythrose 4-phosphate was used to determine

the amount of D-erythrose 4-phosphate in the original solution. The three D-erythrose 4-phosphate fractions showed identical t.l.c. properties, and ^{1}H- and ^{13}C-n.m.r. spectra (Tables I and II) that were identical except for the different equilibrium concentrations of monomers and dimers.

In a ^{1}H-n.m.r. spin-saturation experiment (100 MHz, D_2O, 0.24M, external Me_4Si), irradiation at δ 5.67 (0.2 V) caused the signal at δ 10.3 to be decreased to 39% of the original intensity at pD 1.4 and to 87% of the original intensity at pD 6.4. Irradiation at δ 10.3 (0.2 V) caused the signal at δ 5.67 to be decreased to 0% of the original intensity at pD 1.4 and to 33% of the original intensity at pD 6.4. The changes in peak width at half height of the signal at δ 10.3 were 7.4 Hz at pD 1.4 and 1.4 Hz at pD 6.4.

D-(*4,4'-*$^2H_2$)- *And* -(*3,4,4'-*$^2H_3$)-*erythrose 4-phosphate* (**1a** *and* **1b**). — Compound **1a** and **1b** were prepared from D-(6,6'-$^2H_2$)- and (5,6,6'-$^2H_3$)-glucose 6-phosphate, respectively, by the method already described for D-erythrose 4-phosphate. The amount of deuterated D-glucose 6-phosphate required for the oxidations on a 3mM scale was determined by the phenol–sulfuric acid method[6].

DL-*Glyceraldehyde 3-phosphate*. — DL-Glyceraldehyde 3-phosphate diethyl acetal barium salt (532 mg; Boehringer–Mannheim) was dissolved in water (25 mL) with a few drops of formic acid added, and the solution passed through Bio-Rad AG-50W X8 (H^+) resin. The acidic eluate was evaporated and the syrup redissolved in water (20 mL). After 48 h at room temperature, only a trace of starting material could be detected by t.l.c. (cellulose, solvent *B*). ^{1}H-N.m.r. and ^{13}C-n.m.r. confirmed that the hydrolysis was essentially complete.

The ^{1}H-n.m.r. data (100 MHz, D_2O, 0.4M, pD ~1.4, external Me_4Si) showed the *aldehyde form* (4%) δ 10.18, very broad singlet; *hydrate aldehyde form* (96%) δ 5.49, d, J 5.5 Hz (1 H), 4.57, m (2 H), 4.24, m (1 H). ^{1}H-N.m.r. (D_2O, 1.2M, pD ~1.4, external Me_4Si) showed the *aldehyde form* (3%); *hydrate aldehyde form* (77%); and *major dimeric form* **11** (*diastereoisomers*, 20%) δ 6.11, d, J 2.0 Hz, and 6.06, d, J 2.3 Hz (1 H), and 5.88, d, J 3.0 Hz (1 H). In a ^{1}H-n.m.r. spin-saturation experiment (D_2O, 1.2M, pD ~1.4, external Me_4Si), irradiation at δ 10.18 (0.2 V) caused the signal at δ 5.49 to collapse to 29% of its original intensity. ^{13}C-N.m.r. (15 MHz, D_2O, 0.4M, pD ~1.4, internal 1,4-dioxane) showed the *hydrated aldehyde form* (~100%) δ 90.16, 74.70, d, J 7.8 Hz, and 67.81, d, J 5.3 Hz. The ^{13}C-n.m.r. spectrum (D_2O, 0.8M, pD ~1, internal 1,4-dioxane) showed the *hydrated aldehyde form* (77%); the *major dimeric form* **11** (16%) see Table IV; and *other forms* (7%) δ 105.06, 92.95, 92.53. Phosphorus heteronuclear decoupling at 24,206.4 Hz collapsed all doublets to singlets.

D-*Glyceraldehyde*. — D-Glyceraldehyde (743 mg; EGA-Chemie) was dissolved in solvent *A* (20 mL) and loaded onto a cellulose column (Whatman CC31, 35 g, solvent *A*) and the eluate collected in 7-mL fractions. The fractions having t.l.c. spots positive to silver nitrate–sodium hydroxide were pooled and evaporated to syrups: fractions 10 and 11 (186 mg); 12 and 13 (363 mg); and 14 (41 mg). The three groups showed t.l.c. and n.m.r. properties identical with the unfractionated material.

^{1}H-N.m.r. (100 MHz, D_2O, external Me_4Si) spectra showed *aldehyde form* δ 10.21, s; *hydrated aldehyde form* δ 5.48, d, J 4.5 Hz; 4.9–3.8, m; and *major dimeric form* **9** δ 5.93, d, J 3 Hz, 5.79, d, J 3 Hz; ^{13}C-n.m.r. (15 MHz, gated 1, D_2O, 1.9M, internal 1,4-dioxane) *hydrated aldehyde form* (37%) δ 90.58, dd, J 162 Hz, 2 Hz, 74.87, and 62.79; *major dimeric form* **9** (18%); see Table IV for selected values, others δ 62.47, 61.43; and *other forms* (45%).

tert-*Butyldimethylsilyl derivatives of* D-*glyceraldehyde*. — To D-glyceraldehyde (434 mg; EGA-Chemie) were added *N*,*N*-dimethylformamide (10 mL), imidazole (1.97 g), and *tert*-butylchlorodimethylsilane (2.17 g), and the solution was stirred for 24 h at room temperature. The mixture was poured into water and extracted with dichloromethane (3 × 10 mL). The extract was washed with water (3 × 100 mL), dried (magnesium sulfate), and filtered. The filtrate was then concentrated to a syrup (1.78 g); g.l.c.: 6 ft × 0.25 in. (i.d.) stainless-steel column packed with 10% Apiezon L on 60–80 Gas-Chrom W untreated, 230 → 300° (Δ4); *peak 1*, 0.8 min (19%); *peaks 2 and 3*, 8.7 and 9.2 min, respectively, (28%); *peaks 4 and 5*, 10.4 and 10.9 min, respectively, (32%); *peaks 6 and 7*, 12.0 min (21%). Column chromatography (silica gel, 40 × 500 mm, solvent, light petroleum) gave on elution with light petroleum a fraction of low polarity (133 mg). Further elution with increasing proportions of ether (20:1, 10:1, 5:1) gave fraction I (827 mg); g.l.c. (as before): *peak 1*, 0.8 min (20%); *peak 3*, 9.4 min (5%); *peaks 4 and 5*, 10.4 and 10.9 min (45%); and *peaks 6 and 7*, 12.0 min (21%). Preparative fractions *A* (colorless solid, 20 mg), *B* (colorless solid, 131 mg), and *C* (pale-yellow syrup, 96 mg) were collected by using the foregoing g.l.c. conditions. Analytical g.l.c. [12 ft × 0.08 in. (i.d.) glass column packed with 3% Dexsil 400 on 80–100 Chromosorb W-AB, 250 → 300° (Δ2)] gave the following compositions for the foregoing fractions: A, *peak 3* (98%), *peak 4* (2%): B, *peak 3* (2%), *peak 4* (90%), *peak 5* (8%); C, *peak 6* (72%), *peak 7* (28%).

Preparative g.l.c. of fraction A. — ^{1}H-N.m.r. (100 MHz, $CDCl_3$, Me_4Si) showed δ 5.01, d, J 1.8 Hz (0.68 H), 5.41, d, J 7 Hz (1.32 H), 4.1–3.5, m (6 H), and 0.88, s (36 H); ^{13}C-n.m.r. (15 MHz, CD_2Cl_2, Me_4Si): δ 99.04 (0.33 C), 91.98 (0.67 C), 85.24 (0.33 C), 73.21 (0.67 C), 64.61 (0.33 C), 63.14 (0.67 C), 26.11 (6 C), 18.72 (2 C), and −5.16 (4 C); *m/z*, (%): 579 ($M^{+\cdot}$ − 57, 4) and 261 (100).

Preparative g.l.c. of fraction B, *compound* **10** (*90% of total*). — The product had m.p. 41–43°; ^{1}H-N.m.r. (100 MHz, $CDCl_3$, Me_4Si) δ 5.33, d, J 1.8 Hz (1 H), 5.18, d, J 2.4 Hz (1 H), 4.0–3.35, m (6 H), and 0.88, s (36 H): ^{13}C-N.m.r. ($CDCl_3$, Me_4Si): see Table IV for selected values: others δ 64.41, d, J 140 Hz, and 62.98 d, J 143 Hz; *m/z*, (%): 579 ($M^{+\cdot}$ − 57, 2.5) and 261 (100).

Anal. ($C_{30}H_{68}O_6Si_4$)C,H.

Preparative g.l.c. of fraction C. — The ^{1}H-n.m.r. spectrum ($CDCl_3$, Me_4Si) showed δ 4.82, d, J 1.2 Hz (0.14 H), 4.59, app. d, J 6.7 Hz with underlying peaks, 4.49, app. dd, 1.9- and 2.7-Hz spacing (0.86 H), 4.05–3.18, m (3 H), and 0.88, s (18 H): ^{13}C-N.m.r.: *major isomer* δ 100.24, 99.75, 74.83 70.04, 69.07, 64.77, 26.05 (12 C), 18.50, 18.26 (4 C), −4.30, −4.46, and −5.19 (8 C). Additional peaks of intensity assigned to the *minor isomer* were δ 100.24, 96.59, 75.73, 71.51, 67.93, 64.77, 26.05

(12 C), 18.50, 18.26 (4C), —4.30, —4.46, and —5.19 (8 C). The mass spectrum showed *m/z*, (%): 636 ($M^{+\cdot}$, 0.07), 579 (15), and 261 (100).

Glycolaldehyde. — Glycolaldehyde (Fluka) was dissolved in D_2O and kept for at least 24 h at room temperature (20°). The ^{13}C-n.m.r. spectrum (15 MHz, gated I, D_2O, 3M, internal 1,4-dioxane) showed: *aldehyde form* (2%) 205.21; *hydrated aldehyde form* (62%) δ 90.52, d, J 163 Hz, 65.39, t, J 143 Hz; and *major dimeric form* 7 (14%, see Table IV for selected values). *Other forms* (22%) showed: δ 104.61, d, J 170 Hz, 97.40, d, J 163 Hz, 95.06, d, J 176 Hz, 90.26, 90.00, d, $J \sim 164$ Hz, 89.35, d, $J \sim 160$ Hz, 72.59, t, $J \sim 150$ Hz, 70.91, t, J 142 Hz, 69.15, td, J 143, 29 Hz, 64.87, 64.35, t, J 142 Hz, and 62.86, td, J 144, 6 Hz.

tert-*Butyldimethylsilyl derivatives of glycolaldehyde.* — Glycolaldehyde (700 mg, Fluka) was dissolved in water (20 mL) and kept for 24 h at room temperature. The water was removed and the residual syrup heated to 30° under vacuum for 1 h, and then dissolved in *N,N*-dimethylformamide (10 mL) and cooled to 0°. While maintaining the temperature at ~0°, *tert*-butylchlorodimethylsilane (3.5 g) and imidazole (3.17 g) were added to the stirred solution. After the reagents had completely dissolved, the solution was warmed to room temperature and stirred for 24 h. The mixture was poured into water at 0° (50 mL) and extracted with dichloromethane (3 × 10 mL). The organic extract was washed with water (3 × 50 mL), dried (magnesium sulfate), and filtered. The filtrate was evaporated to a pale-yellow syrup (4.11 g). Column chromatography (silica gel, 40 × 500 mm, solvent, light petroleum) gave, on elution with light petroleum with increasing proportions of ether (20:1, 10:1, 5:1), one major fraction (606 mg). G.l.c.–m.s. [12 ft × 0.08 in. (i.d.) glass column packed with 3% Dexsil 400 on 80–100 Chromosorb W-AB, 150 → 200° (Δ2)]: *peak 1* 14.7 min (12%), *m/z* (%) 117 (100;) *peak 2* 15.4 min (57%), *m/z* (%) 203 ($M^{+\cdot}$ — $CH_2OSi(CH_3)_2$*tert*-Bu, 8), 117 (100); and *peak 3* 16.0 min (31%), *m/z* (%) 203 ($M^{+\cdot}$ — $CH_2OSi(CH_3)_2$*tert*-Bu, 7), 117 (100). ^1H-(100 MHz) and ^{13}C-N.m.r. (15 MHz) spectra indicated that the mixture contained four components, which could be partially assigned on the basis of intensities and ^1H-n.m.r. decoupling experiments as the individual dimers could not be separated by preparative g.l.c. *Compound 1* (8, 58%) showed ^1H-n.m.r. ($CDCl_3$, Me_4Si): δ 5.57, dd, J 4.0, 2.2 Hz (1 H); 5.18, t, J 3.6 Hz, 1 H; 4.01, dd, J 8.1, 4.0 Hz (1 H); 3.69, dd, J 8.1, 2.2 Hz (1 H); 3.67, d, J 3.6 Hz (2 H); and 0.90, s (18 H); ^{13}C-n.m.r. (gated I, $CDCl_3$, Me_4Si): see Table IV for selected values; others δ 25.89, 25.73, q, J 126 Hz, (6 C); 18.42, 17.86, s (2 C); and —4.22, —5.19, q, J 118 Hz (4 C). *Compound 2* (23%): showed ^1H-n.m.r.: δ 5.52, dd, J 3.0, 2.5 Hz (1 H); 5.09, t, J 4.8 Hz (1 H); 3.84, dd, J 6.5, 2.5 Hz, (1 H); 3.82, dd, J 6.5, 3.0 Hz (1 H); and 3.71, d, J 4.8 Hz (2 H); ^{13}C-n.m.r. (gated I): δ 105.51, d, J 166 Hz; 90.58, d, J 157 Hz or 95.12, d, J 168 Hz; 73.21; and 65.66 or 65.90. *Compound 3* (14%) gave: ^1H-n.m.r. δ 4.88, dd, J 5.7, 3.0 Hz (2 H); 3.55, dd, J 11.5, 3.0 Hz (2 H); and 3.37, dd, J 11.5, 5.7 Hz (2 H); ^{13}C-n.m.r. (gated I): δ 95.12, d, J 168 Hz or 90.58, d, J 157 Hz; and 65.66 or 65.90. *Compound 4* (5%) had ^1H-n.m.r. δ 4.86, dd, J 5.6, 2.5 Hz (2 H); ^{13}C-n.m.r. (gated I): δ 91.5; and 67.2, t, J 145 Hz.

Anal. ($C_{16}H_{36}O_4Si_2$)C,H.

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