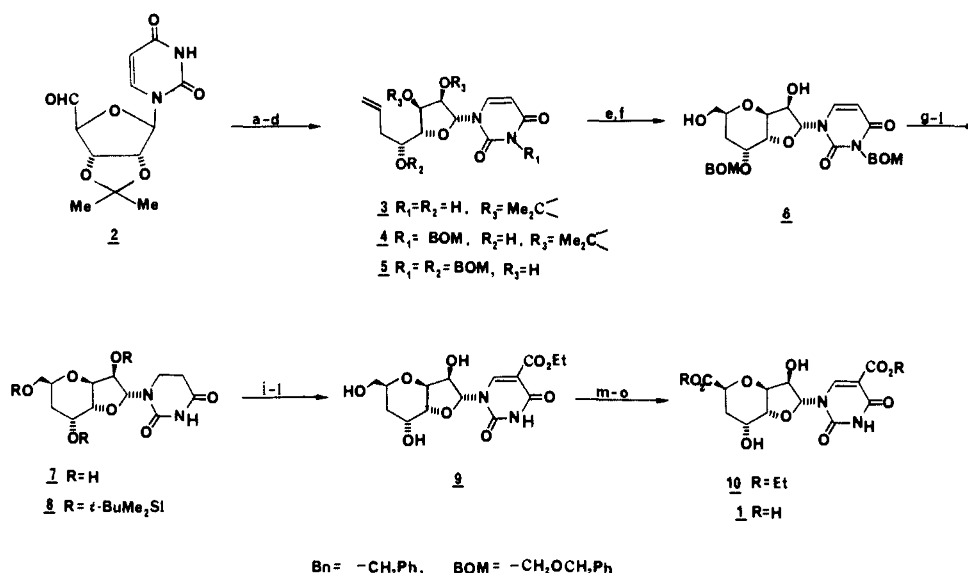
Scheme I[a]



Bn= $-CH_2Ph$, BOM= $-CH_2OCH_2Ph$

[a](a) AllylMgBr, THF, 100 °C (70% both isomers). (b) BOMCl, DBU, DMF, 0 °C (94%). (c) BOMCl, i-Pr_2NEt, THF, 70 °C (85%). (d) THF-HOAc-H_2O (1:2:1) 65 °C (70%). (e) $Hg(OAc)_2$, THF, 36 h, then NaBr. (f) $NaBH_4$, O_2, DMF, (54% from **5**). (g) 20% $Pd(OH_2)$/C, H_2, MeOH, (99%). (h) 5% Rh on alumina, MeOH, (99%). (i) t-$BuMe_2SiCl$, i-Pr_2NEt, DMAP, DMF, (68%). (j) LDA, $ClCO_2Et$, THF, -78 °C. (k) PhSeCl, pyr, CH_2Cl_2, then H_2O_2 (88% from **8**). (l) n-Bu_4NF, THF, (97%). (m) PtO_2, $NaHCO_3$, H_2O, 90 °C. (n) H^+, EtOH. (o) LiOH, H_2O, then Dowex-50 (H^+) (70% from **9**).

well as of its diacetate.[13] Subsequent critical operations involved the introduction of a carboxyl group at C_5 and oxidation at C_8'. Deprotection of **6** and catalytic reduction gave the dihydrouridine derivative **7**. Treatment of the enolate derived from the corresponding silylated nucleoside **8** with ethyl chloroformate[14] gave the corresponding C_5 carboethoxy derivative, which was subjected to an oxidative elimination[15] to reinstate the C_5–C_6 double bond. After desilylation, the resulting triol derivative **9** was then catalytically oxidized[16] to the corresponding half-ester derivative. Saponification gave octosyl acid A as a colorless solid (**1**), mp 285–288 °C dec, $[\alpha]^{25}_D$ +9.8° (c 0.5, N NaOH),[17] whose identity was confirmed by 400-MHz ^1H NMR spectroscopy and comparison with authentic material. On the other hand, esterification of the half-ester gave the diethyl ester **10**, $[\alpha]^{25}_D$ +3.0° (c 1.0, EtOH).

The total synthesis of octosyl acid A from uridine was possible in large measure due to the successful application of the intramolecular alkoxymercuration reaction[11,18] for the construction of the strained dioxahydrindane ring system. The methodology developed in this work should also provide an expedient route to octosyl acid C and other structurally and stereochemically demanding nucleosides such as the ezomycins.[4]

Acknowledgment. We thank the National Scientific and Engineering Council of Canada and the Ministère de l'éducation du Québec for Financial support. We also thank Drs. Phan Viet Tan, A. Ugolini, and P. Beaulieu for assisting in the ^1H NMR spectroscopic studies and Michael Evans for mass spectra. We thank Professor K. Isono for samples of the octosyl acids.

Supplementary Material Available: Spectroscopic data and physical constants for new compounds reported in this paper (21 pages). Ordering information is given on any current masthead page.

(13) ^1H NMR of **6** (400 MHz, $CDCl_3$) δ (multiplicity, integration, assignment, coupling constants) 7.709 (d, 1 H, H-6, J = 8.2 Hz), 7.38–7.24 (m, 10 H, 2 Ph), 5.748 (s, 1 H, H-1'), 5.702 (d, 1 H, H-5, J = 8.2 Hz), 5.474 (s, 2 H, NCH_2O), 4.911 (dd, 2 H, OCH_2O, J = 6.9, 9.5 Hz), 4.693 (s, 2 H, OCH_2Ph), 4.640 (dd, 2 H, OCH_2Ph, J = 11.8, 17.2 Hz), 4.63–4.57 (m, 1 H, H-5'), 4.256 (d, 1 H, H-2', J = 4.6 Hz), 4.034 (dd, 1 H, H-4', J = 2.5, 10.3 Hz), 4.01–3.92 (m, 1 H, H-7'), 3.849 (dd, 1 H, H-3', J = 4.6, 10.3 Hz), 3.794 (dd, 1 H, H-8'A, J = 2.2, 12.2 Hz), 3.526 (dd, 1 H, H-8'B, J = 4.3, 12.2 Hz), 1.85–1.82 (m, 2 H, H-6'). ^1H NMR of the diacetate of **6** (400 MHz, $CDCl_3$) δ 7.51 (d, 1 H, H-6, J = 8 Hz), 7.2–7.4 (m, 10 H, 2 Ph), 5.87 (s, 1 H, H-1'), 5.70 (d, 1 H, H-5, J = 8 Hz), 5.47 (s, 2 H, NCH_2O), 5.34 (d, 1 H, H-2', J = 5 Hz), 4.90 (s, 2 H, OCH_2Ph), 4.89 (s, 2 H, OCH_2Ph), 4.63 (dd, 2 H, OCH_2O, J = 11, 14 Hz), 4.55–4.59 (m, 1 H, H-5'), 4.05–4.15 (m, 3 H, H-7', -8'), 4.02 (dd, 1 H, H-3', J = 5, 10 Hz), 3.88 (dd, 1 H, H-4', J = 3, 10 Hz), 2.07 2.16 (2s, 6 H, 2 OAc), 2.04–2.10 (ddd, 1 H, H-6'e J = 3, 3, 15 Hz), 1.60 (ddd, 1 H, H-6'a, J = 3, 12, 15 Hz).

(14) Hayakawa, H.; Tanaka, H.; Miyasaka, T. *Tetrahedron* **1985**, *41*, 1675.

(15) See, for example: Liotta, D.; Barnum, C.; Puleo, R.; Zima, G.; Bayer, C.; Kesar, H. S., III. *J. Org. Chem.* **1981**, *46*, 2920.

(16) Heyns, K.; Paulsen, H. *Newer Methods of Preparative Organic Chemistry*; Foerst, W., Ed.; Academic Press: New York, 1963; Vol. II.

(17) Reported physical constants for natural octosyl acid A[2] hydrate: mp 290–295 °C dec; $[\alpha]^{25}_D$ +13.3° (c 0.425, N NaOH). There is a discrepancy in the optical rotation value of our synthetic octosyl acid A sample, even though its structure and purity have been ascertained beyond any doubt (see supplementary material). Professor Danishefsky has made a similar observation in his independent synthesis of octosyl acid A (private communication).

(18) Riediker, M.; Schwartz, J. *J. Am. Chem. Soc.* **1982**, *104*, 5842.

Determination of Equilibrium ^{18}O Isotope Effects on the Deprotonation of Phosphate and Phosphate Esters and the Anomeric Effect on Deprotonation of Glucose 6-Phosphate

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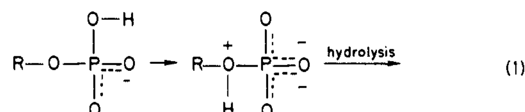
In conjunction with an investigation of the mechanism(s) of phosphate-transfer reactions, we have determined equilibrium ^{18}O isotope effects on the deprotonation of phosphate and phosphate esters. The first step in the hydrolysis of phosphate monoesters is thought to be a preequilibrium proton transfer to the bridge oxygen:[1]



We have determined the secondary kinetic ^{18}O isotope effect on the hydrolysis of glucose 6-phosphate labeled with ^{18}O only in the

(1) Benkovic, S. J.; Schray, K. J. *Enzymes* (*3rd* Ed.) **1973**, *8*, 201. Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313.

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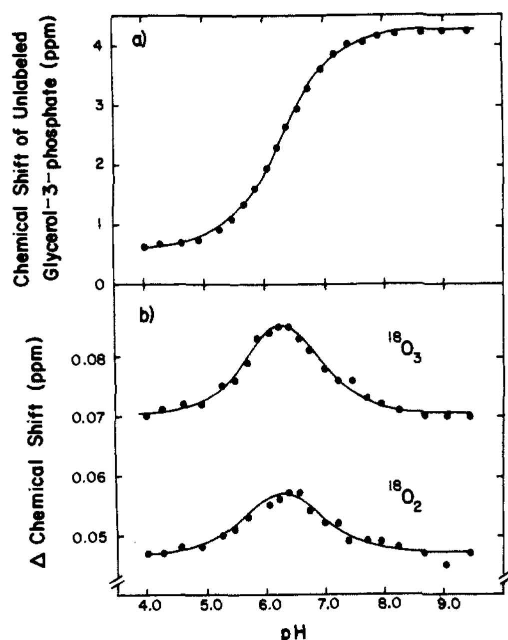

Figure 1. Typical data set for determination of $^{18}K_{eq}$ for the deprotonation of glycerol 3-phosphate. (a) ^{31}P chemical shift vs. pH. (b) The difference in chemical shift between the species containing either three or two ^{18}O and unlabeled glycerol 3-phosphate vs. pH.

nonbridge oxygens,[2] but to calculate the isotope effect on P–O bond cleavage we need to know the equilibrium ^{18}O isotope effect for the first step in mechanism 1. The equilibrium isotope effect introduced in this step should be equivalent to that for deprotonation of a phosphate ester labeled only in the nonbridge positions. Isotope effect theory predicts that ^{18}O should become enriched in the more tightly bonded protonated ester relative to the deprotonated species, so that a small elevation of the pK of the ester should occur upon ^{18}O-substitution.

The pK's of phosphate esters can be determined from the pH dependence of the chemical shift observed in ^{31}P NMR. ^{18}O-substitution causes a very small upfield change in the ^{31}P chemical shift (~0.02 ppm per ^{18}O), and the difference in the pK's of the ^{16}O- and ^{18}O-labeled phosphate esters can be readily determined by monitoring the separation in the peaks for ^{16}O- and ^{18}O-containing species as the solution is titrated through the pK. The separation increases to a maximum at the pK and then returns to its normal value at the end of the titration. This technique was introduced by Ellison and Robinson[3] to determine equilibrium isotope effects on the deprotonation of formic acid (they monitored the ^{13}C NMR chemical shift as a function of pH). We have also used this technique to determine the difference in the pK's of the α and β anomers of glucose 6-phosphate and thus calculate the anomeric effect on the pK.

[$^{18}O_4$]Phosphate (I) and γ-[$^{18}O_3$]ATP (**II**) were synthesized by the method of Hackney et al.[4] Glycerol 3-[$^{18}O_3$]phosphate (III) and glucose 6-[$^{18}O_3$]phosphate (IV) were synthesized by phosphorylating glycerol or glucose with II in the presence of glycerokinase or hexokinase. I, III, and IV were then mixed with equimolar unlabeled phosphate, glycerol 3-phosphate or glucose 6-phosphate. The Na^+, Li^+, or K^+ salts of these preparations[5] were then titrated with HCl (or DCl in D_2O) and the titrations monitored by ^{31}P NMR.[6] To determine the pK accurately, the chemical shifts relative to the external reference and the pH values were fitted to eq 2, where Y is the experimental chemical shift,

$$\log Y = \log [YL + YH(K/H)]/(1 + K/H) \quad (2)$$

YL and YH are the chemical shifts of fully protonated and fully deprotonated species, K is the acid dissociation constant, and H is [H^+]. Values for the degree of protonation at each pH of the ^{16}O compound (x) and the difference in chemical shift between ^{16}O- and ^{18}O-containing compound (ΔY) were then fitted to eq 3,[3] where DH and DL are ΔY values for fully deprotonated and

$$\Delta Y = DH - (DP)x + {}^{18}K_{eq}x(DP + DL - DH)/({}^{18}K_{eq}x + 1 - x) \quad (3)$$

fully protonated species, DP is ($YH - YL$) from the fit to eq 2, and $^{18}K_{eq}$ is the ^{18}O isotope effect on the acid dissociation constant (that is, [K for ^{16}O]/[K for ^{18}O]).

Figure 1 shows representative plots vs. pH of the chemical shift and the difference in chemical shifts for [$^{18}O_3$]- and [$^{18}O_2$]glycerol 3-phosphate relative to unlabeled material.[7] Table I contains the observed equilibrium ^{18}O isotope effects. The isotope effect is linear with the extent of ^{18}O-substitution and is independent both of the nature of the counterion and ionic strength but is 30% higher in D_2O because of the increased stiffness of O–D vs. O–H bonds. These results are similar to those for deprotonation of formic acid[3] ($^{18}K_{eq}$ value for full ^{18}O-substitution, 1.022 in H_2O and 1.029 in D_2O), benzoic acid[8] (1.020 in 20% methanol), p-nitrophenol[9] (1.018), and 2,4-dinitrophenol[9] (1.019).

The value of $^{18}K_{eq}$ for glycerol 3-phosphate was determined at four temperatures and extrapolated to 100 °C on the assumption that $^{18}K_{eq}$ would be unity at infinite temperature. The resulting value of 1.0125 at 100 °C was used for analysis of the kinetic isotope effect data in the following paper.[2]

Table I. Equilibrium ^{18}O Isotope Effects on Deprotonation of Phosphate and Phosphate Esters[a]

compd	temp, °C	counterion	$^{18}K_{eq}$
$HP^{18}O_4^{2-}$	27	Na^+	1.019 ± 0.001
$H_2P^{18}O_4^-$	27	Na^+	1.019 ± 0.001
	65		1.0154 ± 0.0007
$H_2P^{18}O_3{}^{16}O^-$	27	Na^+	1.015 ± 0.002
$H_2P^{18}O_2{}^{16}O_2^-$	27	Na^+	1.010 ± 0.002
$H_2P^{18}O^{16}O_3^-$	27	Na^+	1.004 ± 0.002
$D_2P^{18}O_4^-$	27	Na^+	1.0248 ± 0.0007
glycerol-3-$P^{18}O_3H^-$	7	K^+	1.0174 ± 0.0007
	27		1.0154 ± 0.0009
	46		1.0145 ± 0.0005
	67		1.0139 ± 0.0008
glycerol-3-$P^{18}O_2{}^{16}OH^-$	27	K^+	1.0102 ± 0.0007
glycerol-3-$P^{18}O^{16}O_2H^-$	27	K^+	1.0051 ± 0.0005
glycerol-3-$P^{18}O_3H^-$	27	K^+ (0.58 M KCl)	1.0145 ± 0.0005
glycerol-3-$P^{18}O_2{}^{16}OH^-$	27	K^+ (0.58 M KCl)	1.011 ± 0.001
glycerol-3-$P^{18}O_3H^-$	27	Li^+	1.0152 ± 0.0006
glycerol-3-$P^{18}O_2{}^{16}OH^-$	27	Li^+	1.0106 ± 0.0006
glycerol-3-$P^{18}O_3D^-$	27	K^+	1.022 ± 0.001
glycerol-3-$P^{18}O_2{}^{16}OD^-$	27	K^+	1.015 ± 0.001
glucose-6-$P^{18}O_3H^-$	27	K^+	1.0147 ± 0.0006

[a] Values for mixed ^{16}O and ^{18}O were obtained from compounds incompletely labeled with ^{18}O.

(2) Weiss, P. M.; Knight, W. B.; Cleland, W. W., following article in this issue.

(3) Ellison, L. R.; Robinson, M. J. T. *J. Chem. Soc., Chem. Commun.* **1983**, 745.

(4) Hackney, D. D.; Stempel, K. E.; Boyer, P. D. *Methods Enzymol.* **1980**, *64*, 61.

(5) 10–120 mM, 0.5 mM ethylenediamminetetraacetate. The concentration of the phosphate ester did not affect the observed $^{18}K_{eq}$ value.

(6) ^{31}P NMR spectra were obtained with a Nicolet NT-200 Fourier transform spectrometer operating at 80.99 MHz in the quadrature detection mode and equipped with a variable-temperature control unit. The spectra were the sum of 4–20 scans of ±275 Hz with 4K data points and were externally referenced to 0.18 M H_3PO_4 in D_2O (external lock) or 0.18 M H_3PO_4 in H_2O when the titrations were conducted in D_2O. The data were routinely zero-filled to 8K and resolution-enhanced by a double exponential digital multiplication (DM = 1 in the Nicolet software) of the free induction decays prior to Fourier transformation. The observed $^{18}K_{eq}$ values were the same if only a single exponential multiplication (0.1-Hz line broadening) was used.

(7) The ester was not totally enriched in ^{18}O, so the species containing two ^{18}O was present.

(8) Tanaka, N.; Araki, M. *J. Am. Chem. Soc.* **1985**, *107*, 7780.

(9) Rosenberg, S. Ph.D. Dissertation, University of California, Berkeley, 1978.

This NMR technique has also allowed us to determine the pK's of α- and β-glucose 6-phosphate as 6.193 ± 0.008 (α) and 6.134 ± 0.005 (β).[10] The pK of β-glucose 6-phosphate is lower as the result of an "anomeric effect" of 1.143 ± 0.001 on the acid dissociation constant. The reason for this is likely that formation of a hydrogen bond between the anomeric hydroxyl (pK more acidic than bulk water by ~2 pH units) and the phosphate group stabilizes deprotonation of the latter in the β anomer, but not in the α anomer where such a hydrogen bond cannot readily form.

Acknowledgment. This work was supported by NIH Grant GM 18938. W.B.K. holds a NIH postdoctoral fellowship (GM09677).

Registry No. ^{18}O, 14797-71-8; phosphate, 14265-44-2; glycerol 3-phosphate, 57-03-4; glucose 6-phosphate, 56-73-5.

(10) The chemical shift of the α anomer is upfield from that of the β anomer by 0.027 ppm when both are deprotonated, although the difference is 0.009 ppm in the opposite direction for the protonated esters (these values come from the fits to eq 3). The difference (α upfield of β) reaches as much as 0.134 ppm near the pK.

Secondary ^{18}O Isotope Effects on the Hydrolysis of Glucose 6-Phosphate

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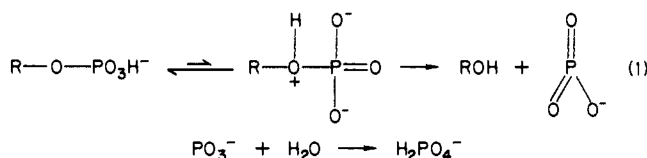
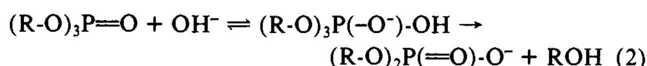
Two limiting mechanisms have been considered for phosphate-transfer reactions. The hydrolysis of monoprotonated phosphate monoesters has been thought to involve a dissociative mechanism with a "metaphosphate" intermediate.[1] This mechanism requires preequilibrium proton transfer to the bridge atom prior to P–O bond cleavage:

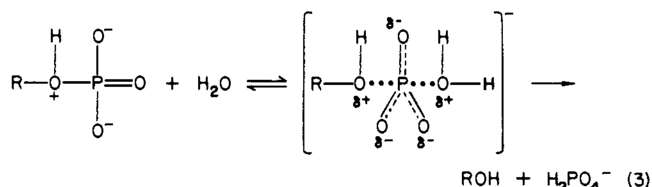




By contrast, reaction of phosphate triesters is thought to involve an associative reaction with a pentavalent intermediate, especially in cases where the eventual leaving group begins in an equatorial position and pseudorotation must occur to permit it to leave from an axial position:[1]

$$(R\text{-}O)_3P{=}O + OH^- \rightleftharpoons (R\text{-}O)_3P(\text{-}O^-)\text{-}OH \rightarrow (R\text{-}O)_2P({=}O)\text{-}O^- + ROH \quad (2)$$

Between these limiting models are S_N2 mechanisms with various axial and equatorial bond orders in the transition state, but no free intermediate. For hydrolysis of a monoprotonated phosphate monoester, for example, an alternate formulation to the metaphosphate mechanism would be



where the positive charge in the transition state might be shared between the two axial oxygens and the phosphorus, depending on the bond orders. Two groups have recently proposed such a mechanism with low axial P–O bond order in place of a metaphosphate mechanism for phosphate transfer from N-phosphorylpyridines to pyridines or primary amines.[2,3]

(1) Benkovic, S. J.; Schray, K. J. *Enzymes (3rd Ed.)* 1973, *8*, 201. Westheimer, F. H. *Chem. Rev.* **1981**, *81*, 313. Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.

Table I. Secondary ^{18}O Isotope Effects on the Hydrolysis of Glucose 6-Phosphate[a]

fractional reactn	isotope effect calculated from residual glucose 6-phosphate[b]	glucose product[c]
0.4898	1.0162	1.0104
	1.0124	1.0120
0.4721	1.0158	1.0122
	1.0131	1.0119
0.480	1.0113	1.0158
	1.0123	

[a] The isotope effects are for substitution of three ^{18}O in the nonbridge positions of the phosphate group but have not been corrected for the lack of isotopic purity of the starting materials. See ref 9 for the corrections needed here. [b] Calculated from the expression $\log (1 - f)/\log [(1 - f)(R_s/R_0)]$, where f is fraction of reaction and R_s and R_0 are ^{13}C content of the CO_2 from C-1 in residual glucose 6-phosphate and initial glucose 6-phosphate, respectively. [c] Calculated from the expression $\log (1 - f)/\log (1 - fR_p/R_0)$, where R_p is the ^{13}C content of C-1 from product glucose.

One potential way to distinguish these mechanisms is by measurement of secondary ^{18}O isotope effects resulting from ^{18}O-substitution in the nonbridge oxygens of a phosphate ester. In an ionized monoester these oxygens have a formal bond order of $^4/_3$ to phosphorus, while in metaphosphate the bond order is $^5/_3$, and a pentavalent adduct contains single bonds. Intuition thus predicts an inverse kinetic isotope effect for a metaphosphate mechanism (after correction for the equilibrium ^{18}O isotope effect on the preequilibrium proton transfer to the bridge oxygen), a normal isotope effect for formation of a pentavalent intermediate, and an isotope effect for a S_N2 reaction that depends on the axial and equatorial bond orders in the transition state.[4]

We have measured secondary ^{18}O kinetic isotope effects resulting from ^{18}O substitution in the three nonbridge oxygens on the hydrolysis of glucose 6-phosphate at 100 °C in 50 mM phthalate, pH 4.5. The reaction involves only P–O bond cleavage, has a half-life of 12 h, and is typical of reactions that are thought to proceed by a largely dissociative "metaphosphate" mechanism.[5,6] This reaction was chosen because of the ease of measuring the isotope effects and because we intend to measure similar isotope effects in enzymatic reactions involving glucose 6-phosphate.

^{18}O isotope effects were measured by the remote label method[7] in which [1-^{13}C]glucose 6-[$^{18}O_3$]phosphate was mixed with [1-^{12}C]glucose 6-phosphate to give material with close to the natural abundance of ^{13}C at C-1.[8] Since there is no isotope effect on the rate of hydrolysis caused by ^{13}C at C-1, any discrimination between the two species of glucose 6-phosphate results from the ^{18}O-substitution. This discrimination is easily measured by separating glucose 6-phosphate and glucose after half-hydrolysis, and degrading them both separately to ribulose 5-phosphate and CO_2 by the action of glucose-6-phosphate and 6-phosphogluconate dehydrogenases (the glucose was phosphorylated to glucose 6-phosphate by hexokinase and MgATP first). The mass ratio in CO_2 was measured with an isotope ratio mass spectrometer.

The observed ^{18}O isotope effect was 1.013 ± 0.002 (see Table I). When this value was corrected for the isotopic purity of the starting materials and the cube root taken,[9] an isotope effect of 1.0046 was obtained for a single ^{18}O-substitution. The equilibrium

(2) Bourne, N.; Williams, A. *J. Am. Chem. Soc.* **1984**, *106*, 7591.
(3) Skoog, M. T.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 7597.
(4) This analysis focuses on bond stretches and ignores the loss or decrease in bending frequencies that results from an exploded transition state with low axial P–O bond order. The quantitive calculations we have made take bending modes into account.
(5) Halman, M.; Degani, Ch. *J. Am. Chem. Soc.* **1966**, *88*, 4075.
(6) Bunton, C. A.; Chaimovich, H. J. *J. Am. Chem. Soc.* **1966**, *88*, 4082.
(7) O'Leary, M. H.; Marlier, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 3300.
(8) Having the mass ratio close to the 1.1% natural abundance value minimizes errors from contaminating CO_2 during the analysis.