$$\log K = A/T + B \log T + C \quad (3)$$

perature, was used to obtain K values for formic acid, acetic acid, and ammonia at 80 °C. The method of least squares was used to obtain values of the constants from data over the range 0–60 °C.[11a] This gave pK_a values of 3.89 and 4.88 for formic and acetic acids, respectively, and a pK_b value of 4.77 for ammonia at 80 °C. The pK_w value for water at 80 °C is 12.62[11b] (on a molarity basis). The pH values were taken as $-\log a_{H^+}$ values and activity coefficients were calculated from the Davies equation[12] (eq 4) by

$$\log \gamma = -0.5739(\mu^{1/2}/(1 + \mu^{1/2}) - 0.2\mu) \quad (4)$$

using the Debye–Hückel constant of 0.5739 at 80 °C.[11c]

From the pH of solutions of cacodylate buffers at 80 °C is calculated a thermodynamic pK_a for cacodylic acid of 6.42 ± 0.02 for ionic strengths (sodium chloride) from 0.004 to 0.30 if the sodium cacodylate concentration is below 0.02 M. As the sodium cacodylate concentration increases to 0.30 M, the calculated pK_a increases to 6.52.

Treatment of Data. A nonlinear least-squares treatment[13] was used to obtain the rate constant that minimized the sum of the unweighted $(mL_{obsd} - mL_{calcd})^2$ values for the runs carried out with about 0.055 M formamide or $(Abs_{obsd} - Abs_{calcd})^2$ values for the runs carried out with about 0.0004 M initial formamide. A simple first-order or second-order rate equation was used for all the runs except those with formate or ammonia buffers and about a 0.055 M initial formamide concentration. The concentrations of formic acid and formate ions in the formate buffers were so much larger than the hydrogen ion (or hydroxide ion) concentration that they were not significantly affected by the small amount of transformation of formic acid to formate ions that accompanied the decrease in acidity of the reaction solution during the reaction. Thus we write differential rate equation shown in eq 5, in which A, B, and C are the initial concentrations of formamide, formic acid, and formate ions, respectively, t is the time, x is the change in formamide concentration, k_2 is the second-order rate constant, and K is the ionization constant of formic acid. The increase in ionic strength causes K to increase slightly during the reaction, but if it is taken as a constant (the value calculated at the average ionic strength), eq 5 can be integrated to give eq 6.

$$dx/dt = k_2KB(A - x)/(C + x) \quad (5)$$

$$k_2KBt = (A + C) \ln [A/(A - x)] - x \quad (6)$$

We calculated k_2 as that value that minimized the sum of the squares of $x_{obsd} - x_{calcd}$. Since we could not solve eq 6 for x, we used an iterative procedure to obtain x_{calcd}. Rate constants in the runs with ammonia buffers were obtained analogously.

Registry No. Formamide, 75-12-7.

(11) (a) Harned, H. S.; Owen, B. B. "The Physical Chemistry of Electrolytic Solutions", 3rd ed.; Reinhold, New York, 1958; pp 663, 758, 763. (b) *Ibid.*, p 645. (c) *Ibid.*, p 165.
(12) Davies, C. W. *J. Chem. Soc.* **1938**, 2093–8.
(13) Hamilton, W. C. "Statistics in Physical Science"; Ronald Press: New York, 1964; Sections 4-1, 5-3.

Model Studies Concerning the First Step in the Hydrolysis of Ribonucleic Acids by RNase A

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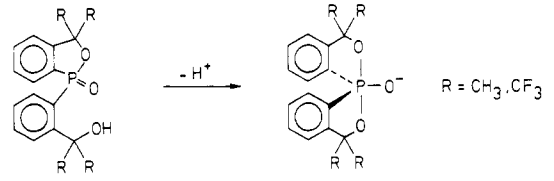
Low-temperature NMR measurements of the reaction of two cyclic oxyphosphoranes with FSO_3H in $CHCl_2F$ are described. An equilibrium is observed of the ring-opened phosphonium ions with the neutral oxyphosphoranes. From the activation parameters it is concluded that the rigidity introduced by a five-membered ring facilitates ring closure of the phosphonium ion, whereas a six-membered ring is less effective. Similarly, ring closure is found to occur in the solvolysis of a bicyclic phosphate where a five-membered ring makes the ring-opened product relatively rigid. This results in exocyclic ester cleavage to a greater extent than predicted by a pseudorotation mechanism. The reactions suggest that intramolecular phosphorylation of the 2'-OH group in RNA is facilitated by the ribose ring.

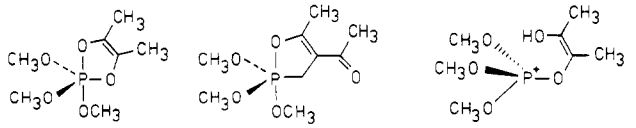
Introduction

It is generally accepted that phosphorylation reactions involve intermediates in which phosphorus is five-coordinated.[1] Examples of these intermediates have recently been isolated by making use of an intramolecular phosphorylation, e.g., in the phosphinate shown in Scheme I.[2] In addition, we have shown that structural factors which promote intramolecular phosphorylation can be conveniently detected by low-temperature NMR measurements of stable, cyclic oxyphosphoranes in strong acid solution.[3] Thus, it was demonstrated that a small amount of acid induces an equilibrium of the phosphorane, e.g., **1** or **2**, with a phosphonium ion, e.g., **1'**. This equilibrium involves a bimolecular proton transfer from the phosphonium ion to a neutral phosphorane molecule[3] (Scheme II). The equilibrium is fast when the ring contains a double bond

Scheme I



Scheme II

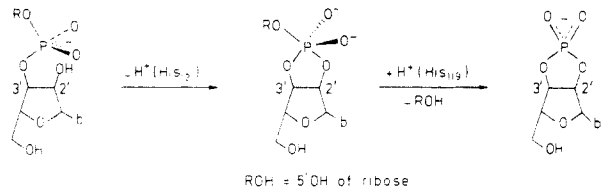


$$1 + {}^*1' \rightleftharpoons 1' + {}^*1$$

(1) (a) Luckenbach, R. "Dynamic Stereochemistry of Pentacoordinated Phosphorus and Related Elements"; G. Thieme: Stuttgart, 1973. (b) Westheimer, F. H. *Pure Appl. Chem.* **1977**, *49*, 1059. (c) Ramirez, F.; Marecek, J. F. *Acc. Chem. Res.* **1978**, *11*, 239.
(2) (a) Granoth, I.; Martin, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 5229. (b) Perozzi, E. F.; Martin, J. C. *Ibid.* **1979**, *101*, 1591.
(3) (a) Castelijns, A. M. C. F.; Schipper, P.; Buck, H. M. *J. Chem. Soc., Chem. Commun.* **1978**, 382. (b) Castelijns, A. M. C. F. Thesis, Eindhoven, **1979**. (c) Castelijns, A. M. C. F.; Schipper, P.; van Aken, D.; Buck, H. M. *J. Org. Chem.* **1981**, *46*, 47.

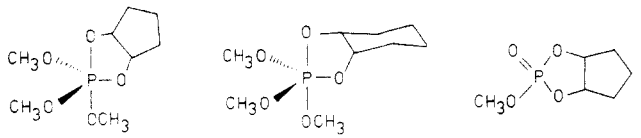
0022-3263/81/1946-3189$01.25/0

Scheme III



(as in 1 and 2) and much slower when the ring is saturated.[3c]

These findings prompted us to consider the mechanistic aspects of a well-known biochemical example of intramolecular phosphorylation, viz., the RNase A catalyzed hydrolysis of ribonucleic acids. The first step in this reaction is known[4] to involve nucleophilic attack by the 2'-OH group of a ribose ring on the vicinal phosphate group, leading to a 2',3'-cyclic phosphate via a five-coordinated intermediate (Scheme III). It seems very plausible that this reaction is facilitated by the rigidity introduced by the ribose ring. Therefore, we were interested in the orientational effect of a five-membered ring in phosphorylation reactions. Here we report the equilibrium measurements of the phosphoranes 3 and 4 with the corresponding



3 4 5

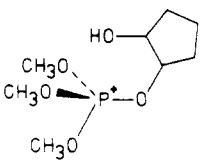
phosphonium ions, 3' and 4', respectively (cf. Scheme II), which allow us to compare the constraint offered by a double bond (compound 1) vs. a five- or a six-membered ring. Furthermore, since we have demonstrated recently[5] that orientational factors in cyclic phosph(on)ates may result in solvolysis with retention of the ring, we investigated the solvolysis of the phosphate 5 which is related to the oxyphosphorane 3 and the 2',3'-cyclic intermediate in the RNase reaction. We anticipated that the five-membered ring might provide sufficient orientation to facilitate reclosure of a ring-opened product. In that case, more exocyclic solvolysis should be observed than predicted by a pseudorotation mechanism.

Results and Discussion

Acid-Induced Equilibria of Phosphoranes and Phosphonium Ions. The phosphoranes 3 and 4 were prepared according to the sulfenate method developed by Denney et al.[6] (Scheme IV).

When a solution of 3 in Freon 21 ($CHCl_2F$) is cooled to −120 °C, line broadening of the ^1H NMR signals is observed, but the signal of the three methoxy groups remains one doublet, indicating that pseudorotation[1a] of this compound remains fast at low temperature.

The addition of less than 1 equiv of fluorosulfonic acid to the Freon solution (see spectroscopic study of the reactions of 3 and 4 with FSO_3H in the Experimental Section) causes the appearance of new signals which can be attributed to the ring-opened phosphonium ion 3' since



3'

the methoxy doublet of this compound is at lower field than the oxyphosphorane doublet. The ^1H NMR spectrum of the mixture of 3 and 3' is temperature dependent: warming of the sample causes broadening of the methoxy doublets of 3 and 3' and coalescence of these signals at −55 °C. This behavior is completely reversible. In the ^{31}P NMR spectrum at low temperature, the resonances of the oxyphosphorane (−41.6 ppm) and the phosphonium ion (+4.1 ppm) can be clearly distinguished. At relatively high temperatures, the spectra reveal the occurrence of an irreversible reaction which produces a phosphate-like compound. Presumably, dealkylation of the phosphonium ion occurs at this temperature.[8]

The line broadening and coalescence phenomena indicate an equilibrium between 3 and 3', just as found for 1 and 1'. As discussed previously,[3] this equilibrium necessarily involves a biomolecular proton-transfer step from 3' to 3, since the acid anion FSO_3^- is a much weaker base than the neutral oxyphosphorane 3. This is supported by the activation parameters for the process (vide infra).

The behavior of 4 is completely analogous to that described for 3, but the coalescence temperature of the methoxy doublets is higher (−15 °C). This can be correlated with a slower equilibrium in case of 4, since the chemical shift difference (phosphorane–phosphonium ion) is similar in both cases.[9]

From the line-shape analysis of the methoxy signals at various temperatures the activation parameters for the observed equilibria can be determined[9,10] (see spectroscopic study of the reaction of 3 and 4 with FSO_3H in the Experimental Section). These parameters are listed in Table I in which the values reported for compound 1[3c] are included. All three equilibria have a rather large negative entropy of activation, indicating a rate-determining bimolecular process which involves the proton-transfer step[3] (Scheme V). Furthermore, it is clear that the $T\Delta S^{\ddagger}$ term is larger than the $\Delta H^{\ddagger}$ term and that the order of $\Delta G^{\ddagger}$ parallels the order of $\Delta S^{\ddagger}$ values; i.e., entropy effects are dominant. Since the phosphonium ion must assume a special conformation to reach the transition state, the entropy of activation can be correlated with the degrees of freedom in the phosphonium ion. Thus, a double bond which completely excludes one rotational degree of free-

Table I. Activation Parameters for Equilibria of Oxyphosphoranes and Phosphonium Ions

compd	$\Delta H^{\ddagger}$, kJ/mol	$\Delta S^{\ddagger}$, J/mol·K	$\Delta G^{\ddagger}$, kJ/mol
1[a]	11.6	−155	51.2
3	12.5	−169	54.3
4	11.0	−184	56.5

[a] Data taken from ref 3c.

(4) (a) Richards, F. M.; Wyckoff, H. W. In "The Enzymes"; Boer, P. D., Ed.; Academic Press: New York, 1978; Vol. IV. (b) Gorenstein, D. G.; Wyrwicz, A. M.; Bode, J. *J. Am. Chem. Soc*, **1976**, *98*, 2308. (c) Holmes, R. R.; Deiters, J. A.; Galluci, J. C. *Ibid.* **1978**, *100*, 7393. (d) Deakyne, C. A.; Allen, L. C. *Ibid.* **1979**, *101*, 3951.

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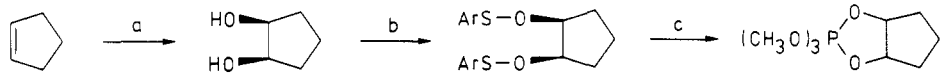
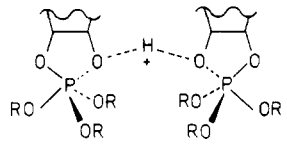
(10) Lowry, T. H.; Schueller Richardson, K. "Mechanism and Theory in Organic Chemistry"; Harper & Row: New York, 1976; p 100.

Scheme IV[a]

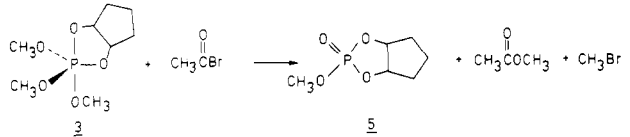


[a] a, $KMnO_4$, water/ethanol, −15 °C (ref 7); b, 2 equiv of ArSCl + 2 equiv of Et_3N, ether, −10 °C (Ar = 4-ClC_6H_4); c, $(CH_3O)_3P$, CH_2Cl_2, −78 °C.

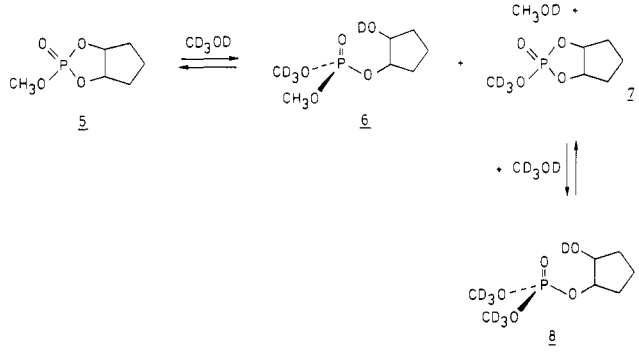
Scheme V



Scheme VI



Scheme VII

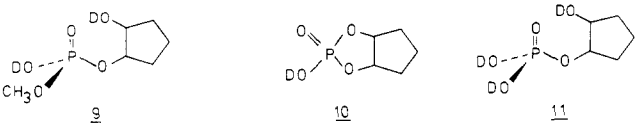


dom results in the least negative $\Delta S^{\ddagger}$ (compound 1). Evidently, the five- and six-membered rings (3 and 4) allow the OH group in the phosphonium ion to move further away from phosphorus, but the five-membered ring offers more rigidity than the six-membered ring. The $\Delta H^{\ddagger}$ values for 1, 3, and 4 are closely related to each other and correspond exactly with that found for the oxonium–water proton exchange processes.[18] Apparently, the bond-making and bond-breaking processes for proton transfer are very similar.

Solvolysis of Cyclic Phosphates. The phosphate 5 was prepared from the corresponding trimethoxyphosphorane 3, using the method of dealkylation with acetyl bromide[11] (Scheme VI). The product was obtained as a mixture of diastereomers as indicated by the presence of two methoxy doublets in ^1H NMR and two signals in ^{31}P NMR (+18.8 and +19.5 ppm).

Solvolysis reactions were carried out at 35 °C in three media: CD_3OD, D_2O, and D_2O buffered with sodium acetate. In deuterated methanol, several simultaneous changes are observed in the ^1H NMR spectrum of 5. A new methoxy doublet and new methine (OCH) peaks appear which are attributed to the ring-opened compound 6. In addition, a singlet from methanol emerges, implying the formation of 7, which may react with more CD_3OD to form 8 (Scheme VII). After 1 day of reaction, an equilibrium has been established in which over 80% of the methoxy groups are present as methanol, while the amounts of 5 and 6 are almost equal as judged by the integrals of their methoxy doublets (~10% each). In addition, the signals from the methine protons indicate that overall 50% of the reaction mixture is ring opened (6 or 8). Thus, it can be concluded that the ratio 5:6:7:8 is approximately 10:10:40:40 in the final mixture.

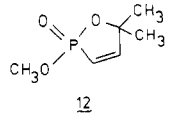
During the hydrolysis of 5 a new doublet and a singlet from methanol are observed in the ^1H NMR spectrum. Hence, both ring opening and exocyclic cleavage occur, yielding the products 9 and 10, respectively. In the



presence of the buffer, 62% compound 9, 33% methanol, and less than 5% starting material are observed after 1 day. Without the buffer, the methoxy doublet from the initially formed 9 decreases again as the reaction proceeds, while the methanol peak increases: at the end of the reaction, about 83% of the methoxy peaks correspond to methanol. These observations strongly indicate that in the unbuffered solution ring opening to 9 is followed by ring closure to form methanol and 10. Furthermore, the signals of the methine protons indicate that 10 reacts further, probably to the ring-opened phosphate 11. The effect of the addition of a buffer (pH ~4.7) may be rationalized by the ionization of the products 9–11 ($pK \simeq 2$). Thus, after ionization of 9 ring closure to form 10 is hampered by the negative charge on the phosphate moiety, which explains the increase of product 9 in the presence of the buffer.

It is important to notice that further solvolysis of the acylic products 6 and 9 to 8 and 11, respectively, is a very slow process compared to the solvolysis of cyclic phosphates[12] (e.g., in a solution of trimethyl phosphate in CD_3OD no methanol formation is observed after 1 day). Hence, the formation of methanol must be explained either by a direct solvolysis with ring retention (e.g., via a pseudorotation mechanism[13]) or by closure of the ring-opened product. The first process, however, does not account for the disappearance of 9 accompanied by the formation of methanol in the hydrolysis reaction. Moreover, unless pseudorotation is favored over direct ring opening, at least 50% ring-opened product would be predicted.[12] Therefore, the 80% methanol formation in the reaction of 5 with CD_3OD must result, at least partially, from ring closure of 6 to yield 7 and CH_3OD.

It can be concluded that in the solvolysis of 5 the orientation offered by the five-membered ring favors a ring opening–ring closure mechanism, which results in exocyclic ester cleavage without the need for pseudorotation. A similar solvolysis with ring retention was recently reported by us[5] for a phosphonate, 12, which is unlikely to react via



the pseudorotation mechanism.[1b] Here, a double bond is responsible for the facile ring closure. According to this

(11) Ramirez, F.; Marecek, J. F.; Ugi, I. *J. Am. Chem. Soc.* **1975**, *97*, 3809.

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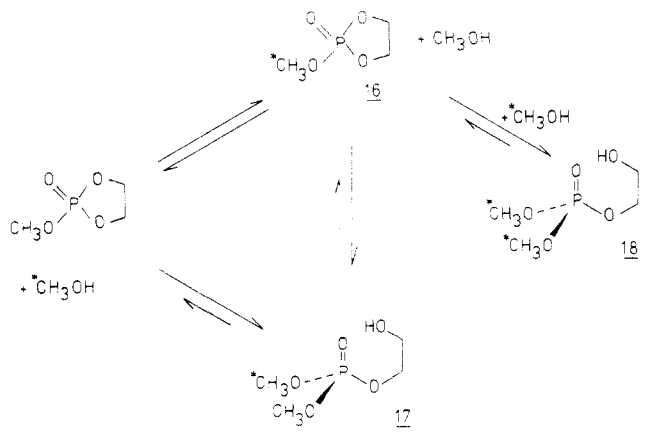
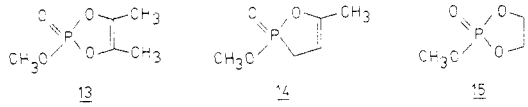
Scheme VIII



view, other compounds having double bonds, such as **13** and **14**, should give ring retention as well. However, it has



been shown[14] that these compounds undergo solvolysis with complete ring opening which can be attributed to the enol → keto tautomerization of the products.

The hydrolysis of methyl ethylene phosphate, **15**, with partial ring retention, has been rationalized by assuming pseudorotation of five-coordinated intermediates.[12] In contrast, the solvolysis of **15** in CH_3OH is reported to yield a ring-opened product exclusively.[15] However, this experiment does not rule out a reaction as shown in Scheme VIII, in which a new cyclic compund (**16**) is formed via pseudorotation or ring closure. Obviously, the equilibria in this case are more in favor of the ring-opened products than in the case of compound **5** (Scheme VII) which reflects the influence of the five-membered ring in **5**. Solvolysis of **15** in CD_3OD would be more conclusive, since this would allow a distinction between products **17** and **18**.

Conclusions

The low-temperature NMR measurements as well as the solvolysis experiments strongly indicate that a phosphate group and a nucleophile as vicinal substituents on a five-membered ring have a suitable orientation for intramolecular phosphorylation. *These results suggest that intramolecular phosphorylation of the 2'-OH group in RNA is strongly facilitated by the presence of the ribose ring.*

In addition, it has become clear that retention of the ring in the solvolysis of cyclic phosph(on)ates may be the result of a ring opening–ring closure mechanism, especially if constraining factors force the leaving group in the ring-opening reaction to remain close to phosphorus. Moreover, some recent reports seem to indicate that ring closure by intramolecular phosphorylation is a very common process.[16,17]

Experimental Section

Apparatus. ^1H NMR spectra were recorded on a Varian T-60A or EM-360A spectrometer. ^{31}P NMR spectra were obtained by using a Bruker HX90 instrument interfaced with a Digilab FT-NMR-3 computer. References were Me_4Si (internal) for ^1H NMR and 85% H_3PO_4 (external) for ^{31}P NMR. Downfield shifts (δ) are designated as positive. Elemental analyses were carried out in our laboratory.

Materials. *cis*-1,2-Cyclopentanediol and *cis*-1,2-cyclohexanediol were prepared according to literature methods[7] in 20 and 33% yields, respectively.

1,2-Cyclopentylene Bis(*p*-chlorobenzenesulfenate). *cis*-1,2-Cyclopentanediol (6.5 g, 0.064 mol) was dissolved in 400 mL of dry ether containing 18 mL of triethylamine (0.13 mol). The solution was stirred vigorously with cooling in ice/salt. *p*-Chlorobenzenesulfenyl chloride (22.9 g) was added dropwise under nitrogen. The salt was removed by filtration, and the ethereal solution was dried over $MgSO_4$ and evaporated. The yellowish solid residue was washed with 100 mL of methanol. After filtration, the solid was dissolved in chloroform and precipitated again by adding pentane: yield 28% of the theory; ^1H NMR ($CDCl_3$) δ 1.8 (m, 6 H, CH_2), 3.97 (m, 2 H, OCH), 7.13 (s, 8 H, aromatic).

1,2-Cyclohexylene Bis(*p*-chlorobenzenesulfenate). The preparation was completely analogous to that of the cyclopentane analogue: yield 49% after crystallization from CCl_4; mp 83–84 °C. Anal. ($C_{18}H_{16}O_2S_2Cl_2$) C, H.

3,3,3-Trimethoxy-2,4-dioxa-3-phosphabicyclo[3.3.0]octane (3). To a solution of trimethyl phosphite (0.04 mol) in 25 mL of dry CH_2Cl_2, cooled to −78 °C, was added 1.5 g of 1,2-cyclopentylene bis(*p*-chlorobenzenesulfenate) (0.039 mol) dissolved in a small amount of CH_2Cl_2. The solution was stirred for 4 h under nitrogen. After filtration under nitrogen in the cold, the solvent was removed and the phosphorane was purified by distillation: bp 53–54 °C (0.03 mmHg); yield 6.2 g (72% of theory); ^1H NMR ($CDCl_3$) δ 1.7 (m, 6 H, CH_2), 3.50 (d, J = 12.5 Hz, 9 H, OCH_3), 4.30 (dm, J = 12 Hz, 2 H, OCH); ^{31}P NMR ($CDCl_3$) −45.3 ppm. Anal. ($C_8H_{17}O_5P$) C, H.

8,8,8-Trimethoxy-7,9-dioxa-8-phosphabicyclo[4.3.0]nonane (4). The preparation was analogous to that of **3**: bp 56 °C (0.01 mm Hg); yield 71%; ^1H NMR ($CDCl_3$) δ 1.6 (m, 8 H, CH_2), 3.57 (d, J = 12.5 Hz, 9 H, OCH_3), 4.27 (dm, J = 13 Hz, 2 H, OCH); ^{31}P NMR ($CDCl_3$) −50.6 ppm. Anal. ($C_9H_{19}O_5P$) C, H.

3-Methoxy-3-oxo-2,4-dioxa-3-phosphabicyclo[3.3.0]octane (5). To a solution of 1.6 g of **3** (7.1 mmol) in 10 mL of dry CH_3CN was added 0.9 g of acetyl bromide. The temperature rose to about 60 °C. The mixture was stirred for 1 h in a nitrogen atmosphere. Evaporation of the solvent yielded 1.3 g of the mixture of diastereomers (100%), which could not be made to crystallize: ^1H NMR ($CDCl_3$) δ 1.85 (m, 6 H, CH_2), 3.77 (d, J = 12 Hz, 3 H, OCH_3, 65%), 3.78 (d, J = 12 Hz, 3 H, OCH_3, 35%), 5.10 (dm, J = 9 Hz, 2 H, OCH); ^{31}P NMR ($CDCl_3$) +18.8 (major isomer), +19.5 ppm (minor isomer). Anal. Calcd for $C_6H_{11}O_4P$: C, 40.46; H, 6.22. Found: C, 38.25; H, 5.63.

Spectroscopic Study of the Reaction of 3 and 4 with FSO_3H. NMR samples in Freon 21 (bp +9 °C) were prepared by condensing the solvent from a lecture bottle into the NMR tube containing the oxyphosphorane, cooled in an ice/salt bath. The concentration of the samples was about 0.8 mol/L. After the NMR tube was immersed in melting pentane (−120 °C), a drop of FSO_3H was added carefully via the wall of the tube. The contents wer mixed by shaking vigorously with occasional cooling. The ^1H NMR spectrum was recoreded in the temperature range from −120 to −10 °C. At relatively high temperatures, the irreversible formation of a phosphate was detected by the appearance of a new methoxy doublet apart from the doublets of oxyphosphorane and phosphonium ion: ^1H NMR (**3** + **3'**, −85 °C) δ 1.8 (m), 3.6 (d, J = 13 Hz, OCH_3, **3**), 4.2 (d, J = 11 Hz, OCH_3, **3'**), 4.5 (dm, OCH); ^{31}P NMR (**3** + **3'**, −51 °C) −41.6 (**3**), +4.1 ppm (**3'**); ^1H NMR (**4** + **4'**, −87 °C) δ 1.6 (m), 3.6 (d, J = 13 Hz, OCH_3, **4**), 4.2 (d, J = 11 Hz, OCH_3, **4'**), 4.5 (dm, OCH); coalescence temperature of the methoxy doublets (T_c) −55 °C (**3**), −15 °C (**4**).

The line width (Δ) of the methoxy doublets below T_c was measured at half-height. The line broadening $\Delta\nu$ was determined from a plot of ln Δ vs. $1/T$ (ref 9). From the plot of ln ($\Delta\nu$) vs. $1/T$, the E_A and the exchange rate, $1/\tau$ (at 248 K), were determined, which were used to calculate the activation parameters according to the following: $\Delta H^* = E_A - RT$; ln $(1/\tau)$ = ln $(RT/N_Ah) + \Delta S^*/R - \Delta H^*/RT$; $\Delta G^* = \Delta H^* - T\Delta S^*$.

Solvolysis Reactions of 5. The solvolyses were carried out in NMR sample tubes, using a concentration of 1 mol/L, at a

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temperature of 35 °C. The reactions were monitored by ^1H NMR. In case of CD_3OD, the integral of the methanol peak was corrected for the small amount of CHD_2OD (quintet) which is always observed in this solvent.

The methine (OCH) resonances of the cyclic compounds were found at about 5.1 ppm, whereas methine peaks around 4.4 ppm were attributed to ring-opened products. ^1H NMR data for the methoxy resonances of the various products are as follows. In CD_3OD: **5**, 3.81 (d, J = 12 Hz), 3.82 (d, J = 12 Hz, 2 isomers); **6**, 3.73 (d, J = 11 Hz); CH_3OD, 3.36 (s). In D_2O: **5**, 3.86 (d, J = 12 Hz), 3.87 (d, J = 12 Hz); **9**, 3.67 (d, J = 11 Hz); CH_3OD, 3.42 (s).

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Registry No. **3**, 77727-50-5; **3'**, 77727-57-2; **4**, 77727-51-6; **4'**, 77727-58-3; **5** (isomer 1), 77727-52-7; **5** (isomer 2), 77790-01-3; **6**, 77727-53-8; **9**, 77727-54-9; *cis*-1,2-cyclopentanediol, 5057-98-7; *cis*-1,2-cyclohexanediol, 1792-81-0; *p*-chlorobenzenesulfenyl chloride, 933-01-7; *cis*-1,2-cyclopentylene bis(*p*-chlorobenzenesulfenate), 77727-55-0; *cis*-1,2-cyclohexylene bis(*p*-chlorobenzenesulfenate), 77727-56-1.

Conformational Analysis of 5-Thio-D-glucose

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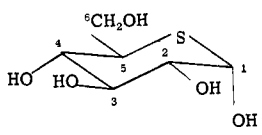
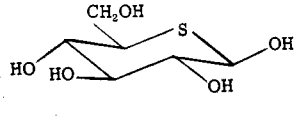
The proton and carbon-13 magnetic resonance spectra of 5-thio-D-glucopyranose have been fully analyzed. This molecule differs from D-glucopyranose in replacement of the ring oxygen by sulfur. The ^1H spectrum showed the presence of two anomers, with an α/β ratio of 85/15. Analysis of the ^1H–^1H vicinal coupling constants showed that the ring of the major anomer, 5-thio-α-D-glucopyranose, is slightly puckered in comparison to that of α-D-glucopyranose. All the axial–axial couplings are smaller in the sulfur heterocycle, and, most critically, $J_{1,2}$ (an axial–equatorial coupling) also is smaller. The electronegativity change for an O to S change would have predicted an increase in $J_{1,2}$. The observed decrease confirms the puckered distortion that is also a property of the unsubstituted heterocycle thiane. The ^{13}C resonances were assigned by a series of selective ^{13}C {^1H} experiments. The resonance positions are readily understood in terms of standard α, β, and γ shielding effects.

Replacement of the ring oxygen atom in D-glucopyranose with sulfur produces 5-thio-D-glucopyranose (**1**). This



1α



1β

material has been found to have a variety of physiological activity. It is effective in killing hypoxic cells, particularly in combination with mild hyperthermia. It inhibits D-glucose transport and sensitizes the hypoxic cells to radiation.[2] It has also been found to be a nontoxic male antifertility agent by inhibiting spermatogenesis[3] and a growth inhibitor for parasites with a high D-glucose requirement.

Differences between D-glucopyranose and 5-thio-D-glucopyranose may be attributed either to the electronic effects of replacement of oxygen by sulfur or to the steric/conformational effects of the altered bond lengths and valence angles. Probably the most effective tool for studying the latter geometric properties in solution is nuclear magnetic resonance spectroscopy. In the present paper we utilize both proton and carbon-13 NMR spectroscopy to explore the conformational changes that occur on replacement of the ring oxygen in D-glucose with sulfur. In earlier studies we have made similar comparisons between the unsubstituted oxane and thiane systems.[4] We will use these latter results as a model for the changes expected in the highly substituted sugar molecules.

Table I. Analysis of the ^1H Spectrum of 5-Thio-D-glucopyranose

proton	anomer	δ-(S)[a,b]	J(S)[b,c]	δ-(O)[a,d]	J(O)[c-e]
H_1	α	5.00	$^3J_{1,2}$ = 3.1	5.25	3.8
	β	4.77	$^3J_{1,2}$ = 9.3	4.65	8.1
H_2	α	3.78	$^3J_{2,3}$ = 9.4	3.54	9.8
H_3	α	3.65	$^3J_{3,4}$ = 8.9	3.73	9.5
H_4	α	3.60	$^3J_{4,5}$ = 9.1	3.42	9.5
	β		$^3J_{4,5}$ = 9.8		9.0
H_5	α	3.23	$^3J_{5,6'}$ = 3.0	3.82	1.5[f]
	β	2.98	$^3J_{5,6'}$ = 3.6	3.49	1.8
$H_{6'}$	α	3.87	$^2J_{6',6''}$ = −11.4	3.98	−12.0
	β				−12.2
$H_{6''}$	α	3.91	$^3J_{5,6''}$ = 5.1	3.90	3.9[f]
	β		$^3J_{5,6''}$ = 6.2		5.2

[a] In parts per million downfield from Me_4Si. [b] For 5-thio-D-glucopyranose. [c] In hertz. [d] For D-glucopyranose, the average from ref 5 and 6. [e] See column J(S) for notation. [f] Because of considerable disagreement between ref 5 and 6, this value was remeasured in our laboratory.

Results

The ^1H spectrum of 5-thio-D-glucopyranose was obtained at 360 MHz in pure D_2O (Figure 1) and in 10/1 Me_2SO-d_6/D_2O. To the latter solvent has been attributed the property of more closely maintaining the anomeric mixture

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