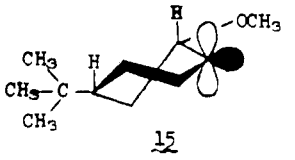
Dry **12** pyrolyzes analogously at 190 °C to **9** (27.5%), **13** (62.2%), and **14** (10.2%). Methoxy migration does not occur in rearrangement of **5** or **6.**

The difference in product composition from thermolyses of **7** and **11** is striking. First, the ratio of **9** from **7** to **9** from **11** is ~3.5:1. More impressive is the occurrence of **13** as the major product from **11;** 13 times more hydrogen migration occurs from C-6 in **6** to give **13** than from C-6 in **5** to **10** even though the repulsive 1,3-diaxial interactions in **13** are greater than in **10.** That **13** is formed in greater percentage than **9** is significant since the methoxy group enhances carbenic migration of other groups from the carbon atom to which it is attached.[3] Finally, and of greatest interest, is that the product proportions from **7** and **11** lead to apparent migratory ratios of H^a from C-2 in **5** to H^e from C-2 in **6** ranging from 35–46:1.[4-6]

These results may be rationalized as follows. In **5,** on the basis of equatorial preference of its *tert*-butyl group and (nearly) sp^2 hybridization of the carbenic center, the methine hydrogen is presumably nearly perpendicular to the filled, nonbonding orbital at C-1, whereas, in **6,** the methine hydrogen is conformationally restricted to an antiplanar orientation[1g] as modified by the diaxial interactions of its methoxy group. In **6,** hydrogen migrates from C-6 (apparently preferentially from an axial position) rather than from the equatorial position at C-2 in spite of extra stabilization of positive charge and relief of 1,3-diaxial interactions from the methoxy group. That little rearrangement of hydrogen from C-6 occurs in **5** and that ring contraction is observed in **6** is consistent with the effect in which the methoxy group enhances migration of other substituents.[3]

The present observations thus imply that, for cyclohexylidenes such as **5** and **6** in chair-like conformation, transition state **2** is more difficult to achieve than **3.** A possible reason for this is that hydrogen bridging is not highly developed in such rearrangements and the transition states reflect much of the structure of the reactant, **1.** Thus the interaction at the vacant orbital of the carbenic center is greater with α-axial than with α-equatorial hydrogen. Such effects may be quite large in systems as in **5** in which hydrogen has marked abilities to migrate because of electronic factors.[7]

A remaining significant and as yet unsolvable question is whether **9** is produced as a minor product from **6** by (1) equatorial rearrangement as for **2** and/or (2) conversion to twist-boat or even inverted chair forms and hydrogen migration from pseudoaxial (as in **15**) or axial positions. Rearrangement



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of equatorial hydrogen in **2** allows preservation of the chair-like structure of the ring system and effective relief of 1,3-diaxial interactions at carbon from which hydrogen migrates. On the other hand, it is unlikely that **6** is conformationally fixed at 190 °C and, at a hypothetical concentration of **15** as small as 2%, migration of α-methine hydrogen (pseudoaxial) ~15 times faster than α-methylene hydrogen will account for **9.** What is clear, however, is that, in cyclohexylidenes in which there are extensive diaxial repulsions, either of the above mechanisms will account for the nearly competitive migration of equatorial and axial α hydrogen.[1k,l]

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References and Notes

(1) (a) J. W. Powell and H. C. Whiting, *Tetrahedron,* **12,** 168 (1961); (b) R. Hoffman, G. D. Zeiss, and G. W. Van Dine, *J. Am. Chem. Soc.,* **90,** 1485 (1968); (c) N. Bodor and M. J. S. Dewar, *ibid.,* **94,** 9103 (1972); (d) O. S. Tee and K. Yates, *ibid.,* **94,** 3074 (1972); (e) H. E. Zimmerman, *Acc. Chem. Res.,* **5,** 393 (1972); (f) J. A. Altman, I. G. Csizmadia, and K. Yates, *ibid.,* **96,** 4196 (1974); (g) A. Nickon, F.-C. Huang, R. Weglein, K. Matsuo, and H. Yagi, *ibid.,* **96,** 5264 (1974); (h) J. A. Altmann, I. G. Csizmadia, and K. Yates, *ibid.,* **97,** 5217 (1975); (i) E. P. Kyba and C. W. Hudson, *ibid.,* **98,** 5696 (1976); (j) J. A. Altmann, O. S. Tee, and K. Yates, *ibid.,* **98,** 7132 (1976); (k) L. Seghers and H. Shechter, *Tetrahedron Lett.,* 1943 (1976); (l) E. P. Kyba and C. W. Hudson, *J. Org. Chem.,* **42,** 1935 (1977); (m) P. K. Freeman, T. A. Hardy, J. R. Balyeat, and L. D. Westcott, *ibid.,* **42,** 3356 (1977); (n) E. P. Kyba and A. M. John, *J. Am. Chem. Soc.,* **99,** 8329 (1977); (o) E. P. Kyba, *ibid.,* **99,** 8330 (1977).

(2) (a) 2-Methoxy-*cis*-4-*tert*-butylcyclohexanone and 2-methoxy-*trans*-4-*tert*-butylcyclohexanone were prepared by prior methods[2b] and converted to their *p*-tosylhydrazones under conditions such that stereochemical integrity was preserved. (b) Private communication, W. Chodkiewicz, Laboratoire de Recherches de Chimie Organique, Paris, France. (c) The *p*-tosylhydrazones were decomposed homogeneously in excess (up to 2.6 equiv) sodium hydride or sodium methoxide. The decompositions in solution or dry were satisfactorily reproducible throughout. (d) Products **10** and **13** were identified by GLC comparison with authentic samples; **9** (stereochemistry unknown) and **14** were hydrolyzed to 3-*tert*-butylcyclohexanone and 3-*tert*-butylcyclopentanecarboxaldehyde, respectively.

(3) W. Kirmse and M. Buschoff, *Chem. Ber.,* **100,** 1491 (1967); K. A. Gould, Ph.D. Dissertation, The Ohio State University, 1975.

(4) Calculated by multiplying the ratio of **9:10** from **5** by the ratio of **13:9** from **6** on the basis that the products formed are related to the rate constants, k_H, for migration of the indicated H by

$$\frac{k_H^a(\text{C-2 in } \mathbf{5})}{k_H^e(\text{C-2 in } \mathbf{6})} = \frac{k_H^a(\text{C-2 in } \mathbf{5})}{k_H^{a+e}(\text{C-6 in } \mathbf{5})} \times \frac{k_H^{a+e}(\text{C-6 in } \mathbf{6})}{k_H^e(\text{C-2 in } \mathbf{6})}$$

and assuming that the overall rate constants for migration of H^a and H^e from C-6 in **5** and **6** are essentially identical because 2-OCH_3 has small transannular steric (*A* value = 0.5–0.7 kcal/mol) and electronic effects.

(5) (a) On the basis that (1) H^a at C-6 in **6** is repelled by 2-$OCH_3{}^a$ at C-2, (2) the transition states for rearrangement of **6** (and **5**) are close to reactant, and (3) movement of H^a on C-6 into alignment with the empty p orbital at C-1 is retarded by 2-$OCH_3{}^a$, conversion to **13** as in **3** may actually be depressed, and the calculated migratory ratio may be smaller than in reality. (b) Steric release of H^a from C-6 in **6** resulting in an enhanced rate of formation of **13** is anticipated to be minor because 2-OCH_3 is small and the reaction transition state is predicted to resemble **6.** Further, such steric releases in **6** would also be expected to result in minor acceleration of formation of **9.** Thus accelerated conversions of **6** to **9** and **13** would be significantly cancelled in their net effects. (c) If it is assumed that the percentage of **9** from **6** has been specifically lowered totally by the conversion to **14,** the migratory ratios still calculate to be 24–34:1.

(6) On the assumption that the rearrangements have identical probability factors, these migratory ratios correspond to activation energy differences of ~3.3–3.5 kcal/mol at 190 °C.

(7) Migration of H^a from C-2 in **5** may be enhanced because 2-$OCH_3{}^e$ is nearly perpendicular to the vacant p orbital at C-1 and thus more capable of inductive electron donation than is 2-$OCH_3{}^a$ (as in **6**) to hydrogen rearrangement.

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Stereochemical Course of Glycerol Kinase, Pyruvate Kinase, and Hexokinase: Phosphoryl Transfer from Chiral [γ(*S*)-^{16}O,^{17}O,^{18}O]ATP

Sir:

We recently reported the stereoselective synthesis of a chiral [^{16}O,^{17}O,^{18}O]phosphate monoester and the independent determination of the absolute configuration at phosphorus in this molecule.[1,2] We now show that the synthetic method can be used to generate [γ-^{16}O,^{17}O,^{18}O]ATP of one configuration at the γ-phosphorus and that this material, when used as a substrate in the glycerol kinase reaction, results in the formation of isotopically labeled *sn*-glycerol 3-phosphate having the opposite configuration at phosphorus. Since we have earlier demonstrated that glycerol kinase, pyruvate kinase, and hexokinase have an identical stereochemical consequence at phosphorus,[3] we may conclude that *the transformations catalyzed by each of these enzymes proceed with inversion of the configuration at phosphorus.*

The synthesis of [γ(*S*)-^{16}O,^{17}O,^{18}O]ATP is outlined in Scheme I, and is an adaption of our earlier approach to chiral [^{16}O,^{17}O,^{18}O]phosphate monoesters.[1] Reaction of [^{17}O]-

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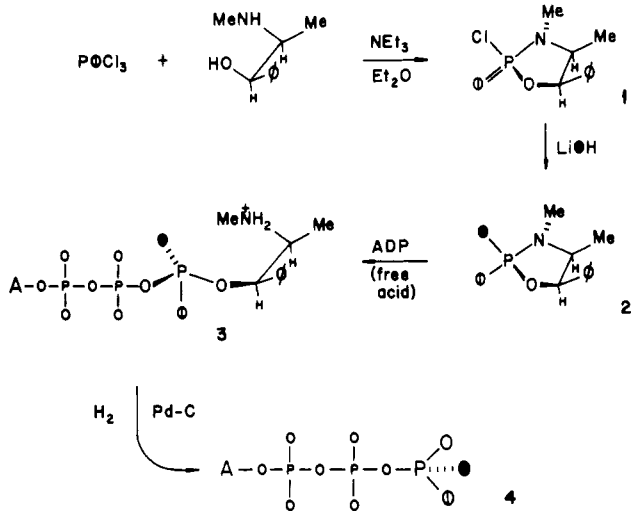
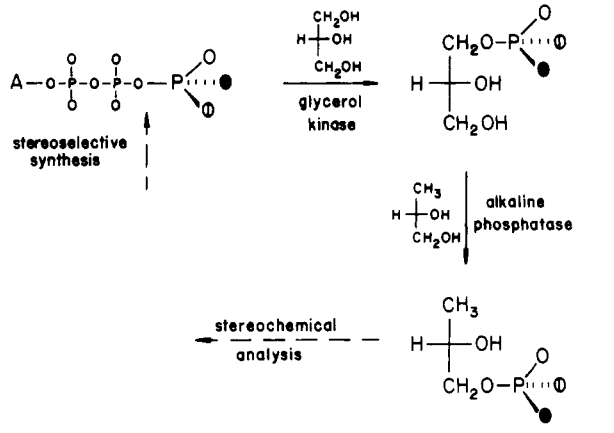
Scheme I. Synthesis of [γ(*S*)-^{16}O,^{17}O,^{18}O]Adenosine Triphosphate (Φ Is ^{17}O, ● Is ^{18}O, and A Is Adenosine)



Scheme II. Steps in the Determination of the Stereochemical Course of the Reaction Catalyzed by Glycerol Kinase



$POCl_3$[4] with (−)-ephedrine gave two epimeric chloro adducts.[5] The major isomer (**1**) was hydrolyzed by Li^{18}OH,[6] with retention of configuration at phosphorus,[1,5,7] to compound **2**. Reaction of **2** with ADP[8] resulted in the formation of **3**, which was purified by ion-exchange chromatography on DEAE-cellulose. The ATP derivative **3** was deprotected by hydrogenolysis[9] and yielded [γ(*S*)-^{16}O,^{17}O,^{18}O]ATP (**4**) in 40% yield (from **1**).

The synthetic [γ(*S*)-^{16}O,^{17}O,^{18}O]ATP was used to elucidate the stereochemical course of the glycerol kinase reaction as shown in Scheme II. Labeled ATP (615 μmol) was incubated with glycerol (40 mmol) and crystalline *E. coli* glycerol kinase (Sigma, 350 units).[10] The product *sn*-glycerol 3-phosphate was isolated in 95% yield after ion-exchange chromatography on Dowex 1 (HCO_3^-). In order to determine the absolute configuration at phosphorus in the glycerol phosphate, phosphoryl group transfer was effected to (*S*)-propane-1,2-diol[11] using alkaline phosphatase.[12,13] Since we have recently shown[12] that this reaction occurs with overall retention at phosphorus, the configuration at phosphorus in the resulting [1-^{16}O,^{17}O,^{18}O]phospho-(*S*)-propane-1,2-diol is the same as in the glycerol phosphate from which it derived. The absolute configuration of the phosphopropanediol was then determined by the following sequence: ring closure, methylation of an exocyclic oxygen, diastereoisomer separation, methanolytic ring opening, and metastable-ion mass spectrometry, as described earlier.[1] It was found that the configuration at phosphorus was *R* (90 ± 8%[14]).

The finding that the phosphoryl group that is transferred from ATP undergoes inversion in the reaction catalyzed by glycerol kinase (and therefore also in those catalyzed by pyruvate kinase and hexokinase[3,15]) restricts the range of possible mechanisms for these enzymes. The single displacement mechanism involving "adjacent" attack at phosphorus (necessarily followed by a pseudorotation[16]) is ruled out, as is a double displacement pathway via a phosphoryl enzyme if the two steps have the same stereochemical course. The only acceptable mechanisms involve either a single "in-line" displacement (or any odd number of "in-line" transfers), or a double displacement where the formation and collapse of the phosphoryl enzyme occur with opposite stereochemistries (i.e., one "adjacent" and one "in-line"). Since there is no evidence for a phosphoryl enzyme intermediate in the glycerol kinase reaction, our result points to a mechanism in which the phosphoryl group is transferred with "in-line" geometry directly between the two enzyme-bound substrates. The present experiments do not, of course, distinguish between associative mechanisms (where the pentacoordinate phosphorus is either a reaction intermediate or a transition state) and dissociative mechanisms (involving the highly reactive species monomeric metaphosphate[17,18]), but the geometrical constraints on substrate positioning at the active site and the stereochemical limitations on the mechanisms of the reactions catalyzed by these three kinases provide a welcome focus for mechanistic postulates in this area.

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References and Notes

(1) S. J. Abbott, S. R. Jones, S. A. Weinman, and J. R. Knowles, *J. Am. Chem. Soc.*, **100**, 2558 (1978).
(2) For a different approach, see P. M. Cullis and G. Lowe, *J. Chem. Soc., Chem. Commun.*, 512 (1978).
(3) G. A. Orr, J. Simon, S. R. Jones, G. J. Chin, and J. R. Knowles, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2230 (1978).
(4) The $H_2{}^{17}O$ used to prepare [^{17}O]$POCl_3$ contained 41.5% ^{17}O, 33.9% ^{16}O, and 24.6% ^{18}O.
(5) D. B. Cooper, C. R. Hall, J. M. Harrison, and T. D. Inch, *J. Chem. Soc., Perkin Trans. 1*, 1969 (1977). The chloro compounds (formed in 9:1 ratio) were separated by preparative TLC on silica, eluted with ethyl acetate.
(6) Li^{18}OH (2 equiv) in dioxane–water (1:1, v/v), room temperature, 10 min. The Li^{18}OH was prepared from Li and $H_2{}^{18}O$ (97.8% atom excess). All intermediates and products were characterized by ^{31}P and ^{1}H NMR.
(7) W. S. Wadsworth, Jr., and R. L. Wilde, *J. Org. Chem.*, **41**, 1264 (1976); M. Bauman and W. S. Wadsworth, Jr., *J. Am. Chem. Soc.*, **100**, 6388 (1978).
(8) The free acid (2 equiv), dimethyl sulfoxide, room temperature, 24 h.
(9) H_2–Pd/C, 3.4 atm, 5 h.
(10) In 32 mM triethanolamine–HCl buffer, pH 8.3, containing 3.8 mM $MgCl_2$ and 10 mM β-mercaptoethanol, room temperature, 1 h.
(11) Enantiomer excess 86%.
(12) S. R. Jones, L. M. Kindman, and J. R. Knowles, *Nature (London)*, **275**, 564 (1978).
(13) The sample of disodium *sn*-glycerol 3-phosphate (586 μmol) was incubated with *E. coli* alkaline phosphatase (Sigma, 9 units) in 0.3 M potassium bicarbonate–potassium carbonate (1:1) (2.4 mL) containing (*S*)-propane-1,2-diol[11] (2.4 mL), $Zn(OAc)_2$ (3.75 μM), and $Mg(OAc)_2$ (375 μM), at room temperature for 50 h.[12] The phosphopropanediol and remaining glycerol phosphate were isolated together by chromatography on Dowex 1 (HCO_3^-). The glycerol phosphate was oxidized by treatment with sodium periodate and the phosphopropanediol purified on Dowex 1 (HCO_3^-). Crystallization of the dicyclohexylammonium salt yielded 1-phospho-(*S*)-propane-1,2-diol containing 20% 2-phospho compound (assessed by ^{1}H NMR).
(14) Based on the known isotopic content,[4,6] the optical purity of the propanediol,[11] the diastereoisomeric purity of the separated syn and anti triesters,[1] and the content of the 2-phospho compound[13] in the sample analyzed.
(15) With the only caveat that the identity of the steroechemical course of these three kinases was demonstrated using ^{18}O-labeled phosphorothioates[3] rather than the [^{16}O,^{17}O,^{18}O]phosphoryl groups used here.
(16) F. H. Westheimer, *Acc. Chem. Res.*, **1**, 70 (1968).
(17) C. H. Clapp and F. H. Westheimer, *J. Am. Chem. Soc.*, **96**, 6710 (1974); C. H. Clapp, A. C. Satterthwaite, and F. H. Westheimer, *ibid.*, **97**, 6873 (1975); A. C. Satterthwaite and F. H. Westheimer, *ibid.*, **100**, 3197 (1978).
(18) G. Lowe and B. S. Sproat, *J. Chem. Soc., Chem. Commun.*, 783 (1978).

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