imposable on that of **6**. A similar correlation was made between **3** and **7**.

Acetate kinase catalyzes stereoselective phosphorylation of one of the oxygen atoms at P_β of ADPβs and pyruvate kinase stereoselectively phosphorylates the other.[12] To confirm the chiral purity at P_β of **4** and **5** prepared synthetically and to establish the orientations of phosphorylation by these enzymes, **4** and **5** were enzymatically phosphorylated to ATPβS,β^{18}O. Scheme III outlines our analytical procedure for determining whether ^{18}O in ATPβS,β^{18}O is bridging or nonbridging. Hydrolysis of **10** in Scheme III occurs with nearly equal partitioning of bridging oxygens into both **11** and **12** (53.1 ± 2.8% into **12** and 46.8 ± 2.8% into **11**). Therefore, if ^{18}O is bridging in ATPβS,β^{18}O, both **11** and **12** isolated according to Scheme III will be enriched in ^{18}O. If it is nonbridging, no ^{18}O will be found in **11**. Table I gives relevant mass spectral data. The ^{18}O enrichment in **4** and **5** was 81.3%; so Table I confirms the ^{31}P NMR data on chiral purity of these compounds. The data also show that acetate kinase catalyzes phosphorylation of the *pro-R* oxygen in ADPβS, i.e., ^{18}O in **4**, and pyruvate kinase catalyzes phosphorylation of the *pro-S* oxygen, i.e., ^{18}O in **5**.

Jaffe and Cohn have recently employed a different approach and reached the same conclusion regarding the absolute configurations at P_β in ATPβS diastereomers.[13]

References and Notes

(1) Supported by Grant No. GM 24390 from the National Institute of General Medical Sciences.
(2) (a) D. A. Usher, E. S. Erenrich, and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 115–118 (1972); K.-F. R. Sheu, and P. A. Frey, *J. Biol. Chem.*, **253**, 3378–3380 (1978); (c) F. Eckstein, V. W. Armstrong, and H. Sternbach, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2987–2990 (1976); (d) G. A. Orr, J. Simon, S. R. Jones, G. J. Chin, and J. R. Knowles, *ibid.*, **75**, 2230–2233 (1978).
(3) The abbreviations are ADPβS, adenosine 5′-(2-thiodiphosphate); ATPβS, adenosine 5′-(2-thiotriphosphate); ADPαS, adenosine 5′-(1-thiodiphosphate); ATPαS, adenosine 5′-(1-thiotriphosphate).
(4) W. A. Murray, and M. R. Atkinson, *Biochem. J.*, 7, 4025–4029 (1968). In hydrolytic workup the unreacted thiophosphoryl chloride was removed by vacuum distillation and sodium acetate (0.50 g) and $H_2{}^{18}O$ (0.5 g, 99% enriched in ^{18}O) were added in place of 10% aqueous barium acetate.
(5) A. M. Michaelson, *Biochim. Biophys. Acta*, **91**, 1–13 (1964).
(6) J. L. Darlix, H. P. M. Fromageot, and P. Fromageot, *Biochim. Biophys. Acta*, **145**, 517–519 (1967).
(7) **2**, $\delta(P_2)$ 43.175 ppm downfield from H_3PO_4; **3**, $\delta(P_2)$ 43.56 ppm downfield from H_3PO_4; both compounds, $\delta(P_1)$ 12.20 ppm upfield from H_3PO_4 ($J_{P_1-P_2}$ = 28.08 Hz).
(8) Acid, pH 2.0 for 20 min at room temperature; base, pH 10.5 for 30 min at 50 °C.
(9) **6** and 7 were synthesized according to Scheme I, substituting AMP for methoxymethylidene-AMP. They were separated by chromatography on a DEAE-Sephadex A-25 column using a $(C_2H_5)_3NH^+HCO_3^-$ gradient.
(10) K.-F. R. Sheu, and P. A. Frey (1977) *J. Biol. Chem.*, **252**, 4445–4448 (1977). ADPαS and ADPβS are well separated by $(C_2H_5)_3NH^+HCO_3^-$ gradient elution from DEAE-Sephadex A-25 columns.
(11) S. J. Benkovic and F. Eckstein (personal communications) have independently assigned the *S* configuration to the α-phosphorus of the diastereomers previously designated ADPαS (A) and ATPαS (A). P. M. J. Burgers and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(12) F. Eckstein, and R. S. Goody, *Biochemistry*, **15**, 1685–1691 (1976).
(13) E. Jaffe, and M. Cohn, *J. Biol. Chem.*, **253**, 4823–4825 (1978).

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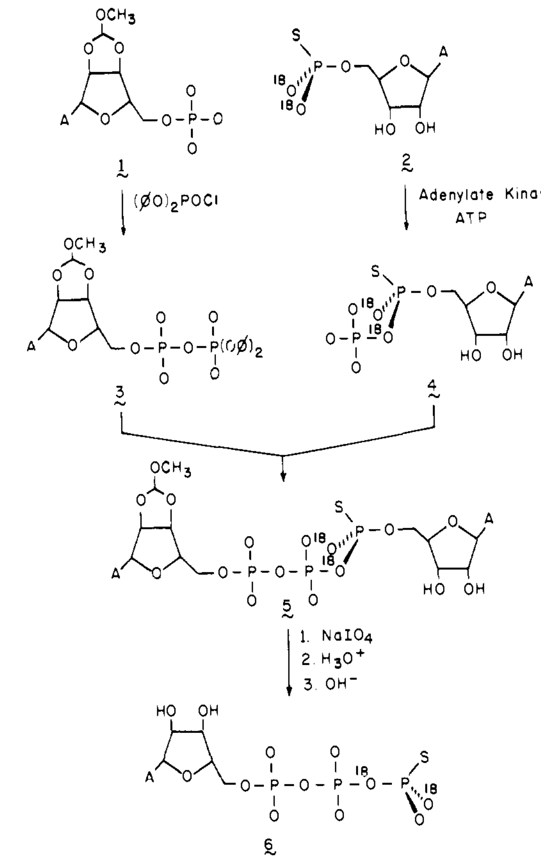
Stereochemical Course of Thiophosphoryl Group Transfer Catalyzed by Adenylate Kinase

Sir:

In recent years the mechanisms of phosphotransferase action have been studied intensively by such techniques as kinetics, radiochemical tracers, and magnetic resonance. These have produced valuable mechanistic information; however, the findings in such experiments are generally determined by the kinetics of the catalytic pathway, including the kinetics for desorption of products. Therefore, for example, the detection of a catalytic intermediate such as a covalent phosphoryl enzyme may be difficult if it exists at a small steady-state concentration.

Scheme I



Stereochemical data on phosphotransferases can give important mechanistic information which is independent of the kinetics. When the phosphate group is chiral and its configurations in the substrate and product can be related, the stereochemical course of the phosphoryl group transfer can be established. Net inversion of configuration is indicative of a single displacement of the phosphoryl group, and net retention is indicative of a double displacement, possibly via a covalent phosphoryl enzyme intermediate. In this paper we report on the synthesis of ATPγS,γ^{18}O[2] with a chiral γ-[^{18}O]phosphorothioate group of known configuration and on its use in showing that [^{18}O]thiophosphoryl group transfer catalyzed by rabbit muscle adenylate kinase occurs with net inversion of configuration of the [^{18}O]phosphorothioate group.

The synthesis of ATPγS,γ^{18}O having the *R* configuration at P_γ, **6**, is outlined in Scheme I. ADPαS,α^{18}O, **4**, having the *S* configuration at P_α is prepared by rabbit muscle adenylate kinase catalyzed phosphorylation of **2** by ATP.[3] **1**[4] was activated to **3** by reaction with diphenyl phosphochloridate, and **3** and **4** reacted smoothly in dimethylformamide-pyridine to produce **5**. The latter compound was not routinely purified but was converted directly to **6** by periodate cleavage of the unblocked ribosyl ring, acid deblocking of the other ribosyl ring, and alkaline elimination of the cleaved nucleoside as described in the preceding paper.[5] The overall yield of **6** from **4** was 55%. In one experiment **5** was purified by DEAE-Sephadex column chromatography, and it gave a ^{31}P NMR spectrum consisting of a P_α doublet 11.44 ppm upfield from H_3PO_4 ($J_{\alpha,\beta}$ = 18.31 Hz), a P_γ doublet 43.21 ppm downfield from H_3PO_4 ($J_{\beta,\gamma}$ = 25.64 Hz), and a P_β doublet of doublets at 24.13 ppm upfield from H_3PO_4.

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Table I. Configuration at P_β of ADPβS,β^{18}O Produced by Adenylate Kinase

phosphorylating system	mass % $^{18}O^a$	
	trimethyl phosphate	trimethyl phosphorothioate
acetate kinase	1.1 ± 0.1	83.0 ± 0.2
pyruvate kinase	20.3 ± 0.2	44.6 ± 0.4

[a] The degradation of ATPβS,β^{18}O to trimethyl phosphate and trimethyl phosphorothioate and the mass analysis of those compounds were as described in the preceding paper.[5]

ATPγS is a reasonably good thiophosphoryl donor substrate for adenylate kinase, which catalyzes eq 1:

$$\text{MgATP} + \text{AMP} \rightleftharpoons \text{MgADP} + \text{ADP} \quad (1)$$

When AMP is thiophosphorylated by **6**, the product is ADPβS,β^{18}O, and the configuration of the β-[^{18}O]phosphorothioate group can be related to that of the γ-[^{18}O]phosphorothioate in **6** by the procedure described in the preceding paper. Thus the configuration at P_γ in compound **6** of this paper is the same as that at P_β in compound **4** and opposite that in compound **5** of the preceding paper.[5] Therefore, if acetate kinase phosphorylates the ^{18}O in ADPβS,β^{18}O produced by adenylate kinase, the configuration is the same as that in **6** (retention) and, if pyruvate kinase phosphorylates this ^{18}O, the configuration is opposite that in **6** (inversion). The data are set forth in Table I which shows that the configuration is opposite that in **6**. We conclude that the reaction occurs with net *inversion* of the configuration of the phosphorthioate group. The least complex interpretation of this result is that the [^{18}O]thiophosphoryl group is transferred directly between the bound donor and acceptor substrates and not via a covalent thiophosphoryl enzyme intermediate.

Our determination of the stereochemical course of adenylate kinase action depends only upon knowledge of the relative configurations of the [^{18}O]phosphorothioate groups prepared in this and the preceding work.[5] The recent assignment of the *S* configuration to P_α of ATPαS isomer A[6] enables us to assign absolute configurations of ATPγS,γ^{18}O and ADPβS,β^{18}O described in this and the preceding paper.

The ^{18}O enrichment in the ADPβS,β^{18}O sample used in Table I was 85.2%. Comparing this with the 83.0% enrichment in trimethyl [^{18}O]phosphorothioate obtained from the ATPβS,β^{18}O sample resulting from acetate kinase catalyzed phosphorylation, it appears that thiophosphoryl group transfer by rabbit muscle adenylate kinase occurs with 97.6% inversion. Given the uncertainties of experimental error and of the magnitude of stereoselectivity exhibited by acetate kinase in the phosphorylation of ADPβS,β^{18}O, this cannot be distinguished from 100% inversion.

Orr et al. have recently prepared ATPγS,γ^{18}O of unknown P_γ configuration and shown that three phosphotransferases catalyze [^{18}O]thiophosphoryl transfer with complete stereospecificity and the same but unknown stereochemical consequences.[7] The present work represents the first synthesis of ATPγS,γ^{18}O with known configuration and the first delineation of the stereochemical course of catalysis by a phosphotransferase.

References and Notes

(1) Supported by Grant GM 24390 from the National Institute of General Medical Sciences.
(2) The abbreviations are ATPαS, adenosine 5′-(1-thiotriphosphate); ADPαS, adenosine 5′-(1-thiodiphosphate); AMPαS, adenosine 5′-phosphorothioate; ATPγS, adenosine 5′-(3-thiotriphosphate).
(3) K.-F. R. Sheu, and P. A. Frey, *J. Biol. Chem.*, **252**, 4445–4448 (1977). AMPαS,α^{18}O was converted to ATPαS,α^{18}O as described and then dephosphorylated to ADPαS,α^{18}O by reaction with excess glucose in the presence of hexokinase.
(4) J. L. Darlix, H. P. M. Fromageot, and P. Fromageot, *Biochim. Biophys. Acta*, **145**, 517–519 (1967).
(5) J. P. Richard, H.-T. Ho, and P. A. Frey, *J. Am. Chem. Soc.*, preceding paper in this issue.
(6) Personal communications from S. J. Benkovic and F. Eckstein. P. M. J. Burgers and F. Eckstein, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
(7) G. A. Orr, J. Simon, S. R. Jones, G. J. Chin, and J. R. Knowles, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2230–2233 (1978).

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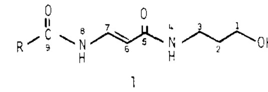
Ultraviolet Chromophores of Palytoxins

Sir:

The palytoxins, exceedingly poisonous substances from marine soft corals of the genus *Palythoa*,[1-3] exhibit ultraviolet absorption maxima at 263 and 233 nm. The λ 263 chromophore of these toxins is associated with a *N*-(3′-hydroxypropyl)-*trans*-3-amidoacrylamide moiety (**1**).[2,4] We report here



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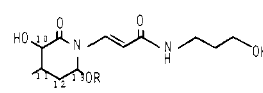
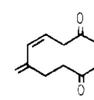
the degradation of palytoxins to **2a** and **5** (isolated and characterized as **6**) which possess the λ 263 and one of the two λ 233 chromophores,[5] respectively.



2a R = H
3a R = Me



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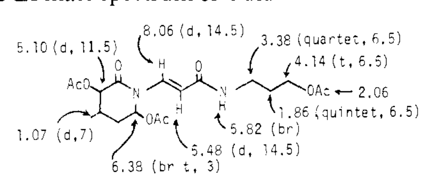
The toxin was oxidized with excess sodium metaperiodate in H_2O at 0 °C and the reaction mixture was extracted with chloroform. The organic material that remained in the aqueous layer was separated by countercurrent distribution (*n*-BuOH, H_2O) to give **2** (λ_{max} 263 nm) as the slowest moving fraction. The ^{1}H NMR spectrum of **2** did not exhibit an aldehydic signal, but **2** was readily converted to **3** when allowed to stand in MeOH-$CHCl_3$ solution. The ^{1}H NMR spectra of **2** and **3** were very similar and both showed doubling of signals for the presence of two closely related compounds.[6,7] The major compounds in **2** and **3** were **2a** and **3a**. Acetylation of **2** and separation of the mixture by TLC on silica gel (20% MeOH-benzene) led to triacetate **4**: UV (EtOH) λ_{max} 257 nm (ϵ 17 300); ^{1}H NMR ($CDCl_3$) 3 H singlets at δ 2.06, 2.09, 2.30. The EI mass spectrum of **4** did not exhibit a molecular ion peak



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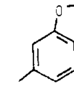

a

but did show small fragment ion peaks at *m/e* 338 and 278 and a very intense peak at *m/e* 134 for successive losses of two HOAc molecules and $CONHCH_2CH_2CH_2OAc$ from the molecular ion[8] to form a (possible structure of *m/e* 134 ion).

The mixture of **5** and other aldehydes in the chloroform fraction was reduced with $NaBH_4$ in 2-propanol and acetylated with acetic anhydride in pyridine. Preparative TLC on silica gel (35% EtOAc–cyclohexane) gave **6**: UV (MeOH) λ_{max} 227 nm; for ^{1}H NMR ($CDCl_3$) see formula; IR ($CHCl_3$) ν_{max} 910 cm^{-1}; mass spectrum m/e (rel intensity) (20 eV), no molecular

