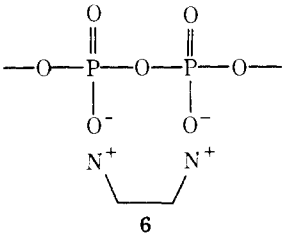
Table I. Relative Values of Equilibrium Constants K^a for the Binding of AMP and ADP by **2** or **3**

Compd	concn, 10^{-4} M		pH 3.0		pH 5.0		pH 8.0	
	Phosphate	**2** or **3**	AMP	ADP	AMP	ADP	AMP	ADP
2	0.2	0.5	(1)	7.7	17	61	27	34
3	0.2	1.0	0.7	0.4	0.0	0.4	0.7	1.4
2	1.0	2.5	(1)	79	12	335	270	1010[b]
3	1.0	5.0	0.6	6	0.9	9	0	6

[a] Relative values of equilibrium constants for extraction: $K = ([AXP]_{CHCl_3}/[AXP]_{aq})_{pH\ i}/([AMP]_{CHCl_3}/[AMP]_{aq})_{pH\ 3}$, for two different phosphate concentrations independently, where X = M or D and i = 3, 5, 8. [b] See note 9.

at pH 3 and 5[8]) or geminate **7** (AMP at pH 8) dianions of phosphate, although the latter interaction occurs to a lesser extent.[9] In marked contrast to the diammonium salt **2**, the monoammonium salt **3** is far less effective at lipophilizing **6** or **7**. Furthermore, the ability of **3** to extract **6** and **7** into chloroform does not depend on the extent of dissociation of phosphates. Thus, the aggregation of two (or three) monoammonium groups is not induced significantly to form the ion pair **8** with the phosphate anion (di- or tri-), probably owing to the unfavorable entropy effect involved.



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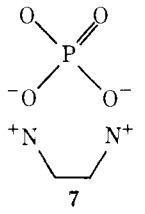

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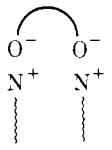

8

The conventional micellar reagent, stearyltrimethylammonium chloride **4** exhibited binding characteristics similar to the new phase transfer reagent **2** reported here. However, **4** and **2** form structurally different ion pairs with the phosphates. Thus, the ADP concentration in the aqueous phase decreased from 1.0 to 0.40 × 10^{-4} M at pH 8 by treatment with the chloroform solution of **4** (5.0 × 10^{-4} M); no trace of ADP was detected in the chloroform solution. The ADP appeared bound at the water-chloroform interface where a thick and opaque third phase was observed. In contrast to **4**, when **2** was employed, the phosphate that disappeared from the aqueous phase was found in the chloroform solution in a quantitative amount. These facts indicate that **2** acts as a typical phase transfer reagent rather than as a micellar reagent.

The highly effective binding of **2** to ADP relative to that of AMP in phase transfer suggests that this novel ammonium salt might be used as a specific carrier of ADP in transport through a liquid membrane. A significant rate difference already has been observed, and the details are currently under investigation.

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(8) These pH's should be valid only in bulk aqueous solution. The presence of an ammonium grouping in close proximity in nonpolar media should shift the pK_a of bound phosphates to a lower value.
(9) When concentrations of diammonium ion and phosphate were high at pH 5 and 8, a relatively slow and minor extraction of phosphate was observed to follow after a very rapid and major uptake of phosphate into chloroform. The final equilibrium value listed in Table I (3rd row) was obtained after long incubation time (usually 3 h). This slow rate process may be correlated with an assumed complex formation between a certain number of ammonium and phosphate ions when both concentrations are relatively high. The trianionic form of ADP could be accommodated in such structured aggregates.

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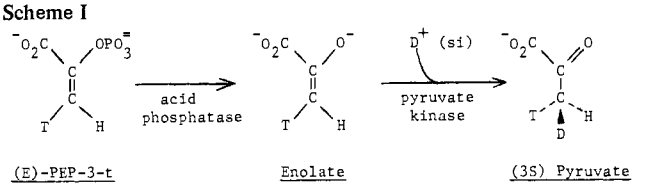
Stereochemistry of Ketonization of Enolpyruvate by Pyruvate Kinase. Evidence for Its Role as an Intermediate[1]

Sir:

Enolpyruvate and enolic forms of other substrates have been proposed as intermediates in many enzymatic reaction mechanisms,[2-8] but alternate proposals such as concerted displacement mechanisms are in active consideration.[9-13] We have recently shown[14] that treatment of phosphoenolpyruvate (PEP) with phosphatase in the presence of large amounts of lactate dehydrogenase gives rise to a transient intermediate which is believed to be enolpyruvate. Judging from the kinetics of this two-enzyme system, the conversion of the intermediate to pyruvate is slow, especially in D_2O ($t_{1/2}$ in $D_2O \simeq 10$ min at 15 °C, pD 6.4; $v_{H_2O}/v_{D_2O} = 6$). If the reaction in D_2O was terminated in acid-H_2O during the steady-state period, the expected amount of pyruvate was found. Mass analysis indicated that formation of the methyl group of the pyruvate occurred subsequent to the inactivation of the enzymes in the primarily H_2O medium as expected if the intermediate was enolpyruvate. It was also observed that pyruvate kinase catalyzed the conversion of the intermediate to pyruvate. The present communication shows that this catalysis is stereospecific and has the same stereospecificity that is observed when pyruvate is generated by pyruvate kinase by the overall reaction: PEP + ADP → pyruvate + ATP.

(*E*)-PEP-*3-t* was prepared as described earlier[4] and converted to enolpyruvate-*3-t* by action of acid phosphatase in D_2O in presence or absence of pyruvate kinase. Scheme I is

Scheme I



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Scheme II

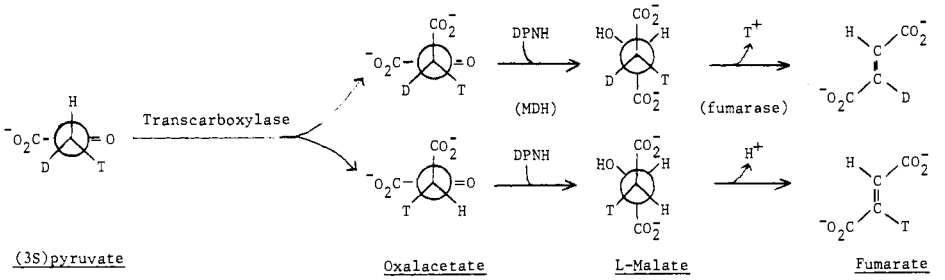

Table I. Stereospecificity of Ketonization by Pyruvate Kinase[a]

expt	precursor of L-malate-*3-t*	fractional release of tritium from L-malate by fumarase: individual	average[c]
1	pyruvate-*3-t*[b]	0.496	0.498 ±
		0.500	0.009
2	(*E*)-PEP-*3-t* + acid	0.484	0.491 ±
	phosphatase	0.479	0.010
		0.495	
		0.497	
		0.497	
3	(*E*)-PEP-*3-t* + ADP +	0.626	0.630 ±
	pyruvate kinase	0.628	0.006
		0.636	
		0.629	
4	(*E*)-PEP-*3-t* + pyruvate	0.618	0.616 ±
	kinase +	0.612	0.006
	acid phosphatase	0.616	

[a] Incubations in 0.5 mL of D_2O at 15 °C contained sodium maleate buffer (50 mM, pD 6.4), $MgCl_2$ (1 mM), PEP (1 mM), and the additions noted. Acid phosphatase of potatoes (2.5 mg) was from Sigma Co. It caused hydrolysis of PEP at ~0.7 μmol/min under the conditions used. Rabbit muscle pyruvate kinase, from Boehringer (0.2 mg ≃ 40 U), was added with $(NH_4)_2SO_4$ (~10 μmol). ADP was present at 1 mM in the incubation lacking phosphatase. [b] This malate was made from achiral pyruvate-*3-t*, transcarboxylase, and MDH. It was purified through silicic acid column. [c] 95% confidence limit.

drawn with the assumption that pyruvate kinase ketonizes enol pyruvate with the same stereospecificity as has been shown with PEP and ADP.[15] When pyruvate production was complete, as judged by assay with lactate dehydrogenase on control samples, $HClO_4$ was added to denature the enzymes and $KHCO_3$ added to neutralize. The precipitate was removed by centrifugation. Pyruvate in the supernatant was converted to malate by action of transcarboxylase with methylmalonyl CoA and malate dehydrogenase (MDH) and reduced diphosphopyridine nucleotide (DPNH), as observed at 340 nm. The L-malate was isolated by ion-exchange chromatography on Dowex-1-Cl^-. Its radiochemical purity was shown to be >96% by a modified procedure[15] in which malate was treated with fumarase followed by MDH plus the 3-acetylpyridine analogue of diphosphopyridine nucleotide and the radioactivity shown to be in water.

The tritium present at the *pro R* position of C-3 of the malate was determined by treatment with fumarase and measurement of the fraction of tritium released to water.[15-17] These steps for determining the chirality of the pyruvate are shown in Scheme II.

As seen in Table I, experiment 1, fumarase labilizes 50% of the tritium of L-malate-*3-t* generated from achiral pyruvate-*3-t* as expected.[6] Experiment 2 shows that pyruvate formed from enolpyruvate nonenzymatically is a racemic mixture. Kinetic studies have also shown that the phosphatase does not catalyze the ketonization.[14] When the (*E*)-PEP-*3-t* was converted to pyruvate by pyruvate kinase and ADP, without acid phosphatase as in experiment 3, the product was (3*S*)-pyruvate in agreement with previous studies.[15] The isotope effect exhibited here by transcarboxylase, which is shown by the ratio of (3*R*)- to (3*S*)-malate-*3-t* formed agreed with the intramolecular discrimination reported.[18] When ADP is omitted there was no production of pyruvate unless acid phosphatase is added. In this case, experiment 4, the pyruvate was formed with the same stereochemistry observed in the overall reaction. This result indicates that pyruvate kinase catalyzes the formation of pyruvate from the intermediate generated by action of phosphatases on PEP by a ketonization mechanism by proton approach to C-3 from the *si* face of enolpyruvate. At the concentration of pyruvate kinase present almost all of the ketonization was enzymatic. This result supports mechanisms in which enolpyruvate is a true intermediate in the overall allylic substitution catalyzed by pyruvate kinase and confirms data showing the separation of enolization and phosphoryl transfer steps. Furthermore this result demonstrates the usefulness of in situ generated enolpyruvate for enzymatic studies.

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(1) This work was supported by U.S. Public Health Service Grants GM-20940, CA-06927, and RR-05539, and by an appropriation from the Commonwealth of Pennsylvania.
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An Intramolecular Diels–Alder Route to Eudesmane Sesquiterpenes

Sir:

The steam volatile oil of hops[1] contains about 15 sesquiterpene hydrocarbons, one of which has been identified as selina-3,7(11)-diene (**1**).[2] This material has never been synthesized and represents a widespread group of compounds known as the eudesmane sesquiterpenes.[3] In general, synthetic

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