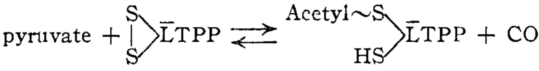
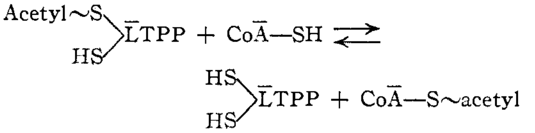
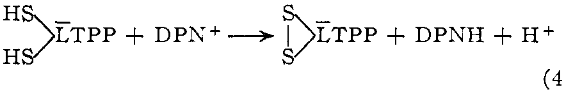
a purified pyruvate apoöxidase preparation from the mutant, shows that reaction 1 comprises reactions 2–4.



$$\mathrm{pyruvate} + \begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} \rightleftharpoons \begin{matrix}\mathrm{Acetyl}\!\sim\!\mathrm{S}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{CO_2} \qquad (2)$$

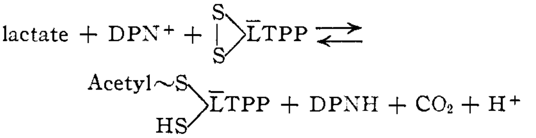


$$\begin{matrix}\mathrm{Acetyl}\!\sim\!\mathrm{S}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{Co\bar{A}{-}SH} \rightleftharpoons \begin{matrix}\mathrm{HS}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{Co\bar{A}{-}S}\!\sim\!\mathrm{acetyl} \qquad (3)$$



$$\begin{matrix}\mathrm{HS}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{DPN^+} \longrightarrow \begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{DPNH} + \mathrm{H^+} \qquad (4)$$

The stoichiometry of reaction 2 is demonstrated in Table I. Its reversibility has been demonstrated spectrophotometrically at 340 mμ by means of over-all reaction 5



$$\mathrm{lactate} + \mathrm{DPN^+} + \begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} \rightleftharpoons \begin{matrix}\mathrm{Acetyl}\!\sim\!\mathrm{S}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{DPNH} + \mathrm{CO_2} + \mathrm{H^+} \qquad (5)$$

which requires lactic dehydrogenase and pyruvate apoöxidase, and is the sum of reactions 6 and 2.

$$\mathrm{lactate} + \mathrm{DPN^+}\ (\mathrm{TPN^+}) \rightleftharpoons \mathrm{pyruvate} + \mathrm{DPNH}\ (\mathrm{TPNH}) + \mathrm{CO_2} + \mathrm{H^+} \qquad (6)$$

Evidence for reaction 3 consists of the demonstration that the acetyl group generated in reaction 2 can be utilized for the synthesis of acetyl sulfanilamide in the presence of pyruvate apoöxidase, CoĀ–SH and the arylamine acceptor enzyme of pigeon liver.[13] The reversibility of reaction 3 has been demonstrated spectrophotometrically at 340 mμ by means of over-all reaction 7, which requires lactic dehydrogenase pyruvate apoöxidase, phosphotransacetylase and a catalytic amount of CoĀ–SH, and is the sum of reactions 6, 2, 3 and 8

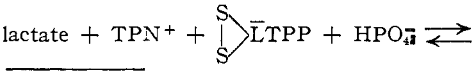
TABLE I

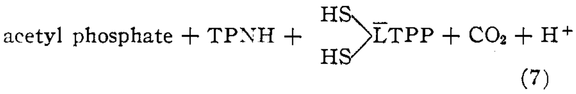
STOICHIOMETRY OF REACTION OF PYRUVATE WITH $\begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}$

(Δ in micromoles)

Pyruvate	CO_2	–SH	Acetylmercaptan
−3.8	+3.88	+3.71	+3.29

The reaction mixture contained 150 units[3] of pyruvate apoöxidase (specific activity, 1500 units/mg.), 6.5 μmoles[8] of DL-$\begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}$, 20 μmoles of potassium pyruvate, 10 μmoles of $MgCl_2$,[9] 60 μmoles of tris-(hydroxymethyl)-aminomethane buffer (*p*H 7.4). Final volume, 1.2 ml. Incubation, 10 min. at 25° in an atmosphere of N_2. Pyruvate was analyzed as the 2,4-dinitrophenylhydrazone,[10] –SH by the nitroprusside reaction,[11] and acetylmercaptan by the hydroxamic acid procedure.[12]





$$\mathrm{lactate} + \mathrm{TPN^+} + \begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{HPO_4^=} \rightleftharpoons \mathrm{acetyl\ phosphate} + \mathrm{TPNH} + \begin{matrix}\mathrm{HS}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP} + \mathrm{CO_2} + \mathrm{H^+} \qquad (7)$$

$$\mathrm{Co{-}\bar{A}S}\!\sim\!\mathrm{acetyl} + \mathrm{HPO_4^=} \rightleftharpoons \mathrm{acetyl\ phosphate} + \mathrm{Co\bar{A}{-}SH} \qquad (8)$$

A spectrophotometric demonstration of reaction 4[14] at 340 mμ, in the presence of pyruvate apoöxidase, has been obtained, but a net reversal of this reaction could not be demonstrated. These results suggest that the oxidation–reduction potential of the $\begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}/\begin{matrix}\mathrm{HS}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}$ system is appreciably more negative than that of the DPN$^+$/DPNH system, and therefore the equilibrium of reaction 4 is far to the right. TPN$^+$ will not replace DPN$^+$ in reaction 4.

It is to be noted that TPP does not function in the above reactions and actually inhibits the action of $\begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}$. However, the pyruvate apoöxidase preparation can effect an oxidative decarboxylation of pyruvate as represented by reaction 9. TPP is required for this reaction and its action is inhibited by $\begin{matrix}\mathrm{S}\\ |\\ \mathrm{S}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}$.

$$\mathrm{Pyruvate} + 2\ \mathrm{ferricyanide} + \mathrm{H_2O} \longrightarrow \mathrm{acetate} + \mathrm{CO_2} + 2\ \mathrm{ferrocyanide} + 2\mathrm{H^+} \qquad (9)$$

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[8] 6.5 mg. of a 66% pure preparation.

[9] The pyruvate apoöxidase preparation exhibits a partial requirement for Mg^{++} in reaction 1. The role of this activator in reactions 2–4 will be the object of a separate study.

[10] T. E. Friedemann and G. E. Haugen, *J. Biol. Chem.*, **147**, 415 (1943).

[11] R. R. Grunert and P. H. Phillips, *Arch. Biochem.*, **30**, 217 (1951).

[12] F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

[13] T. C. Chou and F. Lipmann, *ibid.*, **196**, 89 (1952).

[14] The $\begin{matrix}\mathrm{HS}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}$ was obtained by treating $\begin{matrix}\mathrm{acetyl}\sim\mathrm{S}\\ \mathrm{HS}\end{matrix}\!\!>\!\overline{\mathrm{L}}\mathrm{TPP}$ with aqueous mercuric acetate, which catalyzes hydrolysis of the thiol ester linkage.[15]

[15] F. Lynen, *et al.*, *Ann.*, **574**, 1 (1951).

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RECEIVED FEBRUARY 5, 1953

THE IDENTIFICATION OF THE ISOMERIC ADENYLIC ACIDS *a* AND *b* AS THE 2′- AND 3′-ADENOSINE PHOSPHATES, RESPECTIVELY[1]

Sir:

The location of the phosphate moiety in the first pair of isomeric nucleotides discovered and isolated in this Laboratory four years ago (adenylic acids ***a*** and ***b***)[2,3] has generally been regarded as 2′ and 3′, but not necessarily, respectively.[4] The structures of the subsequently isolated isomeric pairs of guanylic,[3] cytidylic[5,6] and uridylic[5] acids have been assumed to be the same as the adenylic acid pair;

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[1] Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

[2] C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

[3] W. E. Cohn, *ibid.*, **72**, 1471 (1950); **71**, 2275 (1949).

[4] D. M. Brown and A. R. Todd, *J. Chem. Soc.*, 44, 52 (1952); D. M. Brown, D. I. Magrath and A. R. Todd, *ibid.*, 2708 (1952).

[5] W. E. Cohn, THIS JOURNAL, **72**, 2811 (1950).

[6] H. S. Loring, *et al.*, *ibid.*, **72**, 2811 (1950).

the specificity of certain enzymes for the ***b*** forms[7] indicates a structural similarity while cytidylic acids ***a*** and ***b*** can be converted by deamination to uridylic acids ***a*** and ***b***, respectively.[8] The demonstrated acid-catalyzed migration of the phosphate group[4,5] has made difficult a decision as to which nucleotide was 2′ and which 3′ in both the previous synthetic[9] and degradative[10] approaches. However, comparison of the physical properties of the various isomeric pairs has led to the hypothesis that the ***b*** isomers are 3′-phosphates and the ***a*** the 2′-phosphates.[11,12]

We have been able to hydrolyze catalytically the N-glycoside linkage of the individual adenylic acid isomers with the hydrogen form of a polystyrene sulfonic acid resin (Dowex 50) at a rate comparable to the rate of isomerization. The advantage of this method of hydrolysis lies in the fact that the ribose phosphates are released from the resin at the time of formation (in contrast to adenine and most of the adenylic acid) and, therefore, little or no isomerization takes place subsequent to their formation. The two ribose phosphates obtained have been separated by a new ion-exchange procedure[13] and the amount of each is found to be proportional to the average amount of each resin-absorbed adenylic acid isomer existing during the hydrolysis period (*ca.* 30 seconds, 100°). Thus each ribose phosphate has been identified as the daughter of one adenylic acid isomer.

The ribose phosphate ***a*** (derived from adenylic acid ***a***) can be converted to a methyl phosphoribopyranoside, which consumes one mole of periodate, and to a ribitol phosphate with a marked optical activity which is enhanced by borate. The reverse properties (no periodate oxidation of the methyl phosphoriboside, no optical activity of the ribitol phosphate with or without borate) were noted for the ***b*** ribose phosphate. The possibility of the 1 or 5 phosphate isomers arising is excluded by the ion-exchange behavior of these substances; the 4 is *a priori* excluded by the furanoside structure of the parent nucleotide.[14] Therefore, the ***a*** ribose phosphate must be the 2 isomer and its parent adenylic acid ***a*** the 2′-phosphate ester of adenosine while the ***b*** ribose phosphate and adenylic acid ***b*** are the 3- and 3′-phosphate esters of ribose and adenosine, respectively. It can thus be concluded that Levene and Harris[15] were dealing with the ***b*** isomer in their earlier structural studies of the purine nucleotides, an inference supported by our present knowledge of the lesser solubility of the ***b*** forms[11] and the earlier methods of purification by crystallization.

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RECEIVED FEBRUARY 16, 1953

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[7] L. Shuster and N. O. Kaplan, *Federation Proc.*, **11**, 286 (1952); W. E. Cohn and E. Volkin, unpublished observations.

[8] D. M. Brown, C. A. Dekker and A. R. Todd, *J. Chem. Soc.*, 2715 (1952).

[9] D. M. Brown, L. J. Haynes and A. R. Todd, *ibid.*, 408 (1950).

[10] D. G. Doherty, Abstracts 118th Meeting, Am. Chem. Soc., 56 (1950).

[11] W. E. Cohn, *J. Cell. Comp. Physiol.*, **38**, Suppl. 1, 21 (1951).

[12] E. Volkin, J. X. Khym and W. E. Cohn, THIS JOURNAL, **73**, 1533 (1951); A. Kornberg and W. E. Pricer, *J. Biol. Chem.*, **186**, 557 (1950); H. S. Loring, *et al.*, *ibid.*, **196**, 821 (1952); L. Cavalieri, THIS JOURNAL, **74**, 5804 (1952).

[13] J. X. Khym and W. E. Cohn, *ibid.*, in press.

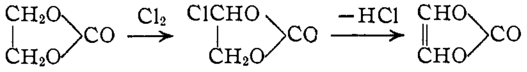
[14] P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **94**, 809 (1932); **97**, 491 (1932); **101**, 529 (1933); *cf.* J. M. Gulland, *J. Chem. Soc.*, 1722 (1938).

[15] P. A. Levene and I. F. Harris, *J. Biol. Chem.*, **101**, 419 (1933) *et ante.*

VINYLENE CARBONATE

Sir:

We wish to report the synthesis of vinylene carbonate, III, by the dehydrochlorination of chloroethylene carbonate, II, formed by the chlorination of ethylene carbonate, I. We believe III to be the first example of a cyclic carbonate of an enediol.



I II III

Vinylene carbonate reacts with 2,3-dimethylbutadiene to form a crystalline adduct, the cyclic carbonate of *cis*-4,5-dihydroxy-1,2-dimethylcyclohexene and is catalytically reduced to ethylene carbonate. Vinylene carbonate also polymerizes to yield clear colorless solid polymers which on hydrolysis yield water soluble polymers. The repeating unit of the hydrolyzed polymers is undoubtedly $-[CHOH]_n-$. Many interesting possible applications of such polymers are apparent.

Particularly striking are the boiling points of the following compounds: ethylene carbonate, 248°; monochloroethylene carbonate, 212°; 1,2-dichloroethylene carbonate, 178°; and vinylene carbonate, 162°.

This work was supported by a grant from the Research Corporation and is being continued.

Chlorination of Ethylene Carbonate.—A stream of chlorine was passed through 303 g. (3.44 moles) of freshly distilled ethylene carbonate at 63–70° in the presence of the ultraviolet light. After 24 hours the gain in weight was 119 g. (3.44 moles for monochloro substitution). Vacuum rectification yielded 28.0 g. (5.2%) of 1,2-dichloroethylene carbonate and 291 g. (69.0%) of monochloroethylene carbonate. Further rectification afforded pure 1,2-dichloroethylene carbonate (b.p. 78–79° at 19–20 mm., 178° at 739 mm.; n^{25}_{D} 1.4610; d^{25}_{4}, 1.5900; MR_D calcd. (Eisenlohr) for $C_3H_2O_3Cl_2$: 26.9. Found: 27.2. *Anal.* Calcd. for $C_3H_2O_3Cl_2$: C, 22.9; H, 1.3; Cl, 45.2. Found: C, 22.9; H, 1.2; Cl, 45.3. Strong strained ring carbonyl absorption at 5.40 μ); pure monochloro-ethylene carbonate (b.p. 106–107° at 10–11 mm., 212° at 735 mm., n^{25}_{D} 1.4530, d^{25}_{4} 1.5082, MR_D calculated for $C_3H_3O_3Cl$: 22.0. Found: 22.0. *Anal.* Calcd. for $C_3H_3O_3Cl$: C, 29.4; H, 2.5; Cl, 29.0. Found: C, 29.6; H, 2.5; Cl, 29.2. Strong strained ring carbonyl absorption at 5.45 μ).

Vinylene Carbonate.—To 30.0 g. of monochloroethylene carbonate in 100 ml. of dry ether at reflux temperature was added dropwise over a 7 hr. period 25.3 g. of triethylamine in 50 ml. of ether. Following refluxing and stirring overnight, the