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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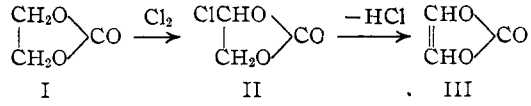
VINYLENE CARBONATE

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

solids were removed and washed with ether. The remaining ether was removed and distillation yielded 12.4 g. (59%) of colorless liquid, b.p. 76–79° at 37 mm. Further rectification afforded pure vinylene carbonate, b.p. 73–74° at 32 mm., 162° at 735 mm.; m.p. 22°; $n^{25}D$ 1.4190; d^{25}_4 1.3541. MR_D calcd. for $C_3H_2O_3$: 16.7. Found: 16.1. *Anal.* Calcd. for $C_3H_2O_3$: C, 41.9; H, 2.3. Found: C, 42.1; H, 2.4. Infrared analysis showed carbon–hydrogen absorption at 3.12 μ and strained ring carbonyl absorption at 5.48 μ.

Catalytic hydrogenation of vinylene carbonate yielded ethylene carbonate. Identity was proven by infrared absorption analysis and mixed m.p. determination.

Chlorine adds to ethylene carbonate to produce 1,2-dichloroethylene carbonate.

Diels–Alder Reaction.—Vinylene carbonate and 2,3-dimethylbutadiene in dry toluene were sealed under nitrogen in a tube and heated at 170–180° for 10 hr. A distilled (b.p. 145–147° at 4 mm.) sample of *cis*-4,5-dihydroxy-1,2-dimethylcyclohexene was crystallized to yield a colorless solid, m.p. 57.1–57.7°. *Anal.* Calcd. for $C_9H_{12}O_3$: C, 64.3; H, 7.2. Found: C, 64.6; H, 7.4.

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RECEIVED FEBRUARY 12, 1953

LUMINESCENCE IN CELL-FREE EXTRACTS OF LUMINOUS BACTERIA AND ITS ACTIVATION BY DPN[1]

Sir:

The enzyme-catalyzed emission of light by extracts of luminous organisms, when a hot water extract of the same organism is added to a cold water extract which has ceased to glow (the classical "luciferin–luciferase" reaction[2]), although demonstrable in extracts of fireflies,[3,4] *Cypridina hilgendorfii*[5] and other species, has never been conclusively demonstrated in extracts of luminous bacteria. Numerous workers have indeed reported negative results.[6–10]

Some time ago Shoup and Strehler,[11] using a quantum counter[12,13] of nearly ultimate sensitivity as a light-detecting apparatus, found that acetone-dried powders of the luminous bacterium, *Achromobacter fischeri*, will give appreciable light for some minutes after mixing with water. Using the same detector, conditions for more optimal rates of luminescence have been investigated and it has now been found possible to obtain luminescence visible to the naked eye from cell-free water extracts of acetone-dried *A. fischeri*. After the luminescence has disappeared, its reappearance may be effected by adding boiled extracts of acetone-dried bacteria. Moreover, it has been found that diphosphopyridinenucleotide (DPN) is a potent substitute for the boiled bacterial extract, raising the counting rate in a typical experiment from *ca.* 30 cts./15 seconds to *ca.* 100,000 cts./15 seconds almost at once. Reduced DPN ($DPNH^+$) is an even more potent substrate for this luminescence, giving a maximal response immediately. DPN presumably requires some time to be reduced by dehydrogenase systems in the extract.

It thus appears either that DPN is closely linked to the light-emitting system as an electron transport agent and becomes rate limiting in the crude active extracts or, possibly, that DPN is bacterial luciferin.

The high sensitivity of this system to added DPN and $DPNH^+$ (*ca.* 0.01–0.1 μg./ml. gives a measurable response) suggests its possible application as an assay tool analogous to the firefly enzyme in ATP measurement.[14] A study of factors influencing the extract luminescence and its application to bioassay is in progress.

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RECEIVED FEBRUARY 2, 1953

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