COMMUNICATIONS TO THE EDITOR

OXIDATIVE COUPLING OF BUTANES TO OCTANES

Sir:

The simplest method of converting low molecular weight paraffins into higher molecular ones would be a one-step synthesis consisting in the oxidative coupling of two paraffin molecules by means of molecular oxygen, in line with the over-all equation

$$2C_nH_{2n+2} + {}^1/_2O_2 \longrightarrow C_{2n}H_{4n+2} + H_2O \quad (1)$$

Unfortunately, all known methods of oxidation of paraffins lead, at best,[1] to oxidation products of the original paraffin containing the same number of carbon atoms. It was anticipated, however, that if paraffin molecules are pressed so tight that they cannot orient themselves and are forced to react with deficient amounts of oxygen in such close proximity to each other that successive oxidation of the same molecule would become highly improbable, *simply because of restrictions of geometry*, oxidative coupling of two close-lying paraffin molecules might occur. Under ideal coupling conditions the oxygen is forced to react with hydrogen atoms in its immediate vicinity and equal reactivities for primary and tertiary C–H-bonds are to be expected. Thus *isobutane* would couple to yield only: 2,5-dimethylhexane, 2,2,4-trimethylpentane and 2,2,3,3-tetramethylbutane, while *n-butane* would give only: *n*-octane, 3-methylheptane and 3,4-dimethylhexane. *Iso-* and *n-butane* were chosen for our experiments because any likely reaction products can be easily analyzed; furthermore they can be readily compressed to desired loading densities. It was assumed that favorable coupling conditions might prevail at pressures over 20,000 p.s.i. and at 300–350°, *i.e.*, below their thermal cracking range.

Isobutane (99.5+%) containing 4.4 mole % dissolved O_2, was heated in a 30-cc. Aminco Superpressure reactor at 325 ± 5° and at 23,000 p.s.i. pressure for 20–24 hours. It was found that over 80% of the O_2 reacted, forming only traces of CO_2 and CO; H_2O was formed in amounts corresponding to one-fourth of the O_2 consumed. Sixteen identical experiments yielded 9.0 g. of reaction products (b.p. > isobutane). They were divided into: (a) normal oxidation products of isobutane = 75 vol. % and (b) coupling products = 25 vol. %.

(a) consisted of ≃75 vol. % *t*-butanol and 25 vol. % of its degradation products, acetone and methanol.

(b) consisted of octanes, after removal of olefin traces. Microanalysis gave: 81.55% C, 15.17% H, or $CH_{2.22}$ (calcd. for C_8H_{18} = 2.25, for C_8H_{16} = 2.00). Infrared and mass spectra identified the following in vol. % of (b) *2,5-dimethylhexane* 40%, *2,2,4-trimethylpentane* 38%, *2,2-dimethylhexane* 8%, *2,2,3,3-tetramethylbutane present*, other octanes and octanes—possible traces. No masses above octanes were observed in the spectrum.

Identical conditions were used with *n*-butane; 25 vol. % of the product analyzed as follows: *n-octane* 10%, *3-methylheptane* 40–50%, *3,4-dimethylhexane* 30%; other paraffins and possibly olefins 10–20%.

Thus with each butane the three expected octanes were produced. They were also the *only* octanes observed, with the exception of 2,2-dimethylhexane. This abnormal octane is perhaps due to isomerization "*in statu nascendi.*" The amount of water found is also in agreement with equation 1.

The data presented indicate that under the conditions given the usual oxidation paths, although not eliminated, are sufficiently restricted so that the coupling reaction can be readily observed. No attempt to discuss possible mechanisms of this reaction will be made at this time.

The effect of highly restricted geometrical conditions, due to high pressure, on reaction paths, is, of course, not limited to paraffins and can be expected to yield interesting results with other types of compounds.

Acknowledgment is due to the Standard Oil Development Company for the support of this project and to Drs. R. F. Robey and B. E. Hudson, Jr., for mass and infrared analyses and to J. Snyder for some preliminary experiments.

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RECEIVED DECEMBER 31, 1952

(1) Usually complete breakdown to CO, CO_2 and H_2O takes place.

MECHANISM OF ENZYMATIC OXIDATIVE DECARBOXYLATION OF PYRUVATE

Sir:

The generation of active acetate (acetyl CoA[1]) from pyruvate by purified pyruvate oxidase preparations from bacterial[3] and animal[4,5] sources has been formulated as shown in reaction 1. TPN^+ will not replace DPN^+ in this reaction.[4]

$$\text{Pyruvate} + DPN^+ + CoA \longrightarrow \text{Acetyl CoA} + DPNH + CO_2 + H^+ \quad (1)$$

Studies[6] with soluble pyruvate apoöxidase preparations from an *Escherichia coli* mutant which cannot synthesize LTPP[7] reveal that reaction 1 does not proceed in the absence of  LTPP.

An analysis of the role of this coenzyme, employing

(1) The following abbreviations are used: CoA or CoA-SH = coenzyme A; DPN^+, DPNH and TPN^+, TPNH = oxidized and reduced diphospho- and triphosphopyridine nucleotides, respectively;  LTPP and LTPP = oxidized and reduced lipothiamide pyrophosphate (LTPP),[2] respectively; TPP = thiamine pyrophosphate.

(2) (a) L. J. Reed and B. G. DeBusk, THIS JOURNAL, **74**, 3964 (1952); (b) *J. Biol. Chem.*, **199**, 881 (1952).

(3) S. Korkes, *et al.*, *ibid.*, **193**, 721 (1951).

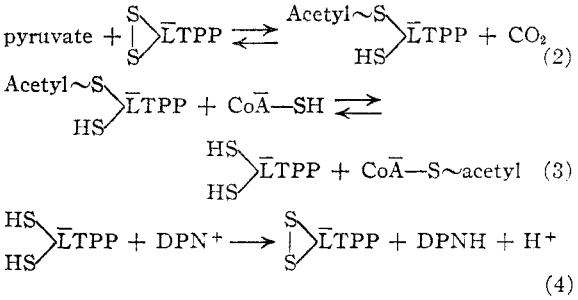
(4) J. W. Littlefield and D. R. Sanadi, *ibid.*, **199**, 65 (1952).

(5) R. S. Schweet and K. Cheslock, *ibid.*, **199**, 749 (1952).

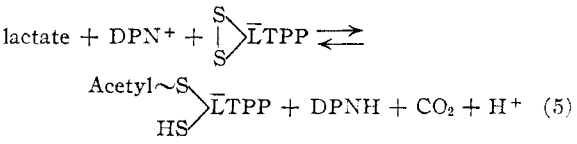
(6) L. J. Reed and B. G. DeBusk, unpublished results.

(7) L. J. Reed and B. G. DeBusk, THIS JOURNAL, **74**, 4727 (1952).

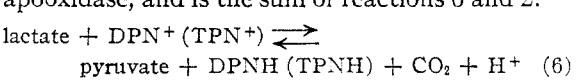
a purified pyruvate apoöxidase preparation from the mutant, shows that reaction 1 comprises reactions 2–4.



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

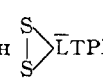


which requires lactic dehydrogenase and pyruvate apoöxidase, and is the sum of reactions 6 and 2.



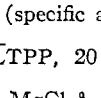
Evidence for reaction 3 consists of the demonstration that the acetyl group generated in reaction 2 can be utilized for the synthesis of acetyl sulfanilamide in the presence of pyruvate apoöxidase, CoĀ–SH and the arylamine acceptor enzyme

TABLE I

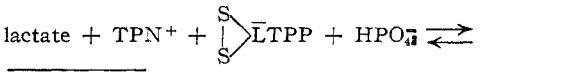
STOICHIOMETRY OF REACTION OF PYRUVATE WITH 

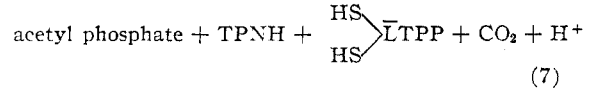
(Δ in micromoles)

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

The reaction mixture contained 150 units[8] of pyruvate apoöxidase (specific activity, 1500 units/mg.), 6.5 μmoles[8] of DL-  , 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]

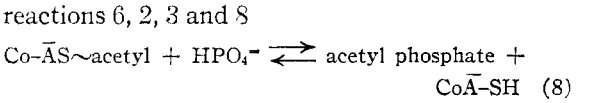
of pigeon liver.[13] The reversibility of reaction 3 has been demonstrated spectrophotometrically at 340 mμ by means of over-all reaction 7, which requires lactic dehydrogenase



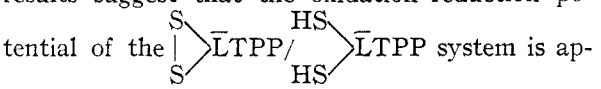


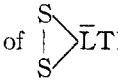
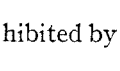
(7)

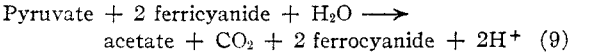
pyruvate apoöxidase, phosphotransacetylase and a catalytic amount of CoĀ–SH, and is the sum of reactions 6, 2, 3 and 8



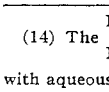
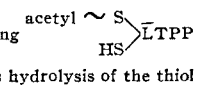
(8)

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  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  . 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  .



(9)

(14) The  was obtained by treating  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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(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).

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;

(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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OAK RIDGE, TENNESSEE WALDO E. COHN

RECEIVED FEBRUARY 16, 1953

(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.

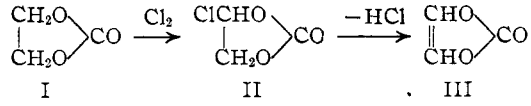
(14) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **94**, 809 (1932); **97**, 491 (1932); **101**, 529 (1933); *cf.* J. M. Gulland, *J. Chem. Soc.*, 1722 (1938).

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

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

(1) Work performed under USAEC contract No. W-7405-eng-26.

(2) E. N. Harvey, "Bioluminescence," Academic Press, Inc., New York, N. Y., 1952.

(3) W. D. McElroy, *Proc. Natl. Acad. Sci. U. S.*, **33**, 342 (1947).

(4) W. D. McElroy and B. L. Strehler, *Arch. Biochem.*, **22**, 420 (1949).

(5) E. N. Harvey, *Am. J. Physiol.*, **42**, 318 (1917).

(6) E. N. Harvey, *ibid.*, **41**, 449 (1916).

(7) E. N. Harvey, *ibid.*, **77**, 449 (1916).

(8) I. M. Korr, *Biol. Bull.*, **68**, 347 (1935).

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