enzyme preparations from kidney and that this process is stimulated by any of a number of cytidine nucleotides, including CDP-choline.[3] The amounts of labeled inositol incorporated ranged from 1–7 millimicromoles.

We have observed a much more extensive incorporation (75–150 millimicromoles) of free inositol catalyzed by well-washed, dialyzed microsomes from guinea pig liver in the presence of 0.01 *M* $MnCl_2$ in TRIS buffer of *p*H 7.4. Under these conditions, there is no requirement for cytidine nucleotides nor for an added source of metabolic energy. At low concentrations of $MnCl_2$, however, or in phosphate buffer, the reaction is stimulated by the same range of cytidine nucleotides as reported by Agranoff. It appears that this reaction, and probably the reaction observed by Agranoff, *et al.*,[2] is an enzymatic exchange of free inositol with inositol monophosphatide present in the enzyme particles and that the cytidine nucleotide effect is related to the binding of manganese by the enzyme. These experiments therefore provide little information about the pathways involved in the *de novo* synthesis of inositol monophosphatide.

TABLE I

CONVERSION OF L-α-GLYCEROPHOSPHATE-P^{32} TO INOSITOL MONOPHOSPHATIDE

Each tube contained 0.5 ml. of a dialyzed 20% homogenate of guinea pig liver in 0.05 *M* phosphate buffer of *p*H 7.4, 0.5 μmole of L-α-glycerophosphate-P^{32} (1.7×10^6 counts/μmole), 1.0 μmole of CoA, 0.1 μmole of oleic acid, 5.0 μmoles of ATP (added in portions), 3 μmoles of $MnCl_2$ and 3 μmoles of $MgCl_2$ in a total volume of 1.42 ml. The system was incubated at 37° for 2 hours. The lipides were extracted, washed and then hydrolyzed in methanol–chloroform containing 10% aqueous 10 *N* H_2SO_4. The inositol monophosphate fraction was isolated by chromatography on Dowex-1 formate and counted.

	Additions	L-α-Glycerophosphate-P^{32} converted to inositol monophosphatide (mμmoles)
1	None	0.1
2	1 μmole inositol	0.2
3	1 μmole CTP	4.8
4	1 μmole inositol + 1 μmole CTP	40.5
5	1 μmole inositol + 1 μmole GTP	0.2
6	1 μmole inositol + 1 μmole ITP	0.8
7	1 μmole inositol + 1 μmole UTP	0.0
8	1 μmole inositol + 1 μmole CDP-choline	1.3

It has now been found that the phosphorus moiety of inositol monophosphatide is derived from L-α-glycerophosphate and that the conversion of L-α-glycerophosphate-P^{32} to inositol monophosphatide P^{32} specifically requires CTP and free inositol (Table I). In contrast to the exchange reaction, which is stimulated by CDP-choline,[2] the conversion of L-α-glycerophosphate-P^{32} to inositol monophosphatide is not stimulated by CDP-choline.

The biosynthesis of inositol monophosphatide is thus like that of lecithin and phosphatidylethanolamine in that it requires CTP but is strikingly different in that the phosphorus of lecithin and phosphatidylethanolamine is not derived from glycerophosphate.[4] The present evidence is consistent with a route of formation involving either the hypothetical compound cytidine diphosphate diglyceride, which has been suggested by Agranoff, *et al.*,[1] as a possible intermediate, or cytidine diphosphate glycerol.[5]

(3) Abbreviations: ATP, CTP, GTP, ITP and UTP are the 5′-triphosphates of adenosine, cytidine, guanosine, inosine and uridine, respectively; CDP-choline = cytidine diphosphate choline; CMP = cytidine-5′-phosphate; CoA = coenzyme A; Tris = tris-(hydroxymethyl)-aminomethane.

(4) E. P. Kennedy, *Ann. Rev. Biochem.*, **26**, 119 (1957).

(5) J. Baddiley, J. G. Buchanan, B. Carss, A. P. Mathias and A. R. Sanderson, *Biochem. J.*, **64**, 599 (1956).

DEPARTMENT OF BIOCHEMISTRY
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OXIDATION OF SECONDARY PHOSPHINES TO SECONDARY PHOSPHINE OXIDES

Sir:

Secondary phosphines, like most derivatives of trivalent phosphorus, are easily oxidized. Indeed the lower alkyl secondary phosphines inflame spontaneously when exposed to the air.[1] Air oxidation of diphenylphosphine proceeds more gently, and the product has been reported to be diphenylphosphinic acid.[2] Phosphinic acids also result from the oxidation of secondary alkyl phosphines with strong oxidizing agents such as nitric acid.[3]

$$R_2PH \longrightarrow \left[\begin{matrix} O \\ \| \\ R_2PH \end{matrix}\right] \longrightarrow \begin{matrix} O \\ \| \\ R_2POH \end{matrix}$$

Although secondary phosphine oxides have been considered as possible intermediates, their isolation from such reactions has heretofore been thought impossible.[4] Moreover, secondary phosphine oxides, prepared by treating dialkyl phosphites with aliphatic[5,6,7] and aromatic[8] Grignard reagents or by reducing phosphinyl chlorides with lithium aluminum hydride,[6] have been found to be relatively stable toward oxidation.[5] Therefore, it seemed reasonable that if secondary phosphine oxides occurred as intermediates during the oxidation of secondary phosphines, they could be isolated by careful control of conditions.

We found this to be the case. Thus, crystalline secondary phosphine oxides were obtained by treating solutions of di-*n*-butylphosphine, di-*n*-octylphosphine, and bis-(2-cyanoethyl)-phosphine in isopropyl alcohol with air at 70°. Di-*n*-butylphosphine oxide, m.p. 66° (calcd. for $C_8H_{19}OP$: P, 19.08. Found: P, 18.84) was characterized by its infrared spectrum and by its reaction with chloral hydrate in refluxing isopropyl alcohol to give 1-hydroxy-2,2,2-trichloroethyldi-*n*-butylphosphine oxide, m.p. 132–133° (calcd. for $C_{10}H_{20}Cl_3O_2P$:

(1) A. W. Hofmann, *Ber.*, **4**, 605 (1871); **6**, 292 (1873).

(2) C. Dorken, *ibid.*, **21**, 1505 (1888).

(3) A. W. Hofmann, *ibid.*, **5**, 104 (1872); **6**, 303 (1873); A. R. Stiles, F. F. Rust and W. E. Vaughan, THIS JOURNAL, **74**, 3282 (1952).

(4) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 137.

(5) R. H. Williams and L. A. Hamilton, THIS JOURNAL, **74**, 5418 (1952).

(6) R. H. Williams and L. A. Hamilton, *ibid.*, **77**, 3411 (1955).

(7) R. C. Miller, J. S. Bradley and L. A. Hamilton *ibid.*, **78**, 5299 (1956).

(8) B. B. Hunt and B. C. Saunders, *J. Chem. Soc.*, 2413 (1957).

P, 10.00. Found: P, 9.78). Di-*n*-octylphosphine oxide, m.p. 85–6° (lit.[6] m.p. 85°) was characterized by its reaction with acrylonitrile to give 2-cyanoethyldi-*n*-octylphosphine oxide, m.p. 51–52° (lit.[7] m.p. 53.4–54.2°). Bis-(2-cyanoethyl)-phosphine oxide, m.p. 98–99°, (calcd. for $C_6H_9N_2OP$: C, 46.15; H, 5.80; P, 19.84. Found: C, 46.32; H, 5.95; P, 19.94) was characterized by reaction with chloral hydrate to give bis-(2-cyanoethyl)-1-hydroxy-2,2,2-trichloroethylphosphine oxide, m.p. 159–160° dec. (calcd. for $C_8H_{10}Cl_3N_2O_2P$: C, 31.65; H, 3.32. Found: C, 31.76; H, 3.35). Additional examples and experimental details will be given in a subsequent publication.

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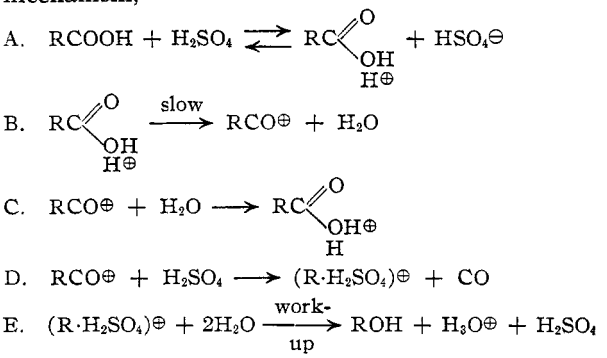
ISOTOPIC EVIDENCE FOR THE MECHANISMS OF DECARBONYLATION OF THREE CARBOXYLIC ACIDS IN SULFURIC ACID

Sir:

As reaction mechanisms are often determined from the form of the observed acid catalysis, it is significant that the mechanism[1] of decarbonylation of triphenylacetic acid proposed on this basis is not supported by isotopic evidence. Complete equilibrium of the oxocarbonium ion with water[1] and carbon–carbon bond cleavage as the rate determining step[1] are not supported since: (I) this decarbonylation in oxygen-18 enriched 95.5% sulfuric acid yielded carbon monoxide having an oxygen-18 enrichment of only about one-fifth that of the sulfuric acid,[2] and (II) no measurable isotope effect was found in the decarbonylation of triphenylacetic-2-C^{14} acid.

For the decarbonylation of formic acid the mechanism,[3]



accounts for the facts: (III) log of pseudo-first order rate constant linearly related to H_0[3]; (IV) Large carbon-14 isotope effect (k_{12}/k_{14} = *ca.* 1.09),[4] (V) secondary deuterium isotope effect (k_H/k_D = *ca.* 1.5) with formic-*d* acid, undoubtedly due to stretching of the carbon–hydrogen bond during the slow step, B, and (VI) carbon monoxide of normal isotopic composition formed during reaction in oxygen-18 enriched sulfuric acid. Step C fails to occur because of its unfavorable competition with D, the rapid proton transfer to the sulfuric acid.

For the triphenylacetic acid decarbonylation the scheme, A, B, C, D, E, adequately accounts for facts I and II. Some back reaction of the oxocarbonium ion with water, C, can occur by favorably competing with D which is an attack by sulfuric acid on the hindered number 2 carbon atom with ejection of carbon monoxide, and which is understandably slower than the analogous proton transfer in the formic acid decarbonylation. Since the oxygen-18 study indicates that C is slower than D, however, the mechanism is closer to that of formic acid[3] than to the other extreme proposed by Deno and Taft.[1] The reported[5,6] non-integral slope of the plot of log k *vs.* H_0 may be due to the intermediate character of the mechanism with neither B nor D strictly rate controlling.

The proposed mechanism[6] of decarbonylation of benzoylformic acid can explain the results of isotopic studies: (VII) the large isotope effect[7] with benzoylformic-1-C^{14} acid (k_{12}/k_{14} = *ca.* 1.1) due to carbon-14–oxygen bond cleavage in the rate step, VIII. A smaller effect (k_{12}/k_{14} = *ca.* 1.036) with benzoylformic-2-C^{14} acid, probably due to the effect of isotopic substitution at the number 2 carbon on the equilibrium constant of the reversible protonation involving the *alpha*-keto group, and IX. unenriched carbon monoxide from decarbonylation of benzoylformic acid in oxygen-18 enriched sulfuric acid, reasonable by analogy with the formic acid decarbonylation mechanism.

Non-radioactive carbon monoxide from decarbonylation of benzoylformic-2-C^{14} acid confirmed an earlier report[8] that the carbon monoxide came only from the carboxyl group.

Helpful suggestions of John D. Roberts and F. A. Long are acknowledged.

(1) N. C. Deno and R. W. Taft, THIS JOURNAL, **76**, 248 (1954).

(2) Although the two oxygen positions in carboxylic acids are not exactly equivalent, they should rapidly equilibrate through the symmetrical form, R—C(OH)(OH)⊕. Hence, the proposed[1] equilibrium involving the oxocarbonium ion and water should result in completely enriched carbon monoxide.

(3) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, N. Y., 1940, p. 283.

(4) G. A. Ropp, A. J. Weinberger and O. K. Neville, THIS JOURNAL, **73**, 5573 (1951); H. Eyring and F. W. Cagle, Jr., *J. Phys. Chem.*, **56**, 889 (1952).

(5) L. P. Hammett, *Chem. Revs.*, **16**, 67 (1935).

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(7) B. Fingerman and M. Lemmon, Bio-Organic Chemistry Report, Radiation Lab., Univ. of California, Berkeley, Calif., 1958, UCRL-8204.

(8) K. Banholzer and H. Schmid, *Helv. Chim. Acta*, **39**, 548 (1956).

(9) Chemistry Division, Oak Ridge National Laboratory, Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

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INTRAMOLECULAR HYDROGEN BONDING INVOLVING DOUBLE BONDS, TRIPLE BONDS AND CYCLOPROPANE RINGS AS PROTON ACCEPTORS

Sir:

We wish to report evidence which demonstrates the occurrence of intramolecular hydrogen bonding between proton donors and unsaturated linkages, including cyclopropane rings, as proton acceptors. Recently, similar observations have been reported for intramolecular interactions between hydroxyl