carbon tetrachloride and norbornylene formed under benzoyl peroxide initiation. Elution with benzene–ether (4:1) yielded an oil which sublimed slowly at room temperature. Sublimation at 110–115° (0.25 mm.) afforded 0.143 g. (10% yield) of the cyclic phthalate of *exo*-2-*syn*-7-bicyclo[2,2,1]-heptanediol (II), m.p. 138–140°. A second sublimation raised the melting point to 141.5–143°; ultraviolet absorption in ethanol, λ_{max} 231 mμ (log ϵ 3.91), 279 (3.22), 285 (3.20); infrared absorption (potassium bromide pellet) 1737, 1712, 1300, 1270, 1148 and 1125 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{14}O_4$: C, 69.74; H, 5.46. Found: C, 69.53; H, 5.62.

Carbon Tetrachloride–Norbornylene Addition Product (IV).—A solution of 1.095 g. (0.117 mole) of norbornylene and 0.272 g. (0.00113 mole) of benzoyl peroxide in 125 ml. of reagent carbon tetrachloride was heated at reflux for 19 hr. The acrid-smelling mixture was washed with aqueous sodium carbonate solution, with water and then dried over magnesium sulfate. Concentration followed by distillation yielded 1.61 g. (56% yield) of a colorless liquid, b.p. 132–135° (9 mm.), n^{30}D 1.5298. Vapor phase chromatography on a silicone–firebrick column afforded a single uniform peak, suggestive of the presence of a single isomer.

Anal. Calcd. for $C_8H_{10}Cl_4$: C, 38.74; H, 4.06; Cl, 57.19. Found: C, 39.01; H, 4.13; Cl, 56.88.

In a separate experiment, 10 mg. (4.13 × 10^{-5} mole) of benzoyl peroxide sufficed to convert 1.17 g. of norbornylene in 125 ml. of carbon tetrachloride into the 1:1 adduct in 50% yield, b.p. 130–133° (10 mm.), n^{29}D 1.5263. Only a faint cloudiness developed after several hours at 25° upon subjection of the liquid to 2% alcoholic silver nitrate solution.

Stability of 2,3-Norbornyl Phthalate to Conditions of Formation.—A solution of 0.058 g. of *exo*-2,3-norbornyl phthalate (I), 6.5 mg. of benzoyl peroxide and 0.257 g. of norbornylene in 25 ml. of carbon tetrachloride was heated at reflux for 20 hr. The colorless acrid-smelling solution was cooled and concentrated under reduced pressure, yielding a semi-solid residue. The residue was treated with ether and filtered yielding 0.042 g. (73%) of recovered cyclic phthalate of m.p. 201.5–204°. Concentration of the ether filtrate, chromatography on 2 g. of Florisil, and elution with petroleum ether–benzene (9:1) yielded the 1:1 solvent addition product. Elution with benzene–ether (1:1) afforded 0.006 g. of *exo*-2,3-norbornyl phthalate, m.p. 193–198°, total recovery 0.048 g. (83%). None of the 2,7-norbornyl phthalate could be isolated or detected.

Alkaline Hydrolysis of the Norbornyl Cyclic Phthalates. A. 2,7-Isomer.—A mixture of 0.16 g. of cyclic phthalate, m.p. 138–140°, 1 g. of sodium hydroxide and 10 ml. of 75% ethanol was heated at reflux for 12 hr. The slightly yellow mixture was cooled, diluted with 3 ml. of water saturated with sodium chloride and extracted with seven 30-ml. portions of ether. Concentration of the dried ether solution and two sublimations of the residual solid at 130–135° (15 mm.) afforded 57 mg. (73% yield) of white solid, m.p. 163–168°, which gave a negative periodic acid test. Recrystallization from ether–petroleum ether yielded pure diol of m.p. 172–174°, *exo*-2-*syn*-7-bicyclo[2,2,1]heptanediol, no depression upon admixture with an authentic sample prepared by the method of Walborsky and Loncrini.[10c]

The alkaline phase was diluted with 40 ml. of water, acidified with hydrochloric acid and extracted with ethyl acetate. Concentration of the dried organic phase yielded 64 mg. (62%) of phthalic acid, identified by sublimation to phthalic anhydride.

B. 2,3-Isomer.—A sample of 0.171 g. of cyclic phthalate of m.p. 204–205° was subjected to the above hydrolytic conditions. Three sublimations at 105–110° (15 mm.) afforded pure *cis-exo*-2,3-bicyclo[2,2,1]heptanediol, m.p. 137–139°, mixture m.p. of 136–138.5° with an authentic sample prepared by the oxidation of norbornylene by permanganate.[15]

The alkaline phase was worked up in the manner described previously, yielding 95 mg. of phthalic acid identified by conversion to phthalic anhydride.

Kinetics.—The rate of reaction of phthaloyl peroxide with olefin was followed by the method described previously[3] (iodometric analysis for peroxide).

Acknowledgment.—We wish to thank the Research Corporation for a Frederick Gardner Cottrell grant which was of great assistance to this research.

[15] K. B. Wiberg and K. A. Saegebarth, THIS JOURNAL, **79**, 2822 (1957).

CAMBRIDGE 39, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

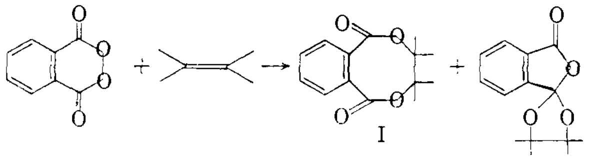
Cyclic Diacyl Peroxides. VI.[1] Reaction of Phthaloyl Peroxide with Diarylacetylenes

BY FREDERICK D. GREENE AND WILLIAM W. REES[2]

RECEIVED JULY 24, 1959

Phthaloyl peroxide reacts with diphenylacetylene and with di-*p*-methoxyphenylacetylene in carbon tetrachloride by a process involving over-all consumption of two moles of peroxide per mole of acetylene. The principal products from acid hydrolysis of the product mixture from peroxide and di-*p*-methoxyphenylacetylene are anisil, isolated in 32% yield, and phthalic acid. Kinetic evidence is presented for rate-determining formation of a one-to-one adduct of peroxide and acetylene followed by rapid reaction of adduct with peroxide. Possible complications in the tolan reactions are considered with reference to the reaction of phthaloyl peroxide with tetraphenylethylene and with a series of olefinic and non-olefinic compounds.

Phthaloyl peroxide previously has been shown to undergo a stereospecific reaction with olefins in a process that is kinetically first order in peroxide and first order in olefin to afford mixtures of products in which cyclic phthalates (I) and lactonic *ortho*-esters (II) are major components.[3] In this paper the results of an investigation of the reaction of phthaloyl peroxide with two compounds possessing a carbon–carbon triple bond are presented.



Results and Discussion

The acetylenic compounds employed in this study were diphenylacetylene (tolan) and di-*p*-methoxyphenylacetylene. The reaction of phthaloyl peroxide with the acetylenes was carried out in carbon tetrachloride at 80°. With both acetylenes orange-red coloration developed during the reaction. The infrared spectra of the crude

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[1] Part V, F. D. Greene and W. W. Rees, THIS JOURNAL, **82**, 890 (1960).

[2] General Electric Fellow, 1957–1958. This article is based upon a portion of the Ph.D. thesis of W. W. Rees, 1958.

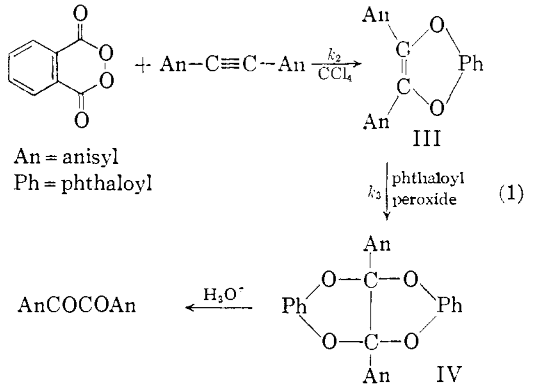
[3] (a) F. D. Greene, THIS JOURNAL, **78**, 2250 (1956); (b) F. D. Greene and W. W. Rees, *ibid.*, **80**, 3432 (1958).

product mixtures showed absorption at 1858 and 1790 cm.$^{-1}$ (phthalic anhydride) and at 1780 cm.$^{-1}$ (lactonic *ortho*-ester carbonyl) and weak absorption at 1730–1720 cm.$^{-1}$ (cyclic phthalate carbonyl). The complex mixture was subjected to acid hydrolysis from which anisil was isolated in 32% yield. The hydrolytic conditions employed were such that any anisoin present would have remained unchanged. Phthalic acid was isolated in 76% yield. The conversion of the acetylene by phthaloyl peroxide to material of the oxidation state of anisil rather than anisoin corresponds to the over-all oxidation of one mole of acetylene by two moles of phthaloyl peroxide. This formulation is supported by kinetic evidence. The rate of disappearance of phthaloyl peroxide is not in accord with a second-order process involving one-to-one reaction, over-all, of acetylene and peroxide but is in close accord with a process involving reaction of phthaloyl peroxide and acetylene in a slow step followed by rapid reaction of the one-to-one adduct with a second molecule of phthaloyl peroxide (eq. 1, k_2 rate-determining, k_3 rapid). Use of the second order equation applicable to this case gave good correlation and enabled

$$\log \frac{a(b - 2x)}{b(a - x)} = \frac{b - 2a}{2.303} k_2 t$$

a = [acetylene]; b = [phthaloyl peroxide]

determination of the reaction rate constants.[4] The results of a kinetic run with diphenylacetylene and with di-*p*-methoxyphenylacetylene are depicted in Fig. 1. The kinetic data are summarized in Table I. Pertinent relative reactivity data are summarized in Table II. The acetylenes are several hundred fold less reactive toward phthaloyl peroxide than the corresponding olefins, in accord with the lower reactivity of acetylenes *vs.* olefins in most electrophilic additions.[5] With both the acetylenes and the olefins the *p*-methoxy group produces substantial acceleration in rate.



Two aspects of the peroxide-acetylene reaction warrant further consideration: (1) the nature of III in eq. 1; and (2) the complexity of the product mixture. Some information pertinent to these questions is provided by a qualitative study of the rate of disappearance of phthaloyl peroxide in the presence of several substrates. The data are summarized in Table III.

TABLE I

RATE OF REACTION OF PHTHALOYL PEROXIDE WITH DIPHENYLACETYLENE AND WITH DI-*p*-METHOXYPHENYLACETYLENE IN CARBON TETRACHLORIDE

Cpd.	Initial concn. acetylene, *M*.	Initial concn. peroxide, *M*.	Temp., °C.	k_2, M^{-1} sec.$^{-1}$
Di-*p*-methoxyphenylacetylene	0.0120	0.0120	80	12×10^{-3}
	.00725	.0145	80	11×10^{-3}
	.0148	.0140	45	2.3×10^{-3}
Diphenylacetylene	.0898	.0123	80	4.4×10^{-5}

TABLE II

COMPARISON OF REACTIVITY OF PHTHALOYL PEROXIDE TOWARD ACETYLENIC AND RELATED OLEFINIC COMPOUNDS IN CARBON TETRACHLORIDE AT 80°

Compound	Rel. *k*
Di-*p*-methoxyphenylacetylene	1.6
(*p*-Methoxytolan)[a]	(0.1)
trans-*p*-Methoxystilbene	74
Diphenylacetylene	0.006
trans-Stilbene[b]	1.9
cis-Stilbene[b]	1.0

[a] Estimated from tolan and dimethoxytolan by use of σ^+ (see ref. 3b). [b] Data of ref. 3b.

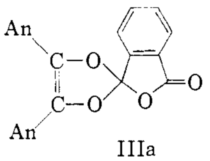
TABLE III

PERCENTAGE CONSUMPTION OF PHTHALOYL PEROXIDE (0.00945 *M*) IN PRESENCE OF SUBSTRATE (0.015 *M*) IN CARBON TETRACHLORIDE AT 80°

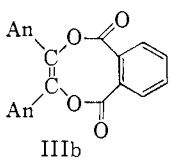
No.	Substrate	2 hr.	4 hr.	9 hr.	24 hr.	48 hr.
1	None[a]	0.53	0.95	1.8	4.6	8.9
2	Cyclohexene[b]	46	66	87		
3	Dihydropyran	90	94			
4	Dimethylresorcinol[c]		72	80		
5	1-Iodopentane[d]		35	69	85	
6	Fluorene		6.5	22	48	
7	2-Cyclohexenyl hydrogen phthlate		3.2	11	26	49
8	Ethyl orthoformate			6.2	35	68
9	Dibenzyl ether			8	31	49
10	*trans*-Stilbene-α,β-diol diacetate			8.1	24	

[a] Calcd. from $k_1 = 5.36 \times 10^{-7}$ sec.$^{-1}$. [b] Calcd. from $k_2 = 6.83 \times 10^{-3}$ M^{-1} sec.$^{-1}$ (ref. 3b). [c] Yellow coloration developed. [d] Purple coloration developed.

The principal alternatives for the initial one-to-one adduct (III of eq. 1) are IIIa and IIIb.



IIIa



IIIb

The prominence of absorption at 1780 cm.$^{-1}$ and weakness of absorption at 1730–1720 cm.$^{-1}$ in the infrared of the crude product mixture are sug-

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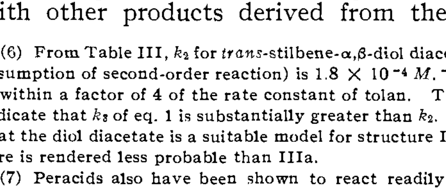
[4] For a general solution of the kinetic situation of the type shown in eq. 1 for the ratio of the two rate constants (k_3/k_2) from a knowledge of the concentrations of two components at a single time, see W. G. McMillan, THIS JOURNAL, **79**, 4838 (1957).

[5] The rate ratio for stilbene to diphenylacetylene in bromination in acetic acid at 25° is 250; P. W. Robertson, W. E. Dasent, R. M. Milburn and W. H. Oliver, *J. Chem. Soc.*, 1628 (1950). See also R. A. Raphael, "Acetylenic Compounds in Organic Synthesis," Academic Press, Inc., New York, N. Y., 1955, p. 33.

gestive that IIIa rather than IIIb is the intermediate. The rapidity of reaction of dihydropyran (no. 3, Table III) and the slowness of *trans*-stilbene-α,β-diol diacetate (no. 10), a compound electronically related to IIIb, are also more in accord with structure IIIa for the initial adduct rather than with structure IIIb.[6]

The apparent complexity in product mixture derived from phthaloyl peroxide and di-*p*-methoxyphenylacetylene may be associated with a number of factors. The great reactivity of phthaloyl peroxide toward the dimethyl ether of resorcinol (no. 4) raises the possibility of some attack on the anisyl rings of the acetylene rather than exclusive attack at the triple bond.[7] A second complicating feature is the possibility of homolytic fission of the central carbon–carbon bond of compounds of type IV. This question has been examined in the potentially simpler system based on reaction of phthaloyl peroxide with tetraphenylethylene. Unlike the reaction of phthaloyl peroxide with olefins such as the stilbenes in which the rate is cleanly first order in each component throughout the course of the reaction and in which the solution remains colorless, the reaction of phthaloyl peroxide with tetraphenylethylene exhibits an autocataylsis in the rate of disappearance of peroxide; and as the reaction proceeds, the reaction mixture becomes colored both in the presence and in the absence of oxygen. The infrared spectrum of the crude product mixture shows strong absorption at 1780 cm.$^{-1}$ and negligible absorption at 1730 cm.$^{-1}$. From an acidic hydrolysis of the crude reaction mixture derived from a solution initially equimolar in olefin and peroxide was isolated benzpinacolone in 25% yield and tetraphenylethylene in 38% yield of the initial amount. The finding of unreacted tetraphenylethylene is an expected result in view of the autocatalysis noted in rate of disappearance of peroxide. These data are suggestive of the reaction of phthaloyl peroxide and tetraphenylethylene, in the manner established for the stilbenes,[3a] to form a one-to-one adduct (V) which possesses a bond (bond "a") prone to undergo homolytic fission. The autocatalysis in rate of disappearance of phthaloyl peroxide may be associated with reaction of the peroxide with the diradical produced by breaking of bond "a" or with other products derived from the diradical.



V

(2)

Efforts to isolate benzophenone, a possible oxidation product derivable from V, were unsuccessful. (Benzophenone was shown to be stable to the experimental conditions.)

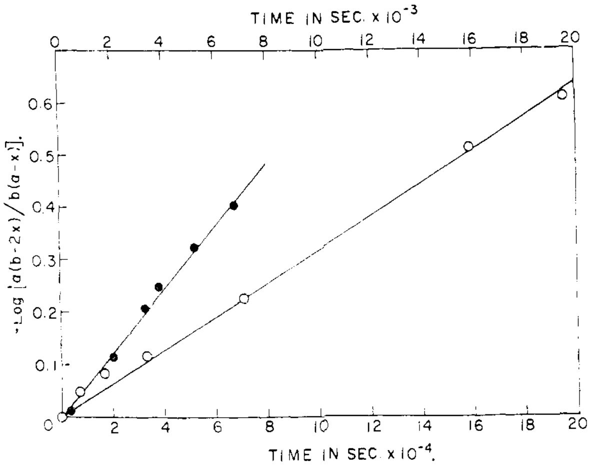


Fig. 1.—Reaction of phthaloyl peroxide with diphenylacetylene (open circles, time scale at bottom of graph) and with di-*p*-methoxyphenylacetylene (solid circles, time scale at top of graph).

The data of Table III also point up other possible complications in reactions of phthaloyl peroxide. The reactivity of dibenzyl ether, fluorene and ethyl orthoformate, although substantially less than that of olefins, is suggestive of oxidative attack on hydrogen located at activated positions. Attention is also directed to the rapid consumption of phthaloyl peroxide in the presence of 1-iodopentane (no. 5). Although benzoyl peroxide is stable in methyl iodide and in ethyl iodide,[8] di-*o*-nitrobenzoyl peroxide decomposes three hundred times more rapidly in methyl iodide than in chloroform containing iodine.[8]

Experimental

Phthaloyl peroxide was prepared by the method of Russell.[9] Material of at least 97% purity, obtained by recrystallization from methylene chloride, was used throughout.

Diphenylacetylene was prepared from stilbene dibromide by the method of Smith and Falkof,[10] m.p. 63.5–65° (lit. m.p. 60–61°).

Tetraphenylethylene was prepared by the method of Bachmann.[11] After several recrystallizations from benzene–methanol, material of m.p. 225–226° was obtained (lit. m.p. 224°).

Di-*p*-methoxyphenylacetylene.—A solution of 4.55 g. (0.0114 mole) of 4,4'-dimethoxystilbene dibromide[12] and 7.5 g. of potassium hydroxide in 50 ml. of ethanol was heated at reflux for 50 hr. The dark mixture was cooled, diluted with 30 ml. of water, and filtered. Recrystallization once from carbon tetrachloride and twice from acetic acid–water afforded 1.07 g. (40% yield) of di-*p*-methoxyphenylacetylene, m.p. 141.5–143° (lit.[13] m.p. 142°).

Reaction of Phthaloyl Peroxide with Di-*p*-methoxyphenylacetylene.—A solution of 0.378 g. (2.3 mmoles) of phthaloyl peroxide and 0.270 g. (1.13 mmoles) of di-*p*-methoxyphenyl-

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[6] From Table III, k_2 for *trans*-stilbene-α,β-diol diacetate (calcd. on assumption of second-order reaction) is 1.8×10^{-4} *M.*$^{-1}$ sec.$^{-1}$. This is within a factor of 4 of the rate constant of tolan. The kinetic data indicate that k_3 of eq. 1 is substantially greater than k_2. To the extent that the diol diacetate is a suitable model for structure IIIb, this structure is rendered less probable than IIIa.

[7] Peracids also have been shown to react readily with methoxylated aromatic compounds; see S. L. Friess, A. H. Soloway, B. K. Morse and W. C. Ingersoll, This Journal, **74**, 1305 (1952).

[8] J. E. Leffler, R. D. Faulkner and C. C. Petropoulos, *ibid.*, **80**, 5435 (1958). The iodo compound was included in the series of Table III at the suggestion of Dr. John E. Leffler.

[9] K. E. Russell, *ibid.*, **77**, 4814 (1955).

[10] L. I. Smith and M. M. Falkof, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., p. 350.

[11] W. E. Bachmann, This Journal, **56**, 449 (1934).

[12] P. Hoering and K. P. Gralert, *Chem. Ber.*, **42**, 1204 (1909).

[13] H. Wiechell, *Ann.*, **279**, 337 (1894).

acetylene in 100 ml. of carbon tetrachloride was heated at 45° for 114 hr. The solvent was removed under reduced pressure and the orange-brown residue was hydrolyzed in 50 ml. of a 3% hydrochloric acid solution of 50% aqueous dioxane at 25° for 3 hr. and at reflux for 14 hr. Dilution with water afforded a dark solid which upon trituration with 80% ethanol yielded 0.092 g. (32%) of crude anisil, m.p. 120–123°. Chromatographic purification on silica gel and recrystallization from ethanol afforded material of m.p. 129.5–131°, mixed m.p. 129.5–131.5°. Extraction of the aqueous hydrolysis layer with ethyl acetate, removal of solvent, and trituration with chloroform afforded 0.29 g. (76%) of phthalic acid, identified by sublimation to phthalic anhydride.

Reaction of Phthaloyl Peroxide with Tetraphenylethylene.—A solution of 0.42 g. (2.56 mmoles) of phthaloyl peroxide and 0.85 g. (2.56 mmoles) of tetraphenylethylene in 125 ml. of carbon tetrachloride was heated at reflux for 15.5 hr. Solvent was removed from the yellow-orange solution under reduced pressure. The residue showed strong absorption in the infrared at 1780 cm.$^{-1}$. The residue was hydrolyzed in 40 ml. of 75% aqueous ethanol containing 4.5 ml. of concd. sulfuric acid for 12 hr. at reflux. A light tan solid, 0.80 g., precipitated on cooling and was chromatographed on 22 g. of basic alumina. Elution with 19:1 petroleum ether–benzene yielded 0.325 g. of tetraphenylethylene of m.p. 221–222.5° after one recrystallization from benzene; mixture m.p. showed no depression. Elution with 1:1 petroleum ether–benzene gave 0.219 g. of benzpinacolone of m.p. 180–181° after one recrystallization from benzene–ligroin; mixture m.p. showed no depression. Attempts to obtain evidence for benzophenone were unsuccessful.

Subjection of the crude olefin–peroxide reaction mixture to basic hydrolysis and work-up by the method described above afforded phthalic acid in 60% yield, characterized by conversion to the anhydride.

Reactivity of Phthaloyl Peroxide with Various Substrates.—Six-ml. portions of a solution of phthaloyl peroxide (0.00945 M) and substrate (0.015 M) in carbon tetrachloride were sealed in Pyrex tubes and heated for the requisite time intervals at 80°. Consumption of peroxide was followed by iodometric analysis of 5-ml. aliquots. The data are summarized in Table III.

Kinetics.—The rates of reaction of phthaloyl peroxide with the unsaturated compounds were followed by iodometric analysis for peroxide by the methods described previously.[3] The data are summarized in Fig. 1 and in Tables I, II, and IV.

TABLE IV

RATE OF REACTION OF DI-*p*-METHOXYPHENYLACETYLENE WITH PHTHALOYL PEROXIDE IN CARBON TETRACHLORIDE AT 80°[a]

Time, sec. × 10³	Thiosulfate soln.,[b,c] ml.
0	12.09
0.36	11.40
2.04	7.50
3.24	5.40
3.84	4.71
5.22	3.71
6.78	2.94

[a] k_2 (graphical) = $11.8 \times 10^{-3}\ M^{-1}\ \text{sec.}^{-1}$. Initial concentrations, 0.0120 M. [b] 0.00996 N. [c] Five-ml. aliquots of reaction solution.

Acknowledgment.—We wish to thank the Research Corporation for a Frederick Gardner Cottrell grant which was of great assistance to this research.

CAMBRIDGE 39, MASS.

[CONTRIBUTION FROM THE CLAYTON FOUNDATION BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Studies on the Nature of Protein-bound Lipoic Acid

BY HAYAO NAWA,[1] WILLIAM T. BRADY, MASAHIKO KOIKE AND LESTER J. REED

RECEIVED JULY 13, 1959

Highly purified pyruvate and α-ketoglutarate dehydrogenation complexes (from *Escherichia coli*) containing bound radioactive lipoic acid were oxidized with performic acid and then partially hydrolyzed with 12 N hydrochloric acid (3 hours at 105°). From the hydrolysates was isolated in good yield a ninhydrin-positive, radioactive conjugate which was identified as ε-N-(6,8-disulfoöctanoyl)-L-lysine by degradation and synthesis. The lipoyl moiety in the two complexes therefore is bound in amide linkage to the ε-amino group of a lysine residue. Partial hydrolysis of the oxidized pyruvate dehydrogenation complex under milder conditions (7 days at 37°) yielded three radioactive 6,8-disulfoöctanoylpeptides. Analysis of these peptides indicated that the amino acid sequence about the lysine residue is either Ala.Lys.Asp. or Asp.Lys.Ala.

Early studies on the distribution of lipoic acid indicated that it occurs in tissues largely in association with proteins. Thus, it was not extractable by hot water or by lipid solvents but was released by hydrolysis with acid, alkali or crude proteolytic enzymes.[2–4] Subsequent studies revealed that "bound" lipoic acid is an integral part of CoA- and DPN-linked pyruvate and α-ketoglutarate dehydrogenation systems.[5–9] An investigation of components and conditions required for incorporation[7] of lipoic acid into bacterial apopyruvate dehydrogenation systems and for its release[8] from the holoenzymes indicated that lipoic acid is bound to protein in covalent linkage through its carboxyl group. This paper presents evidence that lipoic acid is bound to the ε-amino group of a non-terminal lysine residue. A preliminary report of this work has appeared.[10]

When *Escherichia coli* is grown aerobically in the presence of lipoic acid-S_2^{35}, the latter substance is incorporated into the pyruvate and α-ketoglutarate dehydrogenation systems. These systems have been isolated as structural units (en-

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