During the investigation of biologically active boron compounds in this laboratory, compounds such as 2-aminoacetic diphenylborinic anhydride were formed by heating butyl diphenylborinate with glycine.

$$(C_6H_5)_2BOC_4H_9 + H_2NCH_2COOH \longrightarrow (C_6H_5)_2BOCCH_2NH_2 + C_4H_9OH$$

The low-molecular weight amino acids, such as glycine, react well without a solvent. A solvent, such as toluene, is more convenient with the higher molecular weight, more soluble amino acids. The white solid products appear to be stable and have not undergone change on storage in air for 4 years. These anhydrides may be recrystallized from alcohol–water without change, but they react readily with ethanolamine to form 2-aminoethyl diphenylborinate.

Experimental

All melting points were obtained on a Fischer-Johns melting point apparatus and were corrected by comparison with standard compounds.

Reaction with Glycine.—A mixture of 0.50 g. (0.0067 mole) of glycine and 1.59 g. (0.0067 mole) of butyl diphenylborinate[3] was boiled and stirred for 5 min. The mixture became solid. Toluene (5 ml.) was added, and the mixture was boiled for a few minutes. After cooling, the solid was collected on a filter and was washed with ether. The anhydride was boiled with 25 ml. of distilled water and was collected on a filter after cooling, 0.82 g., m.p. 244–245°, 51% (based on butyl diphenylborinate). A 0.33-g. portion was dissolved in 5 ml. of 95% ethanol, filtered while hot, cooled, and recrystallized once more from ethanol to yield 0.13 g., m.p. 244–245°.

Anal. Calcd. for $C_{14}H_{14}O_2NB$: C, 70.4; H, 5.91; N, 5.86; mol. wt., 239. Found: C, 70.2; H, 6.0; N, 5.76; mol. wt., 241.

If the anhydride is heated with ethanolamine in alcohol–water solution, 2-aminoethyl diphenylborinate crystallizes upon cooling. The melting point and mixture melting point with 2-aminoethyl diphenylborinate were 187–189°.

If toluene is used as a solvent for this reaction, the insolubility of the glycine is a problem, and the yield is lowered.

Similar compounds were prepared from DL-alanine and L-leucine. These are summarized in Table I.

TABLE I

PREPARATION AND PROPERTIES OF $(C_6H_5)_2BOC(O)CH(NH_2)R$

R	Solvent	Yield, %	M.p., °C.	Analysis, % N— Calcd.	Found
—H	None	51	244–245	5.86	5.76
	Toluene	39			
—CH_3	None	57	231–232	5.54	5.52
—$CH_2CH(CH_3)_2$	Toluene	88	180–181	4.75	4.62

(3) Distilled from the ammonia complex, see ref. 1b.

Hydrolysis of Cysteamine S-Phosphate

STIG ÅKERFELDT

Research Institute of National Defence, Department 1, Sundbyberg 4, Sweden

Received August 26, 1963

In a recent paper in this journal, Dittmer, Ramsay, and Spalding[1] reported on the hydrolysis of cysteamine S-phosphate [S-(2-aminoethyl)phosphorothioate] and they found two rate maxima of hydrolysis, at pH 2–3 and at pH 8–9. The rate of hydrolysis at pH 2–3 was found to be approximately four times as large as that at pH 8–9.

In an earlier paper by Åkerfeldt,[2] cysteamine S-phosphate was reported to have only one rate maximum, at pH 3. The rate profiles for fifteen other compounds containing the $-SPO_3^{-2}$ group also were investigated.[3,4] In all instances a single rate maximum was found and its position was in the pH range 2–4. It could be shown conclusively that the most easily hydrolyzable ionic species of all compounds studied was the monoanion.[3,5]

In view of the finding by Dittmer, *et al.*,[1] of a second rate maximum at pH 8–9 in the case of cysteamine S-phosphate, a re-examination of the hydrolysis of this compound has been carried out. The investigation was performed (a) under experimental conditions practically identical with those used by Dittmer, *et al.*, and using the same analytical procedure as these authors, (b) using the same technique of hydrolysis as in a but using the analytical procedure of Gomori,[6] and (c) using a low ionic strength incubation medium combined with the analytical procedure of Gomori.[6]

The results obtained with a 99% pure preparation of cysteamine S-phosphate (4.0 mmolar) showed practically the same low rate of hydrolysis at pH 7.0, 8.0, and 9.0. The first-order rate of hydrolysis constants at 35.0° were $k_{obsb} = (2.0 \pm 0.2) \times 10^{-5}$ sec.$^{-1}$ at ionic strength of 0.1 M, and $k_{obsb} = (1.0 \pm 0.2) \times 10^{-5}$ sec.$^{-1}$ at ionic strength of 1 M. The existence of a rate maximum from pH 8–9 has thus not been confirmed.

Dittmer, *et al.*, found at 37° and 1 M ionic strength the following rate constants (sec.$^{-1}$): 3.8×10^{-5} (pH 7.02), 13.1×10^{-5} (pH 8.06), and 9.9×10^{-5} (pH 9.08).

The rate constants reported in the present communication are thus lower than those found by Dittmer, *et al.* This implies the presence of an impurity in their preparation of cysteamine S-phosphate, which is the likely explanation for the observed rate increase at pH 8–9.

(1) D. C. Dittmer, O. B. Ramsay, and R. E. Spalding, *J. Org. Chem.*, **28**, 1273 (1963).

(2) S. Åkerfeldt, *Acta Chem. Scand.*, **14**, 1980 (1960).
(3) S. Åkerfeldt, *ibid.*, **15**, 575 (1961).
(4) S. Åkerfeldt, *ibid.*, **17**, 319 (1963).
(5) S. Åkerfeldt, *Svensk Kem. Tidskr.*, **75**, 231 (1963).
(6) G. Gomori, *J. Lab. Clin. Med.*, **27**, 955 (1942).

The Influence of Dicyclohexylcarbodiimide Concentration on the Rate of Phospho Diester Bond Formation

R. V. TOMLINSON AND G. M. TENER[1]

Department of Biochemistry, University of British Columbia, Vancouver 8, British Columbia, Canada

Received August 14, 1963

Dicyclohexylcarbodiimide (DCC) has proven to be an efficient condensing agent for the synthesis of phos-

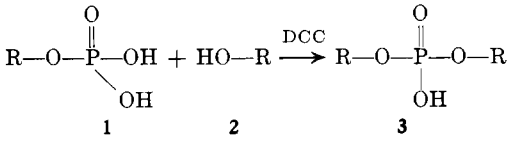
(1) Medical Research Associate, Medical Research Council of Canada. This work has been supported by the U. S. Public Health Grant C-5342.

TABLE I

THE PER CENT OF THE OPTICAL DENSITY[a]

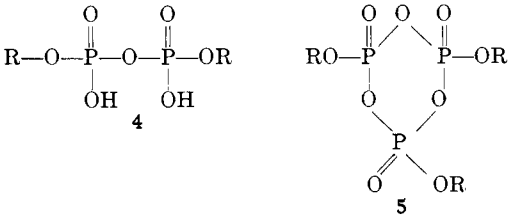
Molar ratio of pT–DDC	1:13 (146 mg. of DCC)			1:65 (731 mg. of DCC)			1:260 (2927 mg. of DCC)		
Reaction products	% pT	% $(pT)_2$	% CEpT	% pT	% $(pT)_2$	% CEpT	% pT	% $(pT)_2$	% CEpT
Time, hr.									
0.5	17	65	20	14	30	56	11	5	84
1	10	54	36	7	9	84	3	2	95
4	6	14	80	1	3	96		3	97
12	1	4	95		2	98		1	99
25	1	2	97		3	97		2	98

[a] At 267 mμ found in thymidylic acid (pT), dithymidine pyrophosphate $(pT)_2$, and the cyanoethyl ester of pT (CEpT) when 0.055 mmole of pT reacted with hydracrylonitrile (5.75 mmoles) in the presence of DCC.



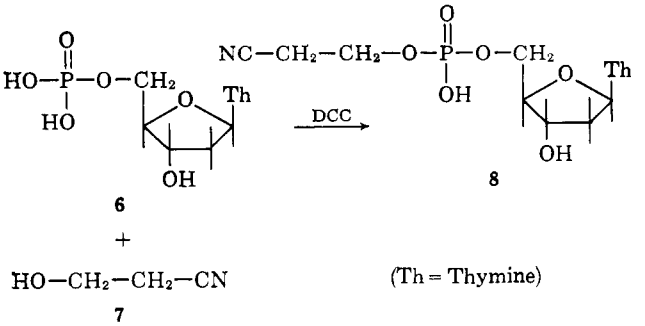
pho diesters (**3**) from a monoalkyl phosphate (**1**) and an alcohol (**2**).[2]

Recently, possible mechanisms for this reaction were suggested.[3,4] The work of Weimann and Khorana[3] showed that **1** reacts rapidly with DCC by way of the pyrophosphate (**4**) to form a trialkyl trimetaphosphate (**5**) which is apparently the initial phosphorylating species. Its formation is complete within a few minutes but formation of the phospho diester is a much slower process.



We now wish to report that the rate of phospho diester bond formation also depends on the concentration of DCC, a fact which would suggest that either a second mechanism must be involved or loss of the metaphosphate (**5**) by anhydride exchange reactions is prevented. The role played by the change in the dielectric constant of the solution in this process is not known. However, for synthetic purposes it is sufficient to note that a large excess of DCC completes the esterification reaction in a short time.

The reaction studied was the esterification of thymidine-5′ phosphate (**6**, pT) with hydracrylonitrile (**7**) to form the β-cyanoethyl ester (**8**, CEpT). The amount of this ester formed as a function of time and DCC concentration was followed chromatographically. The results shown in Table I demonstrate that the esterification was 95% complete after 1 hr. when thymidylic acid and DCC are present in a molar ratio of 1:280, whereas with a lesser ratio (1:13) it had gone only 36%.



In this reaction, the activated intermediates which undergo the nucleophilic attack by the alcohol cannot be only the DCC adduct[5] of pT but also must include poly- or metaphosphates as well. This statement is supported by the finding that dithymidine pyrophosphate $(pT)_2$ is not only isolated after hydrolysis of the reaction mixture at intermediate stages but also serves almost as well as a starting material. The results of these latter studies are shown in Table II. They are similar to those in Table I in that the reaction proceeds faster with higher concentrations of DCC and after an hour is almost complete. It should be noted, however, that the ratio of products, particularly at the short time intervals, is very different in the two cases.

TABLE II

THE PER CENT OF THE OPTICAL DENSITY[a]

Molar ratio of $(pT)_2$–DCC	1:55 (146 mg. of DCC)			1:550 (1460 mg. of DCC)		
Reaction products	% pT	% $(pT)_2$	% CEpT	% pT	% $(pT)_2$	% CEpT
Time, hr.						
0.5	8	73	19	11	18	71
1	6	47	47	4	4	92
4	1	18	81	.5	1.5	98
12		3	97		1	99
25		4	96		1	99

[a] At 267 mμ found in pT, $(pT)_2$, and CEpT when 0.013 mmole of $(pT)_2$ reacted with hydracrylonitrile (5.75 mmoles) in the presence of DCC.

Experimental

Synthesis of β-Cyanoethylthymidine-5′ Phosphate. A. From Thymidine-5′ Phosphate.—Three reactions mixtures were prepared by the following method. A solution containing 0.055 mmole of thymidylic acid in 1 ml. of water and 10 ml. of pyridine was concentrated to dryness. The residue was dissolved in 10 ml. of anhydrous pyridine and again concentrated to dryness. The residue was then dissolved in 5 ml. of anhydrous pyridine and 0.385 ml. (5.75 mmoles) of hydracrylonitrile. DCC was added to give the desired ratio of nucleotide to DCC (146 mg. of DCC for 1:13 ratio, 731 mg. of DCC for the 1:65 ratio, and 2927 mg. for the 1:260 ratio). The reaction was allowed to proceed at room temperature under anhydrous conditions. Aliquots of 0.5 ml. were removed from each reaction mixture at various time intervals and added to 0.5 ml. of water. After 1 hr., these solutions were extracted with three 5-ml. portions of ether and

(2) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212 (1958).
(3) G. Weimann and H. G. Khorana, *ibid.*, **84**, 4329 (1962).
(4) A. R. Todd, *Proc. Natl. Acad. Sci. U. S.*, **45**, 1389 (1959); *Proc. Chem. Soc.* (London), 187 (1961).
(5) M. Smith, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6204 (1958).

the aqueous phase filtered to remove dicyclohexylurea. Samples were spotted onto Whatman No. 40 paper along with standards of thymidylic acid (R_f 0.12), dithymidine pyrophosphate (R_f 0.24), and β-cyanoethylthymidine-5′ phosphate (R_f 0.55) and chromatographed in isopropyl alcohol–water–concentrated ammonium hydroxide (7:2:1). The products were visualized under ultraviolet light and the areas containing the products cut out and eluted with 5 ml. of water. Not more than three components were found in each aliquot. Quantititive data (Table I) were obtained by comparing the ultraviolet absorption of each of these solutions at 267 mμ and the results expressed as the per cent of the component (in terms of its absorption) in the aliquot.

B. From Dithymidine Pyrophosphate.—Similar esterification reactions were carried out using 0.013 mmole of dithymidine pyrophosphate, 5.75 mmoles of hydracrylonitrile, 5 ml. of anhydrous pyridine, and either 146 mg. or 1460 mg. of DCC. The reaction was treated as in A with the results shown in Table II.

Condensation of Aromatic Aldehydes with Acetone-1,3-bis(triphenylphosphonium) Chloride in the Presence of Base[1]

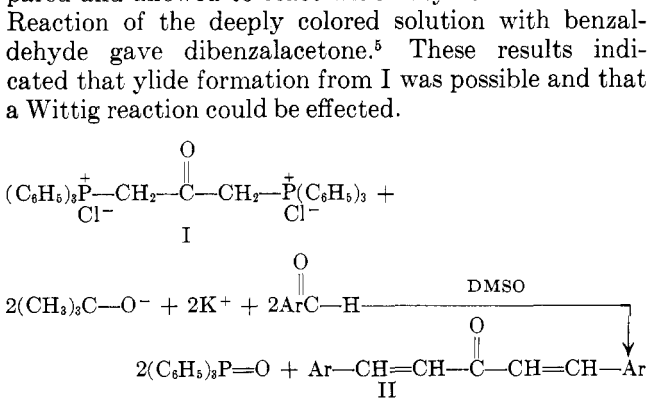
DONALD B. DENNEY[2] AND JOHN SONG[3]

School of Chemistry, Rutgers, The State University, New Brunswick, New Jersey

Received August 12, 1963

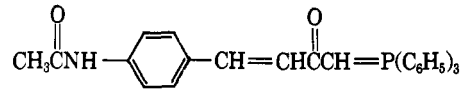
Recently Ford and Wilson[4] have reported that acetone-1,3-bis(triphenylphosphonium) chloride (I) does not give a stable mono- or diylide derived directly from I when treated with aqueous sodium carbonate, but rather undergoes hydrolysis to give triphenylphosphine oxide and acetylmethylenetriphenylphosphorane.

As part of other studies in this laboratory, I was prepared and allowed to react with butyllithium in ether. Reaction of the deeply colored solution with benzaldehyde gave dibenzalacetone.[5] These results indicated that ylide formation from I was possible and that a Wittig reaction could be effected.



It has now been found that I (1 mole) will react with aromatic aldehydes (2 moles) and 2 moles of potassium *t*-butoxide in dimethyl sulfoxide or 2 moles of sodium ethoxide in ethanol to give substituted dibenzalacetones (II). The potassium *t*-butoxide–dimethyl sulfoxide system was found to be the most synthetically useful. The yields of symmetrically substituted dibenzalacetones were di-*m*-nitro, 48%, di-*p*-chloro, 76%, and di-*p*-methoxy, 37%. It also was found that unsymmetrically substituted dibenzalacetones could be prepared if 1 mole of potassium *t*-butoxide and 1 mole of aldehyde were allowed to react with I followed by addition of another mole of base and a mole of another aldehyde. By this technique the mono-*m*-nitro compound was prepared in 57% yield and the *p*-chloro-*m*-nitro compound was prepared in 67% yield.

An attempt to condense I with 2 moles of *p*-acetamidobenzaldehyde and 2 moles of potassium *t*-butoxide gave no compound corresponding to II. There was isolated the phosphorane (III). Attempts to effect the reaction under more strenuous conditions were not undertaken.



III

Experimental[6]

Preparation of Acetone-1,3-bis(triphenylphosphonium) Chloride (I).—A mixture of 21.3 g. (0.168 mole) of 1,3-dichloroacetone, 112.5 g. (0.43 mole) of triphenylphosphine, and 225 ml. of chloroform was stirred under reflux for 20 hr. The cooled reaction mixture was treated with 150 ml. of dry ether. The precipitate was washed with 100 ml. of ether–chloroform (1:1.5) and dried *in vacuo* to give 103 g. of product. This material was further purified by dissolving it in 400 ml. of warm chloroform followed by precipitation with 300 ml. of dry ether. There was obtained 98 g. (86%) of I, m.p. 260–261, lit.[4] m.p. 266–267°.

Preparation of 1,5-Bis(*m*-nitrophenyl)penta-1,4-dien-3-one.—Sodium ethoxide solution (1.37 *N*, 14.5 ml., 0.02 mole) was added with stirring to a mixture of 5.98 g. (0.01 mole) of I and 3.02 g. (0.02 mole) of *m*-nitrobenzaldehyde. After stirring for 3 hr. at 30°, the precipitate was collected, washed with two 15-ml. portions of ethanol and water, and dried to give 1.4 g. (43%) of bis-*m*-nitrobenzalacetone, m.p. 238–241°, lit.[7] m.p. 238°. The infrared spectrum was commensurate with the assigned structure.

Preparation of 1,5-Bis(*p*-chlorophenyl)penta-1,4-dien-3-one.—A mixture of 2.24 g. (0.02 mole) of potassium *t*-butoxide, 5.98 g. (0.01 mole) of I, and 40 ml. of dimethyl sulfoxide was stirred under nitrogen at room temperature for 1 hr. The resulting solution was treated with 2.81 g. (0.02 mole) of *p*-chlorobenzaldehyde in 10 ml. of dimethyl sulfoxide. The mixture was warmed at 40° for 7 hr. The precipitate which formed after cooling was collected and washed with 5-ml. portions of ethanol and water. There was obtained 2.3 g. (76%) of material, m.p. 193–195°, lit.[8] m.p. 193–194°. The infrared spectrum was commensurate with the assigned structure.

Similarly 1,5-bis(*p*-methoxyphenyl)-1,4-dien-3-one was prepared in 37% yield, m.p. 123–125°, lit.[9] m.p. 125°. The infrared spectrum of this compound agreed with that predicted.

Preparation of 1-*m*-Nitrophenyl-5-phenylpenta-1,4-dien-3-one.—A mixture of 1.12 g. (0.01 mole) of potassium *t*-butoxide, 5.89 g. (0.01 mole) of I in 25 ml. of dimethyl sulfoxide was stirred for 1 hr. under nitrogen. There was added 1.06 g. (0.01 mole) of benzaldehyde and the mixture was warmed at 45° for 2 hr. This mixture was cooled and treated with 1.12 g. (0.01 mole) of potassium *t*-butoxide, and after stirring at 45° for 15 min. 1.51 g. (0.01 mole) of *m*-nitrobenzaldehyde in 15 ml. of dimethyl sulfoxide was added. The mixture was heated at 50° for 3 hr. and then cooled. The precipitate (inorganic salt) which formed on cooling was filtered and discarded. The filtrate was treated with 30 ml. of water and the crystals were collected to give 1.6 g. (57%) of material, m.p. 138–141°. A portion was recrystallized twice from ethanol, m.p. 142–143°, lit.[10] m.p. 140°.

Anal. Calcd. for $C_{17}H_{13}O_3N$: C, 73.20; H, 4.67. Found: C, 72.99; H, 4.89.

(1) Research supported by the National Science Foundation.

(2) Rutgers Research Council Faculty Fellow, 1963–1964.

(3) Recipient of an American Cyanamid Junior Educational Award, 1961–1963.

(4) J. A. Ford and C. V. Wilson, *J. Org. Chem.*, **26**, 1433 (1961)

(5) Unpublished results of C. Dennis Hall.

(6) All melting points are uncorrected. Analyses were by G. Robertson, Florham Park, N. J.

(7) P. Pfeiffer and B. Segal, *Ann.*, **460**, 131 (1928).

(8) R. E. Lutz and T. A. Martin, *J. Org. Chem.*, **14**, 286 (1949)

(9) G. V. Austerweil and R. Polland, *J. Appl. Chem.* (London), **5**, 213 (1955).

(10) H. Kauffmann and H. Burkhardt, *Ber.*, **46**, 3808 (1914).