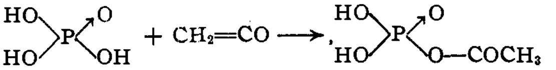
[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

A New Synthesis of Acetyl Dihydrogen Phosphate[1]

BY RONALD BENTLEY[2]

Acetyl dihydrogen phosphate (I) was first synthesized by Lynen[3] who used the catalytic debenzylation of acetyl dibenzyl phosphate. Later, Lipmann and Tuttle[4] described its preparation from silver dihydrogen phosphate and acetyl chloride; in each case the acetyl dihydrogen phosphate was characterized as its sparingly water soluble disilver salt. It has now been found that it may be conveniently and rapidly prepared by acetylating phosphoric acid with ketene, thus confirming the previous observation of Hurd and Dull[5] that "ketene was quantitatively absorbed by phosphoric acid, presumably with the formation of acetylphosphoric acid."

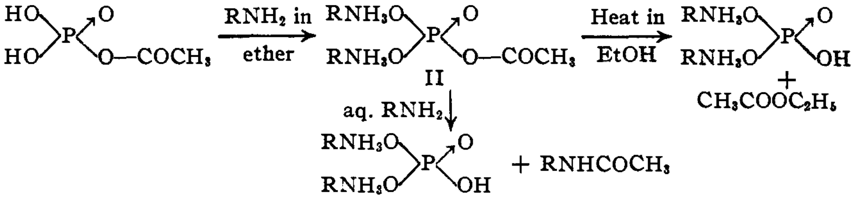


I

Solutions of the free acetyl dihydrogen phosphate were obtained by reaction of the disilver salt with hydrogen sulfide in aqueous suspension or by reaction with ethereal hydrogen chloride. The aqueous solution of the free acid was stable for some hours at 0°; on neutralization with barium hydroxide and addition of silver nitrate, the disilver salt was recovered.

Acetyl dihydrogen phosphate has been widely considered as a possible acetylating agent and also as a phosphorylating agent in physiological systems.[6] It was therefore of interest to study its reactions with various compounds. Aqueous solutions of the sodium salt were found to acetylate ammonia and aniline, but only with low yields. In neutral aqueous solutions no acetylation of γ-phenylaminobutyric acid, choline chloride, guanidine or hydroquinone was observed.

When ethereal acetyl dihydrogen phosphate was treated with aniline, bis-phenylammonium acetyl phosphate (II, R = C_6H_5) precipitated and evaporation of the ethereal mother liquor gave a small amount of acetanilide. Subsequent reaction of the bis-phenylammonium salt with aqueous aniline gave bis-phenylammonium phosphate and acetanilide. Analogous reactions were observed with ammonia.



Dibenzyl hydrogen phosphate reacted smoothly with ketene to yield the mixed anhydride, acetyl dibenzyl phosphate, which was converted to acetyl dihydrogen phosphate using Lynen's method. Acetyl dibenzyl phosphate could also be used as an acetylating agent. With aniline in the absence of solvent there was a vigorous reaction; acetanilide and dibenzyl hydrogen phosphate were obtained. Choline chloride dissolved in glacial acetic acid was also acetylated, in the cold, on addition of acetyl dibenzyl phosphate.

Experimental[7]

Preparation of Disilver Acetyl Phosphate.—In a typical run, sirupy phosphoric acid (85%, 10 ml.) dissolved in dry ether (150 ml.) was treated with a ketene stream for thirty minutes with ice cooling. (A ketene lamp similar to that described by Williams and Hurd[8] was used, which produced about 10 g. of ketene in that time. Ten ml. of 85% phosphoric acid required about 12.5 g. of ketene for reaction with the water and phosphoric acid present.) The ketene was led in through a sintered glass bubbler, with vigorous stirring to avoid high local concentrations. The ether solution was extracted with ice water (3 × 50 ml.; all apparatus used was previously chilled in ice) and the aqueous extract rapidly neutralized (*p*H 7.0) by dropwise addition of saturated barium hydroxide solution, with ice cooling and vigorous stirring. The precipitated barium phosphate was centrifuged off and the solution of barium acetyl phosphate was treated with excess of ice cold 10% silver nitrate solution. The pale creamy precipitate was washed with ice-water, alcohol and ether (16.7 g.). Dilution of the mother liquor with one-third volume of ethanol gave a second crop of crystals (5.0 g.).

For recrystallization the disilver salt (7.2 g.) was ground to a smooth paste with ice water, shaken with ice cold 0.25 *M* sodium chloride (150 ml.—a slight deficiency) and allowed to stand for fifteen minutes. The solution was filtered, and treated with 25% silver nitrate (3 ml.). This first precipitate was rejected, and excess 25% silver nitrate was then added. Disilver acetyl phosphate (5.5 g.) formed white prisms, not having a definite m. p.

Anal. Calcd. for $C_2H_3O_5PAg_2$: Ag, 60.9; P, 8.75. Found: Ag, 60.25; P, 8.7.

Acetyl determinations were performed by repeated steam distillation of 15 mg. samples with water (10 ml.) and concentrated sulfuric acid (2 ml.) in a Kjeldahl type apparatus: 35-ml. portions of distillate were collected until a constant blank titration was obtained. *Anal.*

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(3) F. Lynen *Ber.*, **73B**, 367 (1940).

(4) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **153**, 571 (1944).

(5) C. D. Hurd and M. F. Dull, THIS JOURNAL, **54**, 3427 (1932).

(6) F. Lipmann, *Advances in Enzymol.*, **6**, 242, 257 (1946).

(7) All melting points are uncorrected.

(8) J. W. Williams and C. D. Hurd, *J. Org. Chem.*, **5**, 122 (1940).

Calcd. for $C_2H_3O_5PAg_2$: CH_3CO, 12.2. Found: CH_3CO, 12.7. The acidity produced on warming 40-50 mg. samples with water at 70-75° for two hours was determined by titration of the filtered solution with 0.01 *N* potassium hydroxide.

$$3C_2H_3O_5PAg_2 + 5OH^- = 2Ag_3PO_4 + HPO_4^{=} + 3CH_3COO^- + 2H_2O$$

Calculated per cent. purity, 101.3.

Preparation of Acetyl Dihydrogen Phosphate.—(1) Aqueous solution: Disilver acetyl phosphate (1.5 g.) suspended in ice water, was treated with excess hydrogen sulfide, silver sulfide filtered off, and the filtrate freed from hydrogen sulfide by aeration at 0°. The filtrate was made up to 70 ml.: 40 ml. was immediately neutralized with barium hydroxide solution at 0°, and reprecipitated with silver nitrate (recovery, 0.7 g.). The remaining solution was kept for ninety minutes at 0°, and treated in the same way (recovery, 0.4 g.). The precipitates were recrystallized and the per cent. purity determined by hydrolysis with water as previously described: first precipitate, 104.0%; second precipitate, 100.3%.

(2) Ethereal solution: Finely powdered disilver acetyl phosphate (2.0 g.) was suspended in absolute ether (25 ml.), cooled in ice and ethereal hydrogen chloride (1.33 *N*, 8.0 ml.) added dropwise with vigorous stirring. Stirring was continued for a further ten minutes, the mixture filtered, and the silver chloride washed with a little absolute ether to yield an ethereal solution of acetyl dihydrogen phosphate.

Reaction with Aniline in Aqueous Solution.—Disilver acetyl phosphate (2.0 g.) was converted to sodium salt using 0.25 *M* sodium chloride (45 ml.). The filtered solution was treated with aniline (1.2 ml., a large excess) and acetone (5 ml.) to homogenize the solution. The solution, initially at 0°, was brought to room temperature after two hours, and allowed to stand overnight. Hydrochloric acid (1.068 *N*, 18.0 ml.) was added (*p*H 3.0), and the solution extracted with ether: the dried extract was evaporated to yield white, lath-like crystals, m. p. 114-115° (109 mg., 14% yield); no m. p. depression in admixture with authentic acetanilide.

Reaction with Aniline in Ethereal Solution.—An ethereal solution of acetyl dihydrogen phosphate (from silver salt 2.0 g.) was cooled to 0°, and treated with aniline (2.0 g.) in dry ether (15 ml.). A white precipitate of bis-phenylammonium acetyl phosphate (II, R = C_6H_5) (1.7 g.), m. p. 104-105°, soluble in sodium bicarbonate solution with effervescence. (The ethereal mother liquor was extracted with 2 *N* hydrochloric acid to remove excess aniline and yielded a little acetanilide on evaporation.)

Anal. Calcd. for $C_{14}H_{19}O_5N_2P$: CH_3CO, 13.2. Found: CH_3CO, 13.1.

On attempted recrystallization from absolute ethanol, shining plates m. p. 174-175° were obtained (unchanged on recrystallization). With bis-phenylammonium phosphate there was no depression in m. p.

Bis-phenylammonium acetyl phosphate (320 mg.) was dissolved in ice-water and aniline (1.0 ml.) and acetone (3.0 ml.) added. The mixture was placed in an ice-bath, allowed to warm up to room temperature, and kept overnight. A white crystalline precipitate formed (170 mg.), m. p. 174-175°, recrystallizing from ethanol in plates; no depression in admixture with bis-phenylammonium phosphate. The mother liquor was made acid to congo red, extracted with ether, and the dried extract evaporated; the white solid was recrystallized from chloroform-petroleum ether to form white needles (66 mg., including a second crop), m. p. 111-112°. No m. p. depression in admixture with acetanilide.

Bis-phenylammonium acetyl phosphate (200 mg.) was treated with 2 *N* ammonia (5 ml.), when an oil separated; the mixture was allowed to stand overnight, extracted with ether, and the aqueous solution evaporated to dryness *in vacuo*. The solid residue was extracted with warm chloroform, filtered, and the filtrate diluted with petroleum ether (b. p. 30-60°). White crystals separated, m. p. 81°, no depression in admixture with acetamide.

Reaction with Ammonia in Ethereal Solution.—Disilver acetyl phosphate (1 g.) was converted to the free acid in ether. The solution at 0° was treated with an excess of dry ethereal ammonia when diammonium acetyl phosphate crystallized in white plates m. p. 128-130°. It did not analyze too well.

Anal. Calcd. for $C_2H_{11}O_5N_2P$: N, 16.1; CH_3CO, 24.5. Found: N, 17.1; CH_3CO, 23.0.

The solid was allowed to stand overnight in a little aqueous ammonia, and evaporated to dryness *in vacuo:* the crystalline solid was extracted with warm ethanol, the extract being evaporated. The residue was twice recrystallized from chloroform-petroleum ether (b. p. 30-60°), to form white crystals, m. p. 82°, showing no depression with an authentic sample of acetamide.

Preparation of Acetyl Dibenzyl Phosphate.—Dibenzyl hydrogen phosphate was prepared substantially as described by Lossen and Köhler[9]; it was found preferable, however, to saponify the tribenzyl phosphate by refluxing for some hours with 10% alcoholic potassium hydroxide. Evaporation of solvent, solution of the residue in water, acidification and recrystallization from chloroform-petroleum ether gave dibenzyl hydrogen phosphate, m. p. 79.5°.

Dibenzyl hydrogen phosphate (1.0 g.) was dissolved in absolute ether containing a little chloroform, and treated with a ketene stream (about 0.5 g., an excess) at ice temperature. Solvent was removed *in vacuo* (bath temperature, 25°) to yield acetyl dibenzyl phosphate as a viscous, neutral oil, in almost the theoretical yield.

Anal. Calcd. for $C_{16}H_{17}O_5P$: CH_3CO, 13.4. Found: CH_3CO, 13.3.

The oil was dissolved in dry ether (50 ml.), palladium charcoal (500 mg.) was added and the solution hydrogenated with dry hydrogen, about 200 ml. being taken up at S. T. P. After filtration, the ether was extracted with water (2 × 15 ml.) and the solution neutralized with barium hydroxide solution at 0°. Precipitated barium phosphate was filtered off, and silver nitrate solution added. Disilver acetyl phosphate was precipitated (300 mg.); per cent. purity as determined by titration of acid produced on hydrolysis, 95.9.

Reaction of Acetyl Dibenzyl Phosphate and Aniline.—A portion of acetyl dibenzyl phosphate was treated with a slight excess of aniline, the mixture warming up considerably. After reaction, chloroform was added, and excess aniline extracted with 2 *N* hydrochloric acid. The chloroform was next extracted with saturated sodium bicarbonate solution; acidification of this extract gave a white solid, which recrystallized from chloroform-petroleum ether to form plates, m. p. 80°; no m. p. depression admixed with dibenzyl hydrogen phosphate. The chloroform solution was washed with water, dried and evaporated *in vacuo:* the residue quickly crystallized, and was recrystallized from chloroform-petroleum ether, forming white needles, m. p. 115°; mixed m. p. with acetanilide, 115°.

Reaction of Acetyl Dibenzyl Phosphate and Choline.—Dibenzyl hydrogen phosphate (1.0 g.) was converted to its acetyl derivative, the oil being held in vacuum for some time to remove any traces of ketene. Choline chloride (500 mg.) was added, and glacial acetic acid (1 ml.) to form a clear solution. After forty-eight hours, the acetic acid was evaporated *in vacuo*, the residue treated with 2 *N* hydrochloric acid (5 ml.) and an insoluble oil, extracted with ether. The filtered aqueous solution was treated with a slight excess of auric chloride solution; the yellow precipitate (0.762 g.) had m. p. 163-164°, unchanged on recrystallization from hot water. (Literature m. p. of acetylcholine aurichloride is 166°; no depression in admixture with an authentic sample.)

Summary

1. Acetyl dihydrogen phosphate may be con-

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[9] W. Lossen and A. Köhler, *Ann.*, **262**, 211 (1891).

veniently prepared by the acetylation of phosphoric acid with ketene in ethereal solution, and is recovered as disilver acetyl phosphate. Similar acetylation of dibenzyl hydrogen phosphate yields acetyl dibenzyl phosphate.

2. Acetyl dihydrogen phosphate will acetylate ammonia and aniline under various conditions. No evidence of phosphorylating reactions could be obtained.

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The Structure of Neoprene. I. The Molecular Weight Distribution of Neoprene Type GN

BY W. E. MOCHEL, J. B. NICHOLS AND C. J. MIGHTON

It has been recognized that synthetic high polymers in general are non-homogenous, particularly in respect to molecular weight. Therefore, the molecular weight of a particular polymer cannot be characterized accurately by a single value and there is required a knowledge of the distribution of molecular weights. Any single molecular weight value for a heterogeneous polymer must perforce be an average value and will differ appreciably with the method of averaging. Although it has been shown recently that the tensile strength of some polymers, *e. g.*, cellulose acetate, depends explicitly on the number average molecular weight regardless of the distribution of molecular weights,[1] other properties of high polymers such as plasticity,[2] are influenced markedly by the heterogeneity of molecular weights. The present investigation of the molecular weight distribution of neoprene (polychloroprene) is a part of an extensive study of the structures of these polymers.

There have appeared recently several new methods for determination of molecular weight distribution[3] but the standard method continues to be that of careful fractionation and examination of each fraction. This method also furnishes polymer samples of sufficient homogeneity that they can be used to calibrate the intrinsic viscosity–molecular weight relationship with an absolute method such as osmotic pressure. The two most common methods for fractionation of high polymers are: (1) precipitation by successive additions of a non-solvent to a solution of the polymer and (2) successive extractions of the polymer with solvent/non-solvent mixtures of increasing solvent concentration. It has been shown that neither method can give a really sharp separation of species[4] but the first method appears to be satisfactory for practical purposes if certain precautions are taken. Thus, it is advisable to use a reasonably dilute solution, precipitate at constant temperature and wash the precipitated fractions to remove low molecular weight material.

Experimental

Materials.—A sample of standard, commercial Neoprene Type GN of plasticity classification[5] P3 and age two months was selected for fractionation. Neoprene Type GN is a polychloroprene polymerized in aqueous emulsion in the presence of sulfur and is stabilized with tetraethyl thiuram disulfide.[6] (Neoprene Type GN is identical with GR-M currently manufactured at Louisville by the Office of Rubber Reserve, Reconstruction Finance Corporation.) To obtain the pure polychloroprene, essentially free from soap residues, stabilizers and adjuvants used in the polymerization, 55 g. of the finely-cut neoprene was dissolved in 500 ml. of thiophene-free, dry benzene, and 500 ml. of C. P. methanol was added slowly with stirring, to precipitate the polymer. Further addition of methanol to the clear, supernatant liquid produced no cloudiness. The polymer was washed twice with 100-ml. portions of methanol and dried at room temperature under vacuum. The dry polymer weighed 50.5 g. In these operations and all subsequent handling, the polymer, its solutions and the fractions were kept under an atmosphere of nitrogen.

The benzene–methanol mixture left after precipitation of the polymer was combined with the methanol wash liquors and evaporated to dryness under vacuum. The residue, consisting of 4.6 g. of dark brown, very viscous oil, was not investigated further.

Fractionation.—The purified polychloroprene prepared as described above (50.5 g.) was dissolved in 5 l. of thiophene-free, dry benzene and to the solution was added 0.5 g. of phenyl-α-naphthylamine to inhibit degradation of the polymer. After a sample (150 ml.) of the solution of whole polymer had been removed for test, fraction A was precipitated by the slow addition of methanol, with mechanical stirring, until the solution became hazy at 25°. It was then warmed gently until it became clear, at about 26–28°. Precipitation of the first fraction required 1200 ml. of methanol. The clear, warm solution was cooled slowly to 25° and maintained overnight at constant temperature, during which time the precipitated polymer settled out as a very viscous liquid containing the high molecular weight polymer in solution. The clear, supernatant solution was siphoned off and the fraction was washed twice with a benzene–methanol mixture of the same concentration as the mixture from which the fraction had precipitated. These washings were added to the main solution and the fraction

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(1) A. M. Sookne and M. Harris, *Ind. Eng. Chem.*, **37**, 478 (1945); P. J. Flory, THIS JOURNAL, **67**, 2048 (1945); *Ind. Eng. Chem.*, **38**, 417 (1946).

(2) R. L. Zapp and F. P. Baldwin, *ibid.*, **38**, 948 (1946).

(3) D. R. Morey and J. W. Tamblyn, *J. Appl. Phys.*, **16**, 419 (1945); R. F. Boyer and R. D. Heidenreich, *ibid.*, **16**, 621 (1945); P. M. Doty, B. H. Zimm and H. Mark, *J. Chem. Phys.*, **13**, 159 (1945); L. H. Cragg and H. Hammerschlag, *Chem. Rev.*, **39**, 79 (1946); D. R. Morey and J. W. Tamblyn, *J. Phys. Colloid Chem.*, **51**, 721 (1947).

(4) G. Gee, *Trans. Faraday Soc.*, **38**, 276 (1942); P. J. Flory, *J. Chem. Phys.*, **12**, 425 (1944); R. L. Scott, *ibid.*, **13**, 178 (1945); D. R. Morey and J. W. Tamblyn, *J. Phys. Chem.*, **50**, 12 (1946).

(5) P3 corresponds to a Williams plasticity range of 116–121; see A. M. Neal and P. Ottenhoff, *Ind. Eng. Chem.*, **36**, 653 (1944), this corresponds approximately to Mooney 2.

(6) A. M. Collins, U. S. Patent 2,264,173.