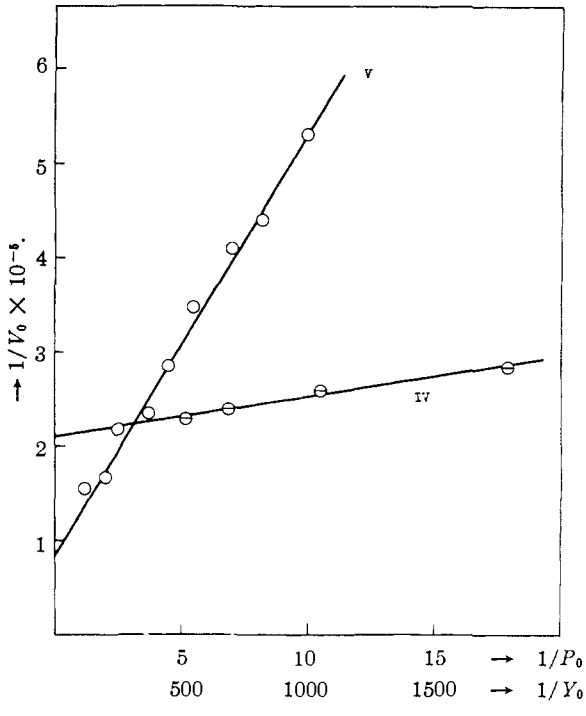

Fig. 2.—Plots of reciprocal initial rates against reciprocal concentrations of piperidine (line IV, ⊖) and benzoic acid (line V, O).

situation can well be represented graphically; the reciprocal initial rate $1/V_0$ varies linearly with reciprocal initial concentrations of diethyl malonate $1/M_0$(Fig. 1), of benzaldehyde $1/B_0$(Fig. 1), of piperidine $1/P_0$ (Fig. 2) and of benzoic acid $1/Y_0$ (Fig. 2). The reaction is accelerated by small amounts of benzoic acid, and the rates without acid catalysts approach zero.

TABLE IV

THE EFFECTS OF *p*-SUBSTITUENTS (INITIAL RATE V_0, MOLE L.$^{-1}$ SEC.$^{-1}$ × 10^6)

	Initial concentration, *M*			
	Benzaldehydes, B_0	Diethyl malonate, M_0	Piperidine, P_0	Benzoic acid, Y_0
In kerosene	0.1975	0.1258	0.0964	0.0014
In *i*-PrOH	0.1975	0.2516	0.1928	0.0014

	Initial rate				
	CH_3O	CH_3	H	Cl	NO_2
In kerosene (99.5°)	1.63	1.65	2.56	2.19	1.52
In *i*-PrOH (83°)	11.3	11.6	19.5	15.5	9.1

Although the effect of *p*-substituents is very small, its mode of action is not simple; both electron-attracting and releasing groups retard the reaction, and a similar effect is recorded for the rate of formation of some Schiff bases.[9] With regard to this, it is of interest to note that the rates of condensation of benzaldehydes with acetic anhydride in kerosene using triethylamine as a catalyst satisfy the Hammett equation with a high positive ρ-value (+2.25).[10]

The Reaction in Isopropyl Alcohol as a Solvent.—Deviation from linearity between the initial velocities and concentrations of the reactants is more remarkable in this solvent than in kerosene. A plot of $1/V_0$ *vs.* $1/M_0$ gives satisfactory straight lines and a plot of $1/V_0$ *vs.* $1/B_0$ is almost linear. But plots of $1/V_0$ *vs.* $1/P_0$ and $1/Y_0$ tend to drift upward with increasing concentrations. In this solvent, the effect of benzoic acid on the rate is very small compared to that observed in kerosene, and the reaction is retarded by excess of the acid.

Acknowledgments.—The authors wish to thank Profs. R. Oda and S. Tanaka for their aid in performing these experiments.

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KYOTO, JAPAN

[CONTRIBUTION FROM THE RADIOISOTOPE SERVICE, VETERANS ADMINISTRATION HOSPITAL AND THE DEPARTMENT OF BIOCHEMISTRY, SCHOOL OF MEDICINE, LOUISIANA STATE UNIVERSITY]

The Synthesis of Citric Acid Phosphate

BY JOE MEYER, ROY J. BOLEN AND IVO J. STAKELUM, JR.

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Citric acid phosphate has been synthesized by phosphorylating triethyl citrate with diphenyl phosphorochloridate, followed by removal of the phenyl groups by catalytic hydrogenolysis and alkaline saponification of the resulting triethyl ester. The calcium and cyclohexylammonium salts were made.

It is well established that citric acid in some form is intimately involved in the biochemistry of bone.[1] Its exact role has yet to be defined. The present report is part of a program designed to investigate this problem and is based on the postulate that citrate *per se* may not be exclusively implicated, but possibly also a form in which the tertiary hydroxyl has reacted to give citric acid phosphate or pyrophosphate. As the calcium salts, these compounds could represent an ideal vehicle for the absorption, transport and deposition of bone salts and perhaps assume a function in other aspects of calcium, phosphorus and citrate metabolism.

The synthesis of citric acid phosphate has not been reported. From the structural standpoint it is unique among organophosphate compounds. The literature offers no other authenticated example of the phosphorylation of a tertiary hydroxyl group. The presence in the molecule of three carboxyl groups further accentuates its unique character.

The present paper describes the synthesis of citric acid phosphate effected by a modification of

(1) T. F. Dixon and H. R. Perkins in "The Biochemistry and Physiology of Bone," Academic Press, Inc., New York, N. Y., 1956, p. 309.

the method used to prepare malic acid phosphate.[2] Triethyl citrate in pyridine was treated with diphenyl phosphorochloridate. The resulting crude triethyl citrate diphenyl phosphate was purified by fractional distillation in a molecular still followed by catalytic hydrogenolysis to remove the phenyl groups. Alkaline saponification served to remove the ethyl groups and the pure citric acid phosphate was obtained by application of ion exchange techniques. The crystalline cyclohexylammonium salt was prepared.

The synthesis of citric acid pyrophosphate has been reported.[3] On the basis of unpublished work, the present authors conclude that the identity of the compound may not yet be considered firmly established. It seems particularly significant that neither citrate nor citric acid phosphate is formed by acid hydrolysis or by the action of bone phosphatase. Chromatographic evaluation of the acid hydrolytic products failed to substantiate the claim that aconitic acid is a major product. These facts, together with the relatively unsatisfactory elemental analysis given by the compound and the possibility of side reactions inherent in the method of synthesis, combine to suggest the need for additional evidence before proper evaluation of the material may be made.

Experimental

Materials.—Diphenyl phosphorochloridate was made in the conventional manner and redistilled at 209–212° (24–25 mm.).[4] Triethyl citrate was the commercial product redistilled at 186° (17 mm.). Pyridine was dried over and distilled from anhydrous barium oxide.

Triethyl Citrate Diphenyl Phosphate.—Diphenyl phosphorochloridate (800 g.) was added to a solution composed of 800 g. of triethyl citrate in 800 ml. of dry pyridine. A deep cherry-red color developed without significant evolution of heat. The reaction flask was incubated at 50° for 5 days with occasional shaking. Solid pyridine hydrochloride began to separate in about 24 hours and progressively increased in quantity. In the presence of an excess of ice, the two-phase system was adjusted to *p*H 7.0 with concentrated sodium hydroxide. The neutral solution was extracted with ether which was then washed with 0.5 *N* sulfuric acid followed with water. After filtering through a bed of anhydrous sodium carbonate, the clear fluid was freed of solvent by vacuum distillation from a warm water-bath. All but the last traces of tenaciously held pyridine were removed by lyophylizing the warmed oil. On the basis of its phosphorus content, the light brown slightly viscous material was 30% triethyl citrate diphenyl phosphate contaminated with unchanged triethyl citrate. The yield was not improved by varying the temperature, increasing the incubation time or altering the relative concentrations of the reactants.

Due to the heat sensitivity of the ester, conventional fractional distillation could not be employed for purification purposes. Molecular distillation proved effective. The large quantities were processed in a laboratory size centrifugal molecular still.[5] With the rotor at 130° (15 μ pressure) a 200-g. fraction of pure triethyl citrate diphenyl phosphate was obtained as a light golden, viscous oil with an index of refraction, $n^{22}D$ 1.5060.

Anal. Calcd. for $C_{24}H_{29}O_{10}P$: C, 56.7; H, 5.7; P, 6.1. Found: C, 56.9; H, 5.7; P, 6.1; inorg. P, 0.0.

Triethyl Phosphocitrate.—Removal of the phenyl groups by hydrogenolysis was effected in the usual manner[6]; 10.3 g. of ester in 100 ml. of 95% ethanol was hydrogenated using 1.5 g. of platinum oxide catalyst added in three portions. The reaction was complete in 24 hours at atmospheric pressure and room temperature. The hydrogen uptake was 3174 ml. or 103% of theory. Removal of the solvent by lyophylizing left a clear faintly yellow viscous oil. The last traces of solvent, water and cyclohexane (from the reduction) were removed by exposing the oil to a continuous high vacuum for 20 hours at room temperature over phosphorus pentoxide.

Anal. Calcd. for $C_{12}H_{21}O_{10}P$: C, 40.4; H, 5.9; P, 8.7. Found: C, 40.2; H, 5.9; P, 8.6; inorg. P, 0.6.

Titration of 11.3 mg. of the product required 6.2 μequiv. of alkali or 98%.

Saponification of Triethyl Phosphocitrate.—In the presence of excess alkali and an adequate amount of calcium ion, the ester groups of triethyl phosphocitrate are easily removed. Conducting the reaction at 0° minimizes the concomitant hydrolysis of phosphate. A solution of 56 mmoles of triethyl phosphocitrate in 250 ml. of water was adjusted to *p*H 8.5 requiring 112 meq. of alkali which is 100% of theory; 348 meq. of additional alkali (approximately twice the theoretical amount) was added to the cold solution together with 224 mmoles of calcium chloride. The mixture was allowed to stand at 0° for 4 hours with occasional stirring. Rough kinetic studies showed that one labile ester group is removed by the time the addition of alkali had been complete. Back titration showed that 162 meq. of alkali had been used which is 96% of theory. The precipitate, collected by centrifugation and suspended twice in ethanol and ether, was dried *in vacuo* over phosphorus pentoxide at room temperature. The resulting calcium citric acid phosphate contained some free citrate and phosphate formed in the saponification step.

Purification of Citric Acid Phosphate.—Standard ion exchange techniques were used to purify the material.[7] In a typical example, 5 g. of the crude salt dissolved in water with the aid of a minimal amount of hydrochloric acid, was freed of calcium by passage through Dowex-50-H^+, adjusted to *p*H 8.5 with ammonia and placed on a Dowex-1-Cl^- column ($1^1/_8'' \times 16''$). The gradient elution technique was used (4 liters water in mixing reservoir: 4 liters 0.5 hydrochloric acid in the acid reservoir), with the column flowing at 5 ml. per minute and 100-ml. fractions collected.[8] Under these conditions, inorganic phosphate was found in fractions 1 through 7, while the desired compound first appeared in fraction 10 (0.12 *N* hydrochloric acid) and was completely eluted by fraction 28 (0.37 *N* hydrochloric acid). The latter fractions were pooled, adjusted to *p*H 8.5 with sodium hydroxide and 30 mmoles of calcium chloride added followed by 2 liters of 95% ethanol. The precipitate was collected and dried as previously described.

The salt was found to be dicalcium phosphocitrate with 8.5% water and 1.0% calcium chloride. Also present was 2.2% sodium, indicating that under the conditions used the carboxyl group which did not react with calcium was only partially (40%) neutralized. The P:C:Ca ratio found was 1:5.95:2.08.

Anal. Calcd.: C, 17.9; H, 1.2; P, 7.7; Ca, 20.8. Found: C, 17.8; H, 1.2; P, 7.7; Ca, 20.8; inorganic P, 0.0.

Cyclohexylammonium Salt.—Following the procedure of Ballou,[9] 252 mg. of the calcium salt in 25 ml. of water (with the aid of dilute hydrochloric acid), was freed of calcium by use of Dowex-50-H^+, and the 125 ml. of effluent adjusted to *p*H 8.0 with freshly distilled cyclohexylamine. After reducing to dryness by lyophylizing, the residue was further dried overnight at 45° *in vacuo* over P_2O_5. The solid was extracted with two 10-ml. portions of absolute ethanol at 45°, 60 ml. of absolute ether was added and the very slight cloudiness removed by filtration. With the aid of dry air, the solution was taken almost to dryness at 45° and then reduced to a white solid *in vacuo*. The material was dissolved in 1 ml. of water, filtered using 0.5 ml. of water to make the transfer quantitative, and two drops of cyclohexylamine added. Sufficient acetone (16 ml.) was then introduced to form a faint cloudiness. On standing at room temperature a cloud of fine oily droplets appeared which partially crys-

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(4) E. Baer in "Biochemical Preparations," John Wiley and Sons, Inc., New York, N. Y., 1952, vol. 2, p. 97.

(5) We are indebted to R. O. Feuge of the Southern Regional Laboratory, Dept. of Agriculture, New Orleans, La., for making the still available.

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(7) W. E. Cohn, THIS JOURNAL, **72**, 1471 (1950).

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TABLE I

HYDROLYSIS OF CALCIUM PHOSPHOCITRATE IN 0.1 N H_2SO_4, 100°

Each aliquot contains 1.09 μmoles esterified P

Time, min.	Inorg. P released, μM	Hydrolysis, %	Citric acid released
0	0	0	0
20	0.32	29	0.29
40	.63	58	.47
60	.80	73	.66
90	.98	90	.81
105	1.09	100	.90

tallized when left at 3° overnight. After collection by centrifugation, the material became completely solidified by stirring with acetone. Two additional crops of 135 and 65 mg. were obtained from the mother liquor by the addition of more acetone. The three dry fractions were pooled, dissolved in 0.5 ml. of water and acetone added until a faint cloudiness developed (approximately 5 ml. required). Within 15 min. at room temperature a heavy precipitate of broad double pointed needles developed which were collected and dried over P_2O_5 *in vacuo*. The yield was 155 mg. or 43%. Additional crystals obtained from the mother liquor were contaminated with a yellow amorphous solid and were discarded. The pure material is the tricyclohexylammonium salt of citric acid phosphate. The melting point of the substance may best be determined by microscopic observation of individual crystals placed on a block preheated to approximately 130°. Under these conditions the compound is seen to melt at 138–140°.

Anal. Calcd. for $C_{24}H_{60}O_{10}PN_3$: C, 50.4; P, 5.4; N, 7.3. Found: C, 50.2; P, 5.4; N, 7.2.

Acid Hydrolysis of Calcium Phosphocitrate.—The phosphate group of citric acid phosphate is moderately labile as shown in Table I. After approximately 35 minutes, 50% of the phosphate was split in 0.1 N sulfuric acid at 100°.

A parallel formation of free citric acid was found by analysis.[10] Milder conditions may produce a 1:1 correlation between the appearance of free citrate and inorganic phosphate. This fact offers a means for the analytical determination of citric acid phosphate. The compound *per se* gives no color in the standard citric acid analytical procedure. The difference between citrate values before and after hydrolysis is a measure of the phosphocitrate present.

Partial Saponification of Triethyl Phosphocitrate.—If alkaline hydrolysis of triethylphosphocitrate is effected in the absence of calcium ion and with only a slight excess of alkali present, partial saponification occurs producing a compound whose calcium salt is remarkably soluble in water. For example, 28.5 mmoles (10.2 g.) of triethyl phosphocitrate was dissolved in 100 ml. of water, the free phosphate group neutralized and 90 meq. of alkali added. This is 5% in excess over that required for complete saponification. After standing overnight at room temperature, 53 meq. of alkali (62% of theory) had been consumed. This indicates that approximately one ester group was intact. When treated on an ion exchange column as previously described, the substance gave the appearance of being a single compound. The purified calcium salt gave a P:C ratio of 1:7.9 indicating the presence of an ethyl group in keeping with the titration data. The P:Ca ratio was 1:1.9 which is approximately the same as for the fully saponified product. However, while the latter compound is soluble in distilled water only to the extent of about 113 mg. %, the former is soluble in excess of 25 g. %. A concentrated solution of the soluble form apparently enters into some sort of reversible polymerization since on standing at room temperature for some hours it may develop a heavy precipitate which is easily redissolved with the aid of acid and remains in solution when the *p*H is adjusted back to 8.5. Critical investigation of these factors was beyond the scope of the present work and is mentioned here to indicate that the physical properties of citric acid phosphate and its derivatives might merit detailed study.

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NEW ORLEANS, LA.

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, DUKE UNIVERSITY]

Carbonyl Addition Condensations of Diphenylmethane Involving Methylene Hydrogen by Alkali Amides. Synthesis of Certain Benzhydryl Type Carbinols[1,2]

BY PHILLIP J. HAMRICK, JR.,[3,2] AND CHARLES R. HAUSER

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Sodium and potassium diphenylmethides prepared from diphenylmethane and sodium and potassium amides in liquid ammonia undergo a reversible addition reaction with benzophenone and certain aromatic aldehydes to form the alkali salts of the corresponding carbinols. Instantaneous acidification of the reaction mixtures produced good yields of the carbinols, whereas gradual neutralization regenerated the starting materials. Replacement of the liquid ammonia with ether precipitated the sodium salt of 1,1,2,2-tetraphenylethanol but not the potassium salt of this carbinol which underwent reversion. Convenient methods of synthesis of several benzhydryl type carbinols are described.

It has previously been shown that diphenylmethane can be metalated at a methylene hydrogen with sodium amide or potassium amide in liquid ammonia and that the resulting alkali diphenylmethide can enter into several types of carbon–carbon condensations. For example, this hydrocarbon has been benzylated almost quantitatively through its sodium derivative in liquid ammonia (equation 1).[4]

$$(C_6H_5)_2CH_2 \xrightarrow[\text{liq. } NH_3]{NaNH_2} (C_6H_5)_2CHNa \xrightarrow{C_6H_5CH_2Cl} (C_6H_5)_2CHCH_2C_6H_5 \quad (1)$$

(red)

Other types of condensations that have been realized with diphenylmethane through its sodium or potassium derivative in liquid ammonia or ether include carbonation,[5] carbethoxylation,[5] benzoylation[5] and conjugate addition.[6]

It has now been found that diphenylmethane can enter into an addition condensation through its

(1) Supported by the National Science Foundation.

(2) Preliminary report given at the 132nd Meeting of the American Chemical Society at New York, N. Y., Sept., 8–13, 1957.

(3) Department of Chemistry, Wake Forest College, Winston-Salem, N. C.

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