

Determination of Citric Acid in the Presence of Interfering Substances.—It is usually necessary to estimate citric acid in the presence of one or more Krebs cycle intermediates. The substances which yield CO₂ when oxidized with ceric sulfate are α -ketoglutaric acid, pyruvic acid, oxalacetic acid, and, to some extent, malic acid. (All of the compounds mentioned, with the exception of malic acid, could actually be determined with ceric sulfate. For each mole of α -ketoglutarate, one mole of CO₂ and one mole of succinate is formed; for each mole of pyruvic acid, one mole of CO₂ and one mole of acetic acid is formed, and for each mole of oxalacetic acid, two moles of CO₂ are formed and one mole of acetic acid.) Fumarate, acetate, succinate and *cis*-aconitate do not interfere. When interfering substances are present, it is best to ether extract the deproteinated sample and isolate the citric acid by paper chromatography.⁴ Citrate is stable and partitions well between *t*-amyl alcohol, formic acid and water. The citric acid spot is eluted with boiling water and quantitatively estimated manometrically, as indicated above.

Determination of Citrate in Biological Material.—Typical results are shown in Table I: 1, 5 and 10 mg. of known citric acid were added to three different flasks, each of which contained 3 ml. of a dialyzed cell-free extract of *Escherichia coli*, prepared according to the method of Utter, *et al.*⁵ The extract was subsequently deproteinated, ether-extracted and appropriate dilutions thereof chromatographed. The eluted citrate bands were boiled down and the quantities determined manometrically with ceric sulfate. Recoveries ranged between 104 and 98.6%.

TABLE I

DETERMINATION OF CITRIC ACID IN BACTERIAL CELL-FREE EXTRACTS

Sample No.	Citric acid added, mg.	Citric acid recovered, mg.	Recovered, %
1	1	1.04	104
2	5	4.93	98.6
3	10	10.00	100

Analytical Range.—The smallest quantity of citric acid which can be determined by the manometric method is limited chiefly by the accuracy of the manometric equipment. Since 14 γ of citric acid is equivalent to $\sim 5 \mu$ l. of CO₂, this amount can be considered the lower limit of the method. The upper limit of this method depends upon the concentration of ceric sulfate. For routine work, 0.5 ml. of saturated ceric sulfate is enough to determine 400 γ of citric acid.

Discussion

The data in Fig. 1 show that a linear relationship was found between microliters of CO₂ liberated and micromoles of citric acid present up to a level of

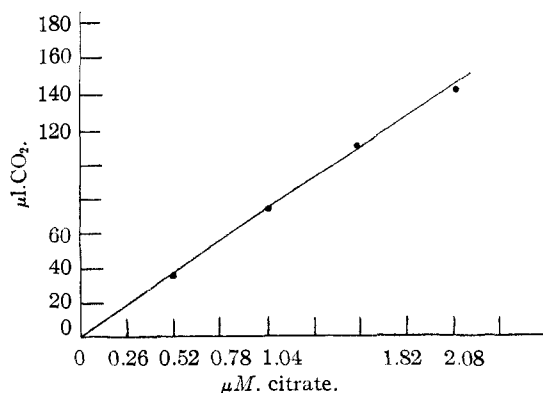
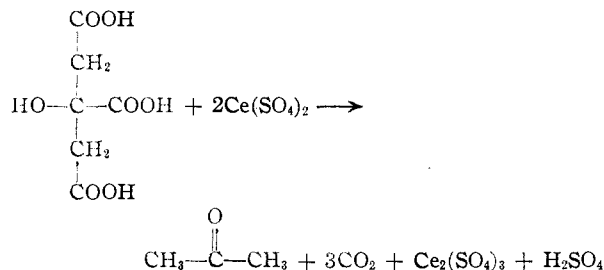


Fig. 1.—Manometric estimation of citric acid with ceric sulfate.

(4) J. W. H. Lugg and B. T. Overell, *Austral. J. Sci. Res.*, **1**, 98 (1948).

(5) M. F. Utter, G. Kalnitsky and C. H. Werkman, *J. Bact.*, **49**, 595 (1945).

2.08 micromoles (400 micrograms) of citric acid. Three micromoles of CO₂ were obtained for each mole of citrate oxidized, in accordance with the probable equation for the oxidation.



It is for this reason that during the determination of citric acid a standard curve need not be constructed. If one divides by three the number of microliters of CO₂ given off during citric acid oxidation, one immediately obtains the number of micromoles of citric acid present.

DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY
WASHINGTON UNIVERSITY MEDICAL SCHOOL
ST. LOUIS, MISSOURI

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A Convenient Synthesis of Orthoformic Esters

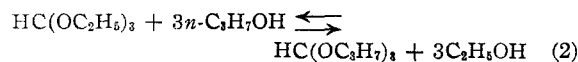
BY ELLIOT R. ALEXANDER¹ AND HIRSH M. BUSCH²

One of the best known syntheses for orthoformates involves the reaction of an alkoxide with a haloform



Thus, as early as 1854, ethyl orthoformate was prepared in this manner by Williamson and Kay.³ Since its discovery the method has been used for the preparation of methyl,⁴ propyl,⁴ isobutyl,⁴ isoamyl,⁵ phenyl,⁵ allyl,⁶ *o*-nitrophenyl,⁷ *n*-butyl⁸ and isopropyl¹ orthoformates. The yields, however, usually range from 20 to 50%.

Although there is another method for the preparation of orthoesters,⁹ it seemed possible that a general procedure could be developed based upon an ester interchange with ethyl orthoformate. It is known that ethyl orthoformate and propyl alcohol give an equilibrium mixture of ethyl orthoformate, *n*-propyl diethyl orthoformate, di-*n*-propyl ethyl orthoformate, *n*-propyl orthoformate and ethyl alcohol.¹⁰ Hence, it seemed possible to obtain the pure alkyl orthoformate by removing the ethyl alcohol as it was formed and thereby displacing the equilibrium to the right (2).



Although this possibility does not seem to have been investigated as a general method, it is interesting

(1) Deceased.

(2) University of Illinois, College of Dentistry, Chicago, Illinois.

(3) A. W. Williamson and G. Kay, *Ann.*, **92**, 346 (1854).

(4) A. Deutch, *Ber.*, **12**, 115 (1879).

(5) F. Tiemann, *ibid.*, **15**, 2686 (1882).

(6) F. F. Beilstein and E. Wiegand, *ibid.*, **18**, 482 (1885).

(7) A. Weddige, *J. prakt. chem.*, [2] **26**, 444 (1882).

(8) P. P. T. Sah and T. S. Ma, *THIS JOURNAL*, **54**, 2965 (1932).

(9) A. Pinner, *Ber.*, **16**, 352 (1883); *ibid.*, **16**, 1643 (1883).

(10) W. H. Post and E. R. Erickson, *THIS JOURNAL*, **55**, 3851 (1933).

TABLE I

Compound ortho- formate	Yield, %	Empirical formula	B.p.		d_{20}^4	n_{20}^D	Analyses, %					
			°C.	Mm.			MR		Carbon		Hydrogen	
							Calcd.	Found	Calcd.	Found	Calcd.	Found
<i>n</i> -Propyl	95.3	C ₁₀ H ₂₂ O ₃	96-97	20	0.8830	1.4078	53.51	53.21				
<i>n</i> -Butyl	86.3	C ₁₃ H ₂₈ O ₃	141	25	.8682	1.4184	67.18	67.34				
Isobutyl	86.0	C ₁₃ H ₂₈ O ₃	118-120	22	.8584	1.4122	67.18	67.23				
<i>s</i> -Butyl ^b	87.5	C ₁₃ H ₂₈ O ₃	115	23	.8632	1.4141	67.18	67.27	69.19	96.05	12.14	12.27
<i>n</i> -Amyl ^b	74.0	C ₁₆ H ₃₄ O ₃	101-103	0.3	.8714	1.4290	81.02	81.17	70.03	69.99	12.48	12.23
Isoamyl	88.2	C ₁₆ H ₃₄ O ₃	81-83	0.3	.8632	1.4251	81.02	81.14				
<i>n</i> -Hexyl ^b	77.4	C ₁₉ H ₄₀ O ₃	127-128	0.35	.8621	1.4344	94.90	95.66	72.07	71.99	12.74	12.75
Benzyl ^b	^a	C ₂₂ H ₂₂ O ₃	1.5645			79.01	78.93	6.63	6.84

^a Yield was not calculated because attempted distillation decomposed some of the product obtained. ^b New compound.

that several high boiling orthoformates (*l*-menthyl,¹¹ bornyl¹¹ and *s*-octyl¹²) have been prepared in this manner.

The modified method of Mkitaryan and Hunter was applied to the preparation of seven orthoformates with yields ranging from 74 to 95%. Experiments were also carried out for the preparation of isopropyl and *t*-butyl orthoformates but in each case the interchange did not occur. The preparation of allyl orthoformate led to a mixture of products that could not be separated by distillation. Benzyl and *n*-octyl orthoformates decomposed on distillation at 0.3 mm. pressure, but the benzyl orthoformate was found to crystallize in cold storage (4 to 10°), m.p. about 8°.

Experimental¹³

In all cases the ethyl orthoformate and the alcohols employed were distilled to constant refractive index before use.

The experiments summarized in Table I were carried out according to the following procedure. It is illustrated here with the preparation of *s*-butyl orthoformate.

***s*-Butyl Orthoformate.**—To a 500-ml. flask was added 74.1 g. (0.5 mole) of ethyl orthoformate and 128.2 g. (2.0 moles) of *s*-butyl alcohol. A "Thermocap" was used and adjusted so that a maximum temperature of the condensing vapor was 79° by controlling the heat to the flask. The mixture was heated for 24 hours (removing the ethyl alcohol as it was formed) and the vapor rectified through a fourteen inch column packed with glass helices. Upon removal of the theoretical amount of ethyl alcohol (69.0 g., 1.5 moles), the mixture was distilled under vacuum. *s*-Butyl orthoformate was obtained as a colorless liquid (see Table I).

(11) V. G. Mkitaryan, *J. Gen. Chem. (U. S. S. R.)*, **8**, 1361 (1938); *C. A.*, **33**, 4222 (1939).

(12) H. Hunter, *J. Chem. Soc.*, **125**, 1389 (1924).

(13) All boiling points are uncorrected.

NOYES CHEMICAL LABORATORY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

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Reactions of Nitrous Acid

By A. T. AUSTIN, E. D. HUGHES, C. K. INGOLD AND J. H. RIDD

It was shown by T. W. J. Taylor, for the aqueous deamination of primary aliphatic amines by nitrous acid,¹ that the reaction rate has the form

$$\text{Rate} \propto [\text{amine}][\text{HNO}_2]^2$$

where [amine] means the concentration of free amine, and [HNO₂] that of undissociated acid, this specification taking adequate care of the dependence of the rate on pH; the equation can be put into other forms, because kinetics cannot locate rapidly transferable protons. Taylor ob-

(1) T. W. J. Taylor, *J. Chem. Soc.*, 1099, 1897 (1928).

served the same kinetic law for the formation of nitrosamines from aliphatic secondary amines.² Abel and his co-workers obtained it again for the reaction of ammonia with nitrous acid.³ Schmid and Muhr obtained it yet again for the diazotization of aromatic primary amines.⁴ The implication is that it is a kinetic law for rate-controlling N-nitrosation.

Of the various interpretations which have been suggested, that of Hammett⁵ has been proved to be correct.⁶ It is simply that the active nitrosating agent in mildly acidic aqueous conditions is dinitrogen trioxide.

Papers by Dusenbury and Powell have just appeared⁷ in which the opinion is expressed that Taylor's rate law is erroneous, and, in particular, that the second ("unnecessary") molecule of nitrous acid which it involves is "non-existent." They do not state that they have repeated Taylor's work. Now it happens that we have repeated nearly the whole of it, and we therefore feel it incumbent on us to report that we have found it to be entirely accurate.

For ammonia and methylamine Dusenbury and Powell observed the rate law

$$\text{Rate} \propto [\text{amine}][\text{HNO}_2][\text{H}^+]$$

But they were working in phosphate buffers; they could have been replacing the usual nitrosating agent, dinitrogen trioxide, by a nitrosyl phosphate, or even in part by nitrosyl chloride when sodium chloride was added to make up the ionic strength. Any such nitrosyl compound of an anion present in excess, if acting as the deaminating agent, could lead to the observed rate law, as also could the nitrous acidium ion, or the nitrosonium ion. The observed law, without other information, does not diagnose mechanism. Schmidt and Muhr's work indicated the presence of a reaction of amines with nitrosyl chloride. We may mention that we have shown that nitrosyl bromide, and also the nitrous acidium ion, can replace the usual dinitrogen trioxide as nitrosating agent in buffer solutions not very dissimilar to those of Dusen-

(2) T. W. J. Taylor and L. S. Price, *ibid.*, 2052 (1929).

(3) E. Abel, H. Schmid and J. Schafranik, *Z. physik. Chem., Boden-stein Festband*, 510 (1931).

(4) H. Schmid and G. Muhr, *Ber.*, **70**, 421 (1937).

(5) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 294.

(6) E. D. Hughes, C. K. Ingold and J. H. Ridd, *Nature*, **166**, 642 (1950); A. T. Austin, E. D. Hughes, and C. K. Ingold, forthcoming paper.

(7) J. H. Dusenbury and R. E. Powell, *THIS JOURNAL*, **73**, 3266, 3269 (1951).