in the methyl substituted 8-quinolinols is the failure of 2-methyl, 2,3-dimethyl, 2,4-dimethyl and 2,3,4-trimethyl-8-quinolinol to precipitate aluminum.

TABLE I

ACIDIC AND BASIC IONIZATION CONSTANTS OF 8-QUINOLINOLS

Substituent	$K_b \times 10^{10}$	$K_a \times 10^{10}$
None[1]	8.32	1.95
3-CH_3	8.71	1.78
2-CH_3[6]	35.5	0.490
4-CH_3[6]	36.4	1.00
3,4-$(CH_3)_2$[6]	63.1	0.892
2,3-$(CH_3)_2$	74.1	.630
2,4-$(CH_3)_2$[6]	159	.252

The ultraviolet absorption spectra of these compounds in acid and base are very similar to 8-quinolinol[8,9] (Table II). The longest wave length of maximum absorption in both acid and base is shortened by the presence of a 2-methyl group and the molecular extinction at this band is highest for the compounds containing a 4-methyl group.

TABLE II

ABSORPTION MAXIMA IN ULTRAVIOLET SPECTRA OF METHYL-8-QUINOLINOLS[a]

Substituent	mμ	mμ	mμ
	A, in 0.1 *N* hydrochloric acid		
2-CH_3[b]	255(44000)	320(3100)	345(1700)
3-CH_3	254(45000)	320(2300)	355(1900)
4-CH_3[b]	250(44000)	315–318(1700)	350–353(2400)
2,3-$(CH_3)_2$	255(45000)	323(3700)	342(1900)
2,4-$(CH_3)_2$[b]	252(48000)	318(3100)	342(2400)
3,4-$(CH_3)_2$	252(40000)	318(2100)	353(2500)
2,3,4-$(CH_3)_3$	255(44000)	316(2500)	346–349(2600)
	B, in 0.1 *N* sodium hydroxide		
2-CH_3[b]	255(30000)		335(3000)
3-CH_3	256–257(30000)		355(2700)
4-CH_3[b]	253(28000)		343(3900)
2,3-$(CH_3)_2$	257(37000)		335(3300)
2,4-$(CH_3)_2$[b]	255(31000)		337(4200)
3,4-$(CH_3)_2$	255(35000)		350–353(3800)
2,3,4-$(CH_3)_3$	255(41000)		340–345(4200)

[a] Figures in parentheses are molecular extinctions.

The solubilities of the methyl-8-quinolinols are with the exception of 8-hydroxyquinaldine very low: for example, a saturated solution of 3-methyl-8-quinolinol has a molarity of 5.4×10^{-4}, of 2,3-dimethyl-8-quinolinol a molarity of 4.8×10^{-4}, as compared to 2.67×10^{-3} for 8-hydroxyquinaldine.

Attempts to correlate various properties of methyl substituted 8-quinolinols with their formation of metal chelates led to the following generalizations: (1) The presence of a group in the 2-position is sufficient to prevent aluminum from forming an insoluble chelate.[2,3] (2) The lower the *p*H at which a metal is precipitated the more insoluble the metal chelate is; this is the same relation observed in the precipitation of metals as hydroxides by ammonia.[10] (3) There appears to be no simple relation between ionic radii of metals and their conditions of chelate formation with 8-quinolinols. (4) The more basic (and less acidic) substituted 8-quinolinols precipitate metal chelates at higher *p*H values.[3]

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NOTES

Manometric Estimation of Citric Acid

BY SAMUEL J. AJL, DONALD T. O. WONG AND DAVID F. HERSEY

A number of methods, enzymatic and chemical, have been described for the determination of small amounts of citric acid. Of the chemical methods, the majority involve the conversion of citric acid to pentabromoacetone, which may be estimated by various gravimetric, titrimetric or colorimetric procedures. The recent modifications[1-3] of this method have simplified it and increased the sensitivity. However, even in the simplified methods, the procedures are quite laborious. The method described in this communication is considerably faster and simpler to carry out.

Citric acid (14–400 micrograms) is oxidized manometrically under controlled conditions with ceric sulfate; the CO_2 produced is a measure of citric acid concentration.

Procedure

Manometric Estimation of Citric Acid.—Warburg flasks of about 20-ml. capacity with or without a center well and a side-arm of 1-ml. capacity are usually employed. The citric acid solution, usually 1 ml., is added to the main compartment. 0.5 ml. of 6 *N* H_2SO_4 is next pipetted into the main compartment to liberate all of the bound CO_2. 0.4 ml. of saturated ceric sulfate (a saturated solution is prepared by heating on a steam-bath an excess of the compound in 4 *N* H_2SO_4 for 6 to 12 hours with occasional stirring) is added to the side-arm. The control vessel is made up in the same way, excepting that 1 ml. of distilled water is placed in the main compartment, in place of the citric acid solution. The bath temperature is adjusted to 30°. After a 10-minute shaking period with the stopcocks open for equilibration, the manometric fluid is adjusted so as to provide a maximum scale for reading and the stopcocks are closed. If equilibration is attained, the content of the side-arm is delivered into the main compartment and the manometers quickly replaced on the bath. Readings are then taken every two or three minutes until the delta values (CO_2 evolution per unit time) of the control and the experimental manometers are equal on two successive readings. The reaction is usually complete in less than 10 minutes.

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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 CO_2 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 CO_2 and one mole of succinate is formed; for each mole of pyruvic acid, one mole of CO_2 and one mole of acetic acid is formed, and for each mole of oxalacetic acid, two moles of CO_2 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 ~ 5 μl. of CO_2, 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 CO_2 liberated and micromoles of citric acid present up to a level of 2.08 micromoles (400 micrograms) of citric acid. Three micromoles of CO_2 were obtained for each mole of citrate oxidized, in accordance with the probable equation for the oxidation.

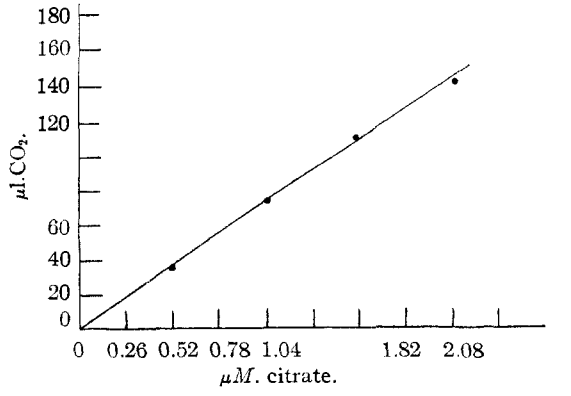
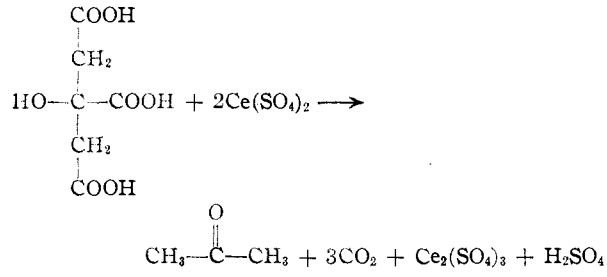


Fig. 1.—Manometric estimation of citric acid with ceric sulfate.



$$HO{-}C(CH_2COOH)_2{-}COOH + 2Ce(SO_4)_2 \longrightarrow CH_3{-}C(=O){-}CH_3 + 3CO_2 + Ce_2(SO_4)_3 + H_2SO_4$$

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 CO_2 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. ALEXANDER[1] AND HIRSH M. BUSCH[2]

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

$$3RONa + HCX_3 \longrightarrow HC(OR)_3 + 3NaX \quad (1)$$

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*-butyl[8] and isopropyl[9] 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).

$$HC(OC_2H_5)_3 + 3n\text{-}C_3H_7OH \rightleftharpoons HC(OC_3H_7)_3 + 3C_2H_5OH \quad (2)$$

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

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