flasks were closed with rubber stoppers since the presence of stopcock grease may complicate the infrared analysis. To each flask was added 1.00 ml. of standard sodium hydroxide solution. At various times flasks were removed, cooled to 0° to stop the reaction, titrated to the phenolphthalein end-point and extracted with about 10 ml. of carbon disulfide. The extract was dried with silica gel, diluted if necessary and analyzed for bromochloroiodomethane and chlorodiiodomethane by infrared measurements.

To calculate $f_I$ from eq. 9, initially, the magnitude of each side of the equation was determined for $f_I$ values at small intervals over the entire range, 0.00 to 1.00, by use of an IBM model 650 data processing machine. After these calculations showed the approximate value of $f_I$, the range and the intervals were decreased in a recalculation.

**Analysis of Haloform Mixtures.**—The infrared analysis was carried out by the general method described previously.[3,9] The Beer–Lambert law was found to hold and the following extinction coefficients were determined at wave lengths of 8.505, 8.615, 9.057 and 9.357 μ, respectively; for bromochloroiodomethane, 59.4 ± 1.7, 5.97 ± 0.1, 173 ± 5 and 1.75 ± 0.03; for chlorodiiodomethane, 7.69 ± 0.16, 59.4 ± 1.5, 10.83 ± 0.37 and 173 ± 5. With measurements being made at four wave lengths it was possible to make two independent calculations of the composition of the haloform mixture. This was done and as shown in Table II there was good agreement between the two sets of values. Additional checks for artifacts due to other materials absorbing at the wave lengths used were made by extracting reaction solutions that had not had time to react appreciably and solutions containing no sodium iodide but in which considerable alkaline hydrolysis of bromochloroiodomethane had taken place. In each case the infrared analysis showed pure bromochloroiodomethane with no appreciable amount of chlorodiiodomethane. Extracts of reaction solutions from which bromochloroiodomethane had been omitted had no appreciable absorption at the wave lengths used for analysis. It was found that the extraction procedure used left practically no haloform in the aqueous solution, showing that preferential extraction of one of the two haloforms could not be affecting the results appreciably.

**Acknowledgments.**—We should like to acknowledge our indebtedness to the Alfred P. Sloan Foundation for a grant in support of this work and to the Rich Electronic Computer Center for the use of their facilities.

ATLANTA, GEORGIA

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[9] J. Hine, R. C. Peek, Jr., and B. D. Oakes, THIS JOURNAL, **76**, 827 (1954).

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[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND CHEMICAL ENGINEERING, THE UNIVERSITY OF TEXAS]

# A Study of *meta* Orientation in the Nitration of Toluene by Isotope Dilution Analysis[1]

BY ROYSTON M. ROBERTS, PHILIP HEIBERGER, JOEL D. WATKINS, HENRY P. BROWDER, JR., AND KENNETH A. KOBE

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The proportion of *m*-nitrotoluene formed by mononitration of toluene with mixed acids at 0°, 30°, 45° and 60° has been determined using isotope dilution analysis.

The determination of isomer distribution among the products of mononitration of toluene has been the subject of a large number of studies. Only a few of the more careful and complete investigations are referred to here.[2–6] The proportion of *m*-nitrotoluene produced is of particular interest not only because of the theoretical implications but also because of its relationship to undesirable trinitrotoluene isomers which contaminate 2,4,6-trinitrotoluene (TNT). *m*-Nitrotoluene has been reported to be formed in amounts varying from 2.5 to 5.1% under widely different conditions of temperature and nitrating media. The methods of analysis used have been indirect and, in general, based upon comparison of unknown mixtures with mixtures of known isomers. These indirect methods have been necessary in the past because of the impossibility of making a quantitative separation of the small amount of *m*-isomer from its closely-similar *o*- and *p*-isomers. This problem is one to which the method of radioactive isotope dilution analysis is ideally suited.

For the nitration of radioactive toluene-1-$C^{14}$ we chose a mixture of concentrated sulfuric and nitric acids, since this is by far the most commonly used nitrating medium. The nitrations were carried out on 2- to 5-gram batches of toluene, using slightly less than the stoichiometric amounts of nitric acid in order to minimize dinitration. Forty per cent. of the sulfuric acid was added to the toluene, vigorous stirring was begun, and the remainder of the sulfuric acid, mixed with the nitric acid, was added dropwise. This procedure avoided the production of appreciable heat of mixing during the addition and kept the toluene always in excess. After the length of time appropriate to reaction at temperatures of 0, 30, 45 and 60°, the reaction mixture was quenched with water and steam distilled. This allowed separation of the mixed nitrotoluenes from any unchanged toluene, dinitrotoluenes and oxidation products. A weighed sample of the mixed nitrotoluenes was diluted with a known amount of non-radioactive *m*-nitrotoluene and the mixture was distilled through an efficient micro-fractionating column in order to recover a pure sample of *m*-nitrotoluene. Known mixtures of *o*-, *m*- and *p*-nitrotoluene were previously distilled through the column to provide the experience and data necessary for successful fractionation. The *m*-nitrotoluene was oxidized by dichromate–sulfuric acid to *m*-nitrobenzoic acid and this material was radioassayed. The proportion of *m*-nitrotoluene present in the mixed nitrotoluenes was calculated from the usual inverse isotope dilution formula; *e.g.*,

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[1] Supported by U. S. Army Ordnance Research under Contract No. DAI-23-072-501-ORD-(P)-6. Taken in part from the Ph.D. thesis of Philip Heiberger, The University of Texas, 1953.

[2] A. F. Holleman, J. Vermeulen and W. J. de Moy, *Rec. trav. chim.*, **33**, 1 (1914).

[3] W. H. Gibson, R. Duckham and R. Fairbairn, *J. Chem. Soc.*, **121**, 270 (1922).

[4] C. K. Ingold, A. Lapworth, E. Rothstein and D. Ward, *ibid.*, **130**, 1959 (1931).

[5] P. de Beule, *Bull. soc. chim. Belg.*, **42**, 27 (1933).

[6] W. W. Jones and M. Russell, *J. Chem. Soc.*, 921 (1947).

Table I

*meta* Orientation in Nitration of Toluene at Different Temperatures

| Temp., °C. | 0 | 30 | 45 | 60 |
|---|---|---|---|---|
| Time for addn. of mixed acids, min. | 90 | 30 | 15 | 8 |
| Additional time stirred at reacn. temp., min. | 90 | 5 | 5 | 2 |
| Yield of mixed nitrotoluenes, % | 100 | 92 | 84 | [a] |
| Isotopic dilution, wt., g. | | | | |
| Mixed nitrotoluenes | 2.97 | 3.11 | 2.07 | 0.830 |
| *m*-Nitrotoluene | 11.08 | 8.09 | 8.07 | 8.06 |
| Radioactivity, μc./mmole | | | | |
| Toluene-1-$C^{14}$ | 3.14 | 2.74 | 3.14 | 3.14 |
| *m*-Nitrobenzoic-1-$C^{14}$ acid | 0.0174 | 0.0357 | 0.0326 | 0.0151 |
| Maximum deviation | ±0.0008 | ±0.0030 | ±0.0055[b] | ±0.0000 |
| *m*-Nitrotoluene, % in mixed nitrotoluenes | 2.08 | 3.44 | 4.18 | 4.70 |

[a] Some product lost by accident before weighing. [b] Average of 8 assays—reproducibility unusually poor in this series.

$$\%\ m\text{-nitrotoluene} = \frac{100x}{y(a/b - 1)}$$

where $x$ is the weight of *m*-nitrotoluene diluent, $y$ is the weight of mixed nitrotoluenes diluted, $a$ is the molar radioactivity of the toluene-1-$C^{14}$ nitrated and $b$ is the molar radioactivity of the *m*-nitrobenzoic acid.

The most likely source of error in the isotope dilution analysis was contamination of the *m*-nitrobenzoic acid by higher-activity *o*- and *p*-nitrobenzoic acid from the corresponding nitrotoluenes. Although we considered that the boiling points of *m*- and *p*-nitrotoluene were far enough apart to allow isolation of a small amount of pure *m*-nitrotoluene by fractional distillation, we made a careful independent check of this separation. A synthetic mixture containing inactive *o*- and *m*-nitrotoluene and radioactive *p*-nitrotoluene was prepared and distilled through the fractionating column. The *m*-nitrotoluene fraction was oxidized and the resulting *m*-nitrobenzoic acid was radioassayed. It was non-radioactive, demonstrating that the separation had been successful. The separation of *m*-nitrotoluene from *o*-nitrotoluene was no more difficult and, in addition, it was expected that any *o*-nitrotoluene present in the *m*-nitrotoluene fraction would be removed in the dichromate–sulfuric acid oxidation step.[7] This was checked by preparing a synthetic mixture of inactive *m*-nitrotoluene and radioactive *o*-nitrotoluene and oxidizing a sample with dichromate–sulfuric acid. The *m*-nitrobenzoic acid obtained was non-radioactive, demonstrating the absence of *o*-nitrobenzoic acid.

The results from nitrations at four different temperatures are given in Table I. The percentage of *m*-nitrotoluene among the mononitrotoluenes ranged from 2.08 at 0° to 4.70 at 60°. A sample of the mixed nitrotoluenes from the 30° nitration was analyzed by infrared spectrophotometry[8] and found to contain 3.45% *m*-nitrotoluene, 44.0% *p*-nitrotoluene, 52.5% *o*-nitrotoluene, trace of 2,4-dinitrotoluene and 0.0% toluene; the value for *m*-nitrotoluene was in excellent agreement with the value from isotope dilution analysis. Some of the previous investigators reported a decreasing proportion of *m*-nitrotoluene at lowered temperatures,[2,4,6] but others found no consistent trend.[3,5]

Our value of 2.08% at 0° is the lowest reported, although some of the nitrations reported previously were carried out at a temperature of −30°. We believe that the extremely high degree of emulsification obtained with a high-speed stirrer in the small scale apparatus may be responsible for the smaller amounts of *m*-nitrotoluene which we found. In some of the preliminary runs in which poorer agitation was used, higher yields of *m*-nitrotoluene were obtained.

## Experimental[9]

**Materials.**—Toluene was Baker and Adamson reagent grade, distilled through the column described below. A constant-boiling fraction distilling at 111° was used. Toluene-1-$C^{14}$ was purchased from Tracerlab, Inc.; it was diluted with the redistilled ordinary toluene. Ordinary *o*-, *m*- and *p*-nitrotoluenes were Eastman Kodak Co. white label; the *o*- and *m*-isomers were redistilled through the same column used for toluene; the *p*-isomer was recrystallized before use. The *o*- and *p*-nitrotoluene-1-$C^{14}$ used for checking the procedures relied upon for separation of *m*-nitrotoluene from the *o*- and *p*-isomers were prepared by nitration of toluene-1-$C^{14}$. The *o*-isomer was purified by distillation and the *p*-isomer by recrystallization. Sulfuric and nitric acids were reagent grade, 96 and 70%, respectively, and were used as received.

**Apparatus.**—The distillation column used in this work was a Podbielniak Series 3300 semi-automatic micro high temperature distillation analyzer, with heli-grid packing, 8-mm. i.d., 24 in. long, rated at 100 plates under total reflux. Its operating characteristics were well established by distilling pure *o*- and *m*-nitrotoluene and mixtures of *o*-, *m*- and *p*-nitrotoluene under reduced pressure, usually at 90 mm. pressure.

The nitration reaction vessel was an 8-in. test-tube with an upturned side-arm through which addition of mixed acids could be made. It was fitted with a motor-driven stirrer consisting of a glass band twisted into a spiral about 2 in. long. The stirring motor operated at 1700 r.p.m., providing excellent emulsification of the heterogeneous reaction mixture. Temperature control (±0.5°) was accomplished by means of a 4-l. water-bath, cooled with ice or heated with a small immersion heater, as required.

**Radioassays** were made by converting organic compounds by wet-combustion[10] to carbon dioxide which was collected in an ionization chamber and counted on a vibrating reed electrometer.[11]

**Nitration of Toluene** at 30°.—A mixture of 5.00 g. of toluene-1-$C^{14}$ (2.74 μc./mmole) and 3.45 g. of 96% sulfuric

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(7) F. Beilstein and A. Kuhlberg [*Ann.*, **155**, 17 (1870)] found that *o*-nitrotoluene was resistant to prolonged boiling with dichromate-sulfuric acid; the small amount of material which did react was apparently completely oxidized.

(8) We are indebted to Mr. B. C. Carlson of U. S. Rubber Co., Joliet Arsenal, for this analysis.

(9) Melting points were determined with calibrated Anschütz thermometers in a Hershberg apparatus. Boiling points are uncorrected.

(10) D. D. Van Slyke and J. Folch, *J. Biol. Chem.*, **136**, 509 (1940); D. D. Van Slyke, J. Plazin and J. R. Weisiger, *ibid.*, **191**, 299 (1951).

(11) O. K. Neville, This Journal, **70**, 3501 (1948).

acid was placed in the reactor and allowed to come to bath temperature. The stirrer was started and a mixture of 4.50 g. of 70% nitric acid and 5.20 g. of 96% sulfuric acid was added dropwise through the side-arm during 30 min. After the reaction mixture had stirred an additional 5 min., about 20 ml. of cold water was added. The quenched reaction mixtures from three such nitrations were poured into 400 ml. of water and the mixture was distilled through a 12-in. Berl-saddle-packed column provided with a Dean–Stark trap for removing the heavier than water layer. No toluene was recovered. (In some nitrations unreacted toluene distilled with the first few milliliters of water and easily was separated from the nitrotoluenes which distilled later.) The mixed nitrotoluenes steam distilled, leaving any di- and trinitrotoluenes in the residue. The nitrotoluenes were extracted into ether, the ether solution was dried with magnesium sulfate and the solution was evaporated on a steam-cone to leave 19.0 g. (92%) of mixed nitrotoluenes.

A 3.11-g. sample of the mixed nitrotoluenes was diluted with 8.09 g. of pure (inactive) *m*-nitrotoluene. This mixture was distilled through the Podbielniak column and the following fractions were collected at 30/1 reflux ratio: 1, 2.38 g., b.p. 137.0–146.9° (90 mm.); 2, 0.77 g., b.p. 146.9–147.3°; 3, 1.31 g., b.p. 147.8°. Fraction 3 corresponded to pure *m*-nitrotoluene, according to previous trial distillations. To a 2.31-g. sample of fraction 3 was added 3.88 g. of sodium dichromate and 8.50 g. of water; 9.70 g. of concd. sulfuric acid was then added dropwise during 8 min. The mixture was heated under reflux and stirred for 45 min. At the end of this time, the reaction mixture was cooled in an ice-bath for two hours. The solid material which separated was collected on a sintered-glass filter and then boiled with benzene, which extracted the organic material away from the inorganic salts. After separating these by filtration, the benzene solution was extracted with 5% sodium hydroxide solution using a gentle swirling motion of the separatory funnel so as to avoid emulsification. The aqueous solution was acidified with sulfuric acid and the crystals of *m*-nitrobenzoic acid were collected on a filter (80% yield of crude material). The acid was recrystallized twice from 1% hydrochloric acid and radioassayed. The average of four assays (10- to 14-mg. samples) was 0.0357 ± 0.0030 μc./mmole, corresponding to 3.43% *m*-nitrotoluene among the nitrotoluenes. The *m*-nitrobenzoic acid was recrystallized once more and re-assayed; the average of two assays (10- and 11-mg. samples) was 0.0356 ± 0.0003μc./mmole. The *m*-nitrobenzoic acid melted at 141.2°.

The nitrations at the other temperatures were carried out similarly on the same amount (5 g.) of radioactive toluene-1-C$^{14}$, except in the 60° nitration only 2.32 g. of toluene was used and the amounts of the other materials were cut down proportionately. Other details and results of these experiments are given in Table I.

**Separation of *m*-Nitrotoluene from *p*-Nitrotoluene by Distillation.**—A mixture was prepared which contained 3.02 g. of *o*-nitrotoluene, 3.00 g. of *m*-nitrotoluene and 6.26 g. of *p*-nitrotoluene-1-C$^{14}$ (0.32 μc./mmole). This mixture was distilled through the Podbielniak column and a cut of 0.80 g. was taken at the *m*-nitrotoluene plateau. This material was oxidized by dichromate–sulfuric acid as described above and a 51.7-mg. sample of the *m*-nitrobenzoic acid obtained was radioassayed. The electrometer reading was not significantly above background. Since a 1% contamination by *p*-nitrobenzoic acid would have given a reading seven times background from this size sample, it was concluded that the separation by fractional distillation was satisfactory.

**Separation of *m*-Nitrotoluene from *o*-Nitrotoluene by Oxidation.**—A mixture of 2.00 g. of *m*-nitrotoluene and 0.05 g. of *o*-nitrotoluene-1-C$^{14}$ (0.09 μc./mmole) was prepared and oxidized by dichromate in sulfuric acid as described above. The *m*-nitrobenzoic acid obtained was recrystallized twice and a 53.4-mg. sample was radioassayed. The electrometer reading was not significantly above background. Since a 1% contamination by *o*-nitrobenzoic acid would have given a reading twice background from this size sample, it was concluded that the *o*-nitrotoluene was satisfactorily separated in the oxidation procedure.

AUSTIN 12, TEXAS

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF BUFFALO SCHOOL OF MEDICINE]

# Fluoroacetyl Phosphate; Preparation and Properties[1]

BY ABRAHAM MARCUS[2] AND W. B. ELLIOTT

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Fluoroacetyl phosphate has been synthesized and compared with acetyl phosphate. The rate of hydrolysis of fluoroacetyl phosphate, like that of acetyl phosphate, is first order with respect to substrate but in contrast to that of acetyl phosphate is minimal at *p*H 2. Both reactions are increased at neutral *p*H by Mg$^{++}$, Ca$^{++}$ or pyridine, but pyridine catalysis is greater with fluoroacetyl phosphate. At acid *p*H, Mg$^{++}$ and Ca$^{++}$ accelerate the hydrolysis of fluoroacetyl phosphate, whereas acetyl phosphate hydrolysis is unaffected. Fluoroacetyl phosphate is much more susceptible to mammalian and bacterial acyl phosphatase than is acetyl phosphate. Possible explanatory mechanisms are discussed.

In a previous report,[3] it was shown that fluoroacetate was enzymatically activated by pigeon liver extracts and could function in condensation reactions which involve both the carboxyl and methyl groups. The latter reactions have also been demonstrated with synthetic fluoroacetyl-coenzyme A.[3,4] During this study it was noted[5] that both FAc-CoA[6] and fluoroacethydroxamic acid (prepared from fluoroacetic anhydride) were much more unstable than the respective acetyl compounds. To obtain additional insight into the chemical and biological properties introduced by the α-F atom, it appeared desirable to seek another fluoroacetyl compound which could be prepared in quantity sufficient for detailed study. The present communication is concerned with the synthesis of fluoroacetyl phosphate and comparison of its chemical properties with those of acetyl phosphate.

## Results

**Synthesis of Fluoroacetyl Phosphate.**—In seeking a convenient method for the preparation of fluoroacetyl phosphate, several reactions were studied for suitability in preparing acetyl phosphate. It was found that Ac-P could be prepared by reaction of either acetic anhydride or a mixed anhydride of acetic acid and ethyl chlorocarbonate[7] with

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[2] Research Fellow of the National Heart Institute, Public Health Service. This work represents a portion of a dissertation submitted by Abraham Marcus to the Graduate School of the University of Buffalo, May, 1956, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] A. Marcus and W. B. Elliott, *J. Biol. Chem.*, **218**, 823 (1956).

[4] R. O. Brady, *ibid.*, **217**, 213 (1955).

[5] A. Marcus and W. B. Elliott, unpublished observations.

[6] The following abbreviations are used: FAc-CoA, S-fluoroacetyl coenzyme A; Ac-P, acetyl phosphate; FAc-P, fluoroacetyl phosphate; tris, tris-(hydroxymethyl)-aminomethane.

[7] T. Wieland and L. Rueff, *Angew. Chem.*, **65**, 186 (1953).