[CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

THE SYNTHESIS OF TOLUENE-1,3,5-C^{14} AND OXALIC ACID-C^{14}; THE MECHANISMS OF THE REACTIONS[1]

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For the purpose of carrying out many types of tracer studies, it is necessary to have aromatic compounds labeled in the ring with isotopic carbon. A useful intermediate for the synthesis of such compounds would be toluene[3]. At the suggestion of Professor Melvin Calvin, the authors have investigated the feasibility of synthesizing toluene-C^{14} by decarboxylating uvitic acid (II), prepared from pyruvic acid-α-C^{14} *via* methyldihydrotrimesic acid (I). A valuable feature of this scheme is the formation of oxalic acid, a useful compound, which should be labeled if the scheme which has been postulated (1) for the condensation of pyruvic acid to methyldihydrotrimesic acid is correct. Although there is no reason to doubt the correctness of the essential features of this postulate, it was thought to be of interest to apply those checks which tracer technique affords, since there are still comparatively few instances in which mechanisms formulated from considerations of the classical types have been directly checked with isotopic carbon. Accordingly, the specific activities of the intermediate compounds have been determined, and the mechanisms of all the reactions involved in the synthesis checked with respect to the fate of the tracer carbon atom.

The reaction sequence is described in Fig. 1, which includes the previously postulated (1) course of the reactions leading to the formation of uvitic acid (equations A and B). It will be shown that equation B is in error with respect to the minor products. Positions expected to be labeled are marked with an asterisk.

The radioactivity measurements are recorded in Table I. The molar specific activity to which reference is made in the heading of the third column is the specific activity of the compound multiplied by its molecular weight. The ratios are calculated with the molar specific activity of the methyldihydrotrimesic acid as the denominator; each ratio has been multiplied by three to obtain integral values. Each value so calculated expresses, for the compound to which it refers, the number of carbon atoms in the molecule which were ketonic carbon atoms in pyruvic acid, assuming that three of the ketonic atoms have been incorporated into methyldihydrotrimesic acid. Why the specific activity of pyruvic acid is not made the denominator of the ratio will be made clear below. The

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[3] *Note added in proof.* Subsequent to the submission of this paper for publication, a synthesis of toluene labeled with carbon 14 in position 1 has been reported by Fields, Leaffer, and Rohan [*Science*, **109**, 35 (1949)].

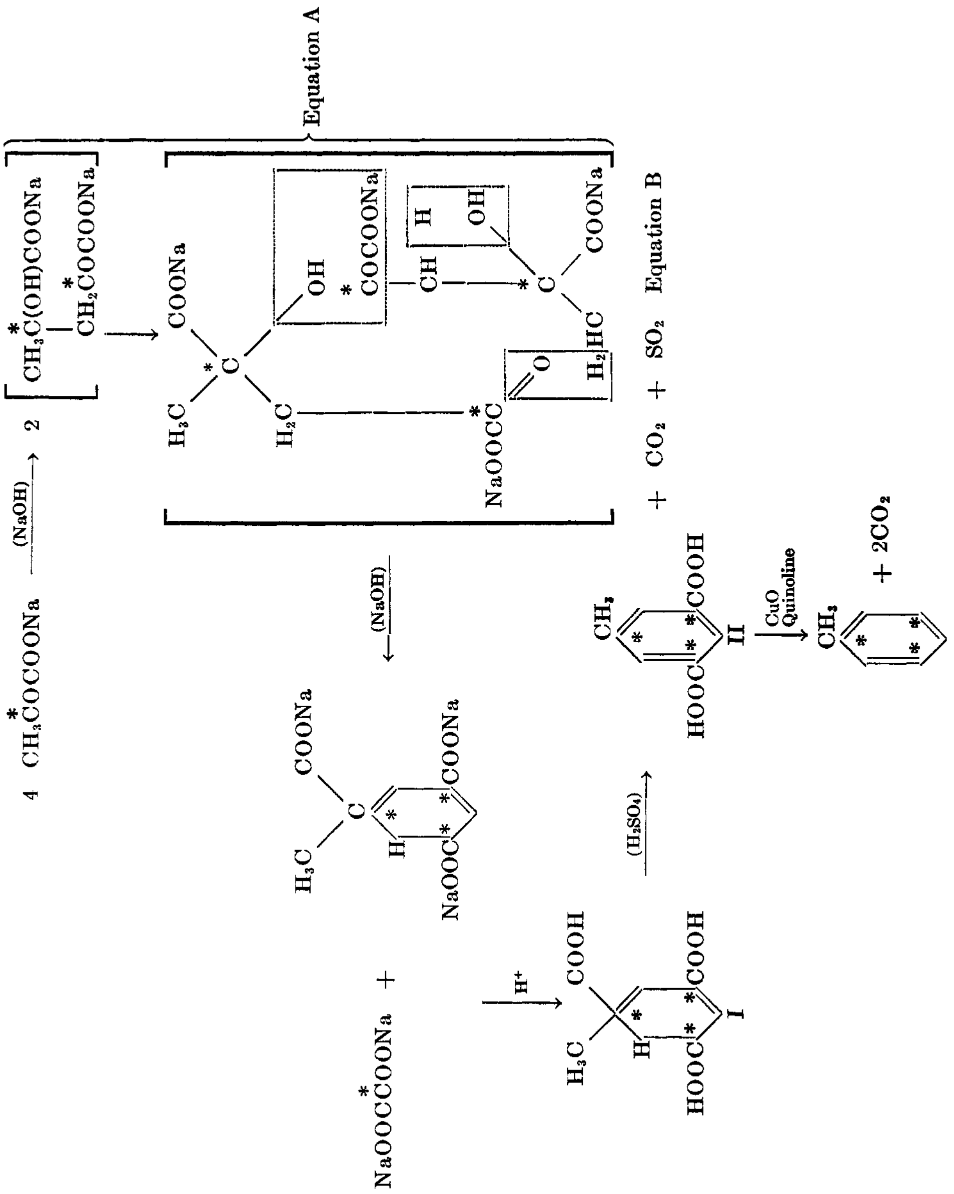

FIGURE 1

ratios show that for each ketonic carbon atom which appears in oxalic acid, three appear in methyldihydrotrimesic acid, and that these three are not lost in the succeeding reactions which lead to toluene. These facts are in accord with the mechanisms implied by the equations in Fig. 1. It is on the strength of this that the assignment of the positions of the tracer atoms has been made.

The molar specific activity ratio for pyruvic acid should be unity; actually, it is 2.08. The explanation of this discrepancy is contained in the activity totals tabulated in the fourth column of Table I. These numbers show that the sum of the total activities of the methyldihydrotrimesic acid, oxalic acid and residual syrup is only 52% of the total activity of the pyruvic acid. Since the total weight of these three substances accounts for substantially all of the pyruvic acid, the radioactive (undiluted) preparation must have contained radioactive impurity, which amounted to about 50% by weight.

TABLE I

RADIOACTIVITY MEASUREMENTS ON THE REACTION PRODUCTS

COMPOUND	SP. ACT. CTS./MIN./MG.	MOLAR SP. ACT. / MOLAR SP. ACT. MDTA	TOTAL ACT. AS % OF INITIAL
Undiluted active pyruvic acid	9420	—	100
Diluted pyruvic acid	427	2.08	100
Methyldihydrotrimesic acid monohydrate	223	3.00	24.5
Oxalic acid dihydrate	150	1.04	11.3
Syrup	464	—	16.5
Barium Carbonate I[a]	66.3	—	8.82
Uvitic acid	302	2.98	23.7
Barium Carbonate II	0.68	~0	0.0022
Barium Carbonate III	0.049	~0	0.0029
Toluene	589	2.98	21.0
Barium Carbonate IV	0.07	~0	0.010

[a] For identification of the source of the barium carbonate specimens, see Experimental section.

Although it is clear from the total activity values in Table I that the impurity did not participate in the reactions under study, it was identified as a matter of interest. A potentiometric titration of the pyruvic acid gave an equivalent weight corresponding to 90% pyruvic acid; the impurity is, therefore, acidic, although only one break was detectable in the titration curve. The fact that the final residue of salts contained a small part (18%) of the missing activity means that the impurity is extracted from acid solution by ether, but only slowly. Since the syrup did not contain an appreciable amount of the activity extracted, the impurity is a volatile substance, which was lost when the syrup was dried in vacuum. That the activity unaccounted for actually was lost from the syrup during its drying was verified by a confirmatory experiment, in which an aliquot of the syrup was assayed without being dried. In this repeat run, substantially all of the initial activity was accounted for; the amount in the methyldihydrotrimesic and oxalic acids was about the same as before, but that in the syrup was much higher.

The method used to synthesize the pyruvic acid[4] suggested that the impurity was acetic acid, as did the observations just recorded concerning its behavior. The identification was made by determining the Duclaux numbers of the acid distillable from the liquor obtained in the confirmatory experiment after removal of methyldihydrotrimesic and oxalic acids. The liquor was freed of chloride by treatment with silver oxide, then was filtered and adjusted to *p*H 3 with sulfuric acid. The Duclaux numbers are given in Table II. The specific activities of the first and last fractions of the distillate were found[5] to be 8.8×10^5 and 9.0×10^5 cts./min./meq. respectively; in contrast to these values the specific activity of the diluted pyruvic acid was 3.26×10^4 cts./min./meq. There was 0.799 meq. of acetic acid in the 50 ml. of distillate collected. This should be about 34% (2) of the acetic acid originally present in the liquor; from this it can be calculated that the liquor contained 7.2×10^5 cts./min. of acetic acid, and the undiluted pyruvic acid contained about 47% acetic acid by weight, a value in satisfactory agreement with the estimate of 50% mentioned earlier.

TABLE II

DUCLAUX NUMBERS FOR THE CONTAMINANT

TOTAL VOLUME OF DISTILLATE, ML.	ACID CONTENT AS % OF TOTAL DISTILLED	% EXPECTED FOR ACETIC ACID
10	20.7	18.7
20	39.9	38.2
30	59.4	57.4
40	79.6	78.6
50[a]	100.0	100.0

[a] The distillation was interrupted when 50 ml. of distillate had been collected because the large amount of sodium sulfate present caused unmanageable bumping.

The Duclaux identification was confirmed by converting the acetic acid to its anilide. To a portion of the Duclaux distillate containing 11.1 mg. of active sodium acetate was added inactive acetic acid equivalent to 334 mg. of sodium

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[4] We are indebted to Mr. R. M. Lemmon for the radioactive pyruvic acid. The method of synthesis, described in outline in another place [Calvin and Lemmon, *J. Am. Chem. Soc.*, **69**, 1232 (1947)] utilizes the following sequence of reactions:

$$CH_3\overset{*}{C}O_2H \rightarrow CH_3\overset{*}{C}OBr \rightarrow CH_3\overset{*}{C}OCN \rightarrow CH_3\overset{*}{C}OCO_2H$$

[5] By evaporating an aliquot of the solution on a glass disc and counting the sodium acetate directly. The amount of sodium acetate was small enough to reduce the self-absorption error to minor proportions.

acetate (both values were determined by titration). Sodium acetate was obtained by evaporating the solution and was converted to acetanilide; the purified derivative had a specific activity of 264 cts./min./mg.; calculated, 212. The discrepancy can reasonably be attributed to a slightly low value in the direct count of sodium acetate.

It was found that in the formation of uvitic acid (equation B) methyldihydrotrimesic acid loses its carboxyl carbon atom as carbon monoxide, not carbon dioxide as reported in the earlier work of Wolff. That the gas evolved is carbon monoxide is shown by the fact that very little barium carbonate, and still less sulfite, was recovered from alkali through which the gas had passed; by passage over hot copper oxide, however, carbon dioxide was formed in essentially quantitative yield.

In order for methyldihydrotrimesic acid to form uvitic acid by loss of carbon dioxide, the extra hydrogen atom attached to the number two position of the ring must be disposed of in some way; the opinion expressed in the earlier work is that a certain amount of di- and tetra-hydrouvitic acid, and perhaps analogous methyltrimesic acids are formed. Such materials were thought to compose the bulk of certain syrups obtained under various decarboxylation conditions, although in general the syrups were difficult to work with and this fact precluded the isolation of pure compounds from them. The high yield of uvitic acid obtained when concentrated sulfuric acid is used as the decarboxylation catalyst is stated (3) to be due to its ability to oxidize the extra hydrogen and thereby prevent the formation of the hydrogenated acids. We have shown, however, that the sulfuric acid does not function in the decarboxylation as an oxidizing agent, yet the yield is quantitative. The analytical method used in the earlier work to identify the evolved gas must have been faulty, and the formation of di- and tetra-hydro acids in the reaction is therefore questioned.

EXPERIMENTAL

The radioactivity measurements were made by converting each organic sample to barium carbonate, which was mounted on a thin aluminum disc and counted with a G-M counter of the end-window type. The techniques employed are described elsewhere (4). All samples were counted long enough to reduce the statistical error to 1% or less.

Methyldihydrotrimesic acid and sodium oxalate from pyruvic acid. A 0.4802 g. portion of pyruvic acid-α-C^{14} which had been prepared[4] three weeks previously and stored in a refrigerator, was diluted with 10.124 g. of freshly distilled inactive pyruvic acid. The mixed acid (0.121 mole) was added dropwise to a solution of 22 g. (0.55 mole) of sodium hydroxide in 39 ml. of water, which was contained in an Erlenmeyer flask cooled in ice. The acid was added slowly to the solution, which was swirled to minimize local heating; the time required was about one-half hour. If local heating was excessive, or if the order of mixing was reversed, the yield was lowered. After the acid had been added, the pale yellow solution was heated 3.5 hours on a steam-bath. During this time, the color became deep orange and a precipitate of sodium oxalate appeared. The mixture was chilled and the sodium oxalate was collected on a sintered glass filter, where it was washed with three 5-ml. portions of 12 *M* sodium hydroxide. The bulk of the product was left in crude form for future use; the total amount of *oxalic acid* present was determined by permanganate titration of an aliquot to be 84% based on pyruvic acid.

To obtain a pure specimen for radioactivity assay, a portion of the crude dium oxalate was converted to barium oxalate. The barium oxalate was dissolved in 3 *M* hydrochloric acid and the solution was evaporated to dryness on a steam-bath; then the residue was transferred to a small sublimation apparatus, and the oxalic acid was recovered by vacuum sublimation. The sublimed acid was crystallized twice from water and dried in air.

Methyldihydrotrimesic acid was precipitated from the filtrate left after removal of sodium oxalate by acidification with concentrated hydrochloric acid. The solution was cooled in ice during the acidification, and the acid was added slowly enough to prevent the temperature from rising above 60° as a precaution against decomposition of the methyldihydrotrimesic acid. A total of 50 ml. of hydrochloric acid was added, and the mixture was filtered. Sodium chloride was washed out of the white solid on the filter with 25 ml. of water in 3–4 ml. portions. The yield of acid, dried in high vacuum at room temperature, was 4.97 g. (67.5%). The principal impurity in the crude product was sodium chloride; purification of a sample for radioactivity assay was accomplished by crystallization from dilute ethanol at a temperature below 60°. The purified acid absorbs atmospheric moisture to form a monohydrate; drying in high vacuum gives the anhydrous compound. The radioactivity measurement was performed on the hydrate.

The filtrate left after removal of the methyldihydrotrimesic acid was subjected to continuous ether extraction for 8 hours. Evaporation of the ether left a syrup. When this was dried in vacuum a brittle brown material was obtained, which weighed 1.60 g.; it readily absorbed moisture from the atmosphere and again became syrupy. The material was burned *in toto* to determine its activity.[6]

The water solution from the ether extraction operation was neutralized with sodium hydroxide and evaporated. The activity of the residue was determined by heating an aliquot in a combustion train until all the carbonaceous materials were burned out of the rather large amount of salt present. The carbon dioxide was recovered as barium carbonate; this is referred to in Table I as Barium Carbonate I.

Uvitic acid from methyldihydrotrimesic acid. The decarboxylation apparatus consisted of a 125-ml. conical flask bearing two ground necks. The center neck was fitted with a compensated dropping-funnel, and the side neck carried a tube to conduct gas out of the flask. The tube extended a short distance into the flask and its end, which was drawn to small diameter, was bent up to serve as a spray trap. Provision was made for introducing a current of nitrogen, purified by passage through a preburner and a carbon dioxide absorber, into the system through the mouth of the dropping-funnel. A stopcock was situated in the line just ahead of the dropping-funnel. After leaving the flask, the gas passed through an absorber containing 1 *M* sodium hydroxide, then over copper oxide heated to 550°, and finally through a second absorber containing alkali. Atmospheric carbon dioxide was excluded from the system by a guard tube attached at the outlet.

Into the flask was placed 4.74 g. of dry crude methyldihydrotrimesic acid, and 20 ml. of concentrated sulfuric acid was placed in the funnel. The apparatus was assembled and the air was swept out with nitrogen. The stopcock was closed and the acid was run into the flask from the dropping-funnel. The mixture was heated in an oil-bath to 120° and decarboxylation commenced. When the initial evolution of carbon monoxide had subsided, the stopcock was opened to admit a current (25 cc./min.) of nitrogen, and the temperature of the bath was raised to 150° where it was held for 2 hours At the end of this time, the reaction mixture was cooled somewhat and poured into 100 ml. of water; this caused the uvitic acid to separate. The mixture was allowed to become hot, for by this procedure a product was obtained which was less difficult to filter than one prepared in the cold. The

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[6] Several experiments were performed to investigate the behavior of this substance on decarboxylation. Subjection of the material to decarboxylating conditions gave, in the most favorable cases, mixtures of volatile liquid products which were largely olefinic in nature. The amounts formed were small and the mixtures were complex; consequently, no use except isotope recovery was made of the syrup.

crude, gray uvitic acid was collected on a sintered glass filter and carefully washed free of sulfate.[7] The yield of crude vacuum dried acid was 3.53 g. (100%).

To obtain a pure specimen for radioactivity assay, an aliquot was crystallized from dilute ethanol, with the use of charcoal, then crystallized again from dilute ethanol and dried under high vacuum. The *neutralization equivalent* of a specimen so purified was 90.5; calculated for anhydrous uvitic acid, 90.1.

To the alkali in each of the absorbers was added excess barium chloride solution and the precipitates were collected. The material from the first absorber weighed 0.149 g. after subtraction of the blank (3.9%). It was shown by permanganate titration to contain 6% barium sulfite; the rest was carbonate. This specimen is referred to in Table I as Barium Carbonate II.

After correction for blank, the barium carbonate obtained from the second absorber weighed 3.689 g. (96.8%). This specimen is referred to in Table I as Barium Carbonate III.

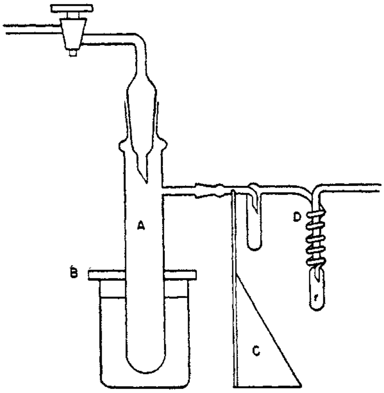

FIGURE 2. DECARBOXYLATION APPARATUS

Toluene from uvitic acid. The apparatus used for the decarboxylation of uvitic acid is shown in Fig. 2. The reaction vessel A is a 2 x 23-cm. Pyrex tube with a side arm, which terminates in a 10/30 ground joint, attached a short distance below the mouth. Provision is made for admitting purified nitrogen through a 24/40 ground joint, whose inner member has an extension which protrudes into the vessel to a point a few millimeters above the side arm. The tube is heated by a salt-bath; a Transite disc B prevents the silicone lubricant in the ground joints from becoming excessively hot. The vessel is connected to the unit D which consists of a fore-trap, whose capacity is about 5 ml., sealed to a second trap consisting of six turns of 7-mm. Pyrex tubing terminating in a 5 ml. receptacle *r*. The outlet of D is connected to a carbon dioxide absorber (not shown) containing 1 *M* sodium hydroxide. A guard tube to exclude atmospheric carbon dioxide completes the train. A Transite shield C protects D from the heat of a thermostatically controlled electric heater used to heat the salt-bath.

Vessel A was charged with 3.44 g. of dry crude uvitic acid, 0.50 g. of powdered cupric oxide, and 10 ml. of freshly distilled synthetic quinoline; it was then connected to the train. The air was swept out of the system with nitrogen, and the stopcock at the entrance to A was closed. A Dry Ice–isopropanol mixture was placed around the spiral trap and the temperature of the contents of A was raised from 150° to 265° over a period of one hour. The stopcock was then opened and a current of purified nitrogen was passed through the

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[7] Unless sulfate is completely removed, the toluene obtained by decarboxylation of the uvitic acid contains sulfur dioxide.

apparatus for 3 hours, while the salt-bath was held at 265 ± 5°. The fore-trap was not cooled; it collected some water and toluene and most of the small amount of quinoline which distilled with them. This partial removal of extraneous substances reduced difficulty from plugs of frozen material in the coils of the spiral trap.

The spiral trap was watched closely and the cooling bath was removed for a brief time, if necessary, to allow plugs to melt and fall into the receptacle. Toward the end of the reaction, toluene in the fore-trap was driven into *r* by stroking the trap with a soft flame.

When the reaction was finished, barium carbonate was precipitated from the absorber solution. The yield was 7.45 g. after correction for blank (98.5%). This specimen is referred to in Table I as Barium Carbonate IV.

The toluene was purified with the aid of the apparatus shown in Fig. 3. This consists of a high vacuum manifold to which are attached vessels D, F and G. A trap H is situated in the line. The unit D contains the crude toluene; the entrance to the fore-trap is closed by a ground glass plug. Vessel F contains about 10 g. of phosphorus pentoxide, and G is a receptacle made by sealing a 3-mm. straight bore stopcock to a 15-ml. graduated conical centrifuge tube. The stopcock and the ground joint by which the receptacle is attached to the manifold are sealed to the centrifuge tube on a lathe, to insure that all the segments are coaxial; it is then possible to insert a long capillary pipet into the tube to withdraw portions of toluene for use, and the receptacle can be used as a storage vessel.

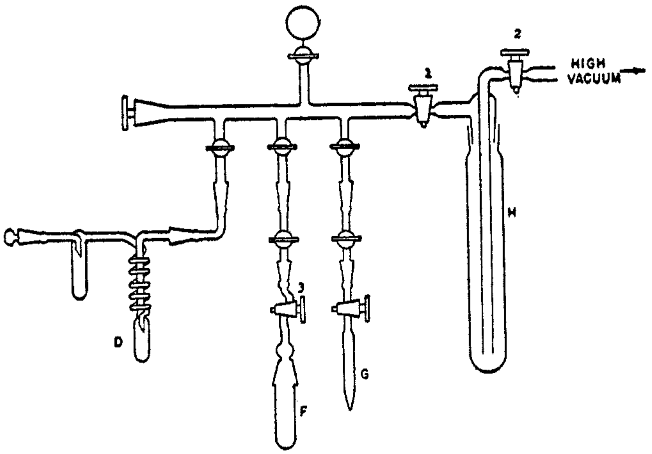

Figure 3. Apparatus for the Purification of Toluene

The contents of the spiral trap were frozen in liquid nitrogen and the pressure in the entire system reduced to 5 microns; stopcock 1 was then closed. The toluene was transferred to F by cooling that vessel with liquid nitrogen while D was allowed to warm up. When most of the toluene had distilled, D was warmed in a bath of water at 40°. A pressure of 50 microns developed during the transfer as the materials in the system released entrained gases; toward the end of the operation these gases interfered with the diffusion of toluene. To obtain the last of the toluene, 1 was opened and the system reevacuated. The residual toluene condensed in H, which was cooled in liquid nitrogen. Stopcock 2 was then closed and the toluene in H was distilled into F.

Stopcock 3 was closed and F was removed from the line. By gently shaking the vessel, the phosphorus pentoxide and toluene were distributed in a layer over the entire inside wall area except the region near the stopcock. The vessel was again attached to the manifold and the toluene was distilled into G. A soft flame was passed a few times over F to drive out the last of the toluene.

The yield was 1.610 g.; 91.5% based on uvitic acid, or 62% based on pyruvic acid. The purity of the product was investigated with a sample prepared in a pilot run with inactive pyruvic acid. The boiling range was 110.6–111.1°; n_D^{22} 1.4978. Mass spectrometric analysis[8] showed the total amount of impurities in the range of mass numbers 0 to 200 to be about 0.2%. Regions scanned with particular attention were those corresponding to benzene, xylene, methylcyclohexane and methylcyclohexene. The height of the toluene peak corresponded to 100.2 ± 0.2% toluene.

SUMMARY

1. A synthesis of toluene labeled with C^{14} in the 1, 3, and 5 positions of the ring is described. Labeled oxalic acid is obtained as a second product. The starting material is pyruvic acid-α-C^{14}; the yields of toluene and of sodium oxalate are 62% and 84%, respectively.

2. Certain aspects of the mechanisms of the reactions involved have been checked by tracer technique, with results consistent with concepts expressed in the literature.

3. The decarboxylation of methyldihydrotrimesic acid has been found to proceed by loss of the carboxyl carbon atom as carbon monoxide, not carbon dioxide. The formation of di- and tetra-hydrouvitic acids in this reaction is therefore questioned.

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(3) Wolff, *Ann.*, **305**, 129 (1899).
(4) Dauben, Reid, and Yankwich, *Anal. Chem.*, **19**, 828 (1947).

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[8] We wish to thank Dr. N. Bauer and the California Research Corporation for the mass spectrometric analysis.