

DETERMINATION OF TRITIUM OR DEUTERIUM IN THE METHYL GROUP OF
LABELED ORGANIC COMPOUNDS

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SUMMARY

The determination of the isotopic content of C-methyl groups in selected tritium and deuterium labeled compounds by modification of the Kuhn-Roth method is presented. Oxidation of the deuterated or tritiated compound using a solution of sodium dichromate and sulfuric acid at 50-85°C allows isotopic assay of the C-methyl group as acetic acid.

Key Words: C-Methyl Group, Tritium, Deuterium, Oxidation

INTRODUCTION

The determination of carbon-14 in the methyl group of organic compounds-¹⁴C by a modified Kuhn-Roth method has been described in a previous paper and found to be isotopically reliable⁽¹⁾. This paper describes an extension of the method to determine the deuterium or tritium content in the methyl group of a homologous series of labeled fatty acids. Further, the present results will at least serve to indicate a greatly broadened range of applications with other classes of compounds containing isotopic hydrogen C-methyl groups.

A solution of sodium dichromate and sulfuric acid is employed to convert tritiated or deuterated compounds to acetic acid. The acid produced is isolated by steam distillation and high vacuum techniques. Finally, the purified acetic acid is assayed for isotopic hydrogen.

Oxidation of tritiated or deuterated compounds with sodium

dichromate and sulfuric acid at temperatures ranging from 120-150°C causes a loss of isotopic hydrogen from the methyl group. This has been shown to be largely due to partial oxidation to an intermediate compound containing a labeled acetyl group attached to a carbon containing group or to a carbon chain. This intermediate compound undergoes an exchange reaction with the "active" hydrogen atoms in the reaction mixture before being oxidized to acetic acid. This results in a loss of tritium or deuterium from the methyl group during the oxidation at 120-150°C.

It has been found that by lowering the reaction temperature to 50-75°C the loss of tritium or deuterium from the labeled methyl groups in organic compounds can be reduced to values below 4%. It is recommended that these conditions (Table IV) be used for the determination of tritium or deuterium in the methyl group of compounds labeled with isotopic hydrogen.

RESULTS AND DISCUSSION

Low molecular weight fatty acids and 1-hexene containing tritium or deuterium have been oxidized to acetic acid at 120-150°C using 80-90 minute reaction times. It can be seen under these conditions, which were used with carbon-14 labeled compounds, that some isotopic hydrogen is lost from the methyl group during the oxidation process. This appears to be largely due to partial oxidation of the isotopic starting material to an intermediate containing a labeled acetyl group attached to a carbon containing group or to a carbon chain. The results are given in Table I.

Because of the loss of isotopic hydrogen from the methyl group during the oxidation of the labeled compounds at 120-150°C, a study involving partial oxidation of n-valeric acid-5-³H was undertaken. In this experiment the starting material was partially oxidized by limiting the amount of oxidizing agent at 120-150°C using 80-90 minute reaction times.

This experiment shows that the loss of tritium from the methyl

Table I. Oxidation of tritiated and deuterated compounds to acetic acid at 120-150°C.

Starting Material	$\mu\text{Ci. of } ^3\text{H/mmole compd.}$	$\mu\text{Ci. of } ^3\text{H/mmole acetic acid}$	Percent Loss of ^3H or ^2H from methyl of acetic acid
Acetic Acid-2- ^3H	5.90	5.70	3.3
Propionic Acid-3- ^3H	15.90	15.10	5.0
n-Butyric Acid-4- ^3H	6.86	6.33	7.7
n-Valeric Acid-5- ^3H	3.25	2.47	24.0
1-Hexene-6- ^3H	11.46	7.50	34.5
Propionic Acid-3- $^2\text{H}_3$			20.0 (^2H)
n-Butyric Acid-2,3,4- $^2\text{H}_7$			21.0 (^2H)

group occurs in the step involving oxidation of the propionic acid-3- ^3H to acetic acid-2- ^3H . The loss of isotopic hydrogen from the methyl group might occur via other intermediates when other isotopic starting materials are employed. The results are given in Table II.

Table II. Partial oxidation of n-valeric acid-5- ^3H .

Starting Material	Compound isolated and assayed after oxidation	Percent loss of ^3H from the methyl group of the compd.
n-Valeric Acid-5- ^3H	Propionic Acid-3- ^3H	0.5
	Acetic Acid-2- ^3H	23.7

If an intermediate is formed during the oxidation containing a labeled acetyl group attached to a carbon containing group or to a carbon chain, then an exchange reaction would provide a partial explanation for the loss of isotopic hydrogen in the acetic acid. Accordingly, non-radioactive fatty acids were oxidized separately in either a tritiated or deuterated medium at 120-150°C using 80-180 minute reaction times. The acetic acid was then isolated, purified, and analyzed for isotopic hydrogen. The results are given in Table III.

Table III. Exchange reaction during oxidation of compounds to acetic acid at 120-150°C.

Starting Material	Percent incorporation of tritium or deuterium from the medium into the methyl group of acetic acid
Acetic Acid	1.5 (³ H)
Propionic Acid	2.4 (³ H)
n-Butyric Acid	8.7 (³ H)
n-Valeric Acid	22.7 (³ H)
n-Valeric Acid	30.9 (² H)
n-Hexanoic Acid	56.9 (² H)
n-Heptanoic Acid	12.9 (² H)

The preceding experimental results indicated that an exchange reaction occurs during the oxidation of the lower fatty acids to acetic acid at 120-150°C using 80-180 minute reaction times. To minimize the exchange reaction, lower reaction temperatures, 50-85°C, and longer reaction times, 90-360 minutes, have been employed. Under these conditions, acceptable results have been obtained for the oxidation of the lower fatty acids and 1-hexene. In addition, these conditions will probably be satisfactory for the C-methyl analysis of other tritium or deuterium labeled compounds. The data are presented in Table IV.

From the experimental results, it can be seen that the determination of tritium or deuterium in the methyl group of organic compounds must be carried out under carefully controlled conditions. Inspection of Table IV indicates that acceptable isotopic results can be obtained by carrying out the oxidation at temperatures ranging from 50 to 75°C using reaction times from 90 to 360 minutes. Finally, the amount of tritium or deuterium that will be lost from the methyl group of acetic acid by oxidation of compounds containing either tritiated or deuterated methyl groups

Table IV. Oxidation of tritiated and deuterated compounds to acetic acid at 50-85°C.

Starting Material	Reaction temperature °C	Reaction time min	Percent Loss of tritium or deuterium from methyl group of acetic acid
Propionic Acid-3- ³ H	65-75	180	0.8
n-Butyric Acid-4- ³ H	65-75	150	2.8
n-Valeric Acid-5- ³ H	65-75	150	2.3
1-Hexene-6- ³ H	75-85	150	12.3
1-Hexene-6- ³ H	50	150	3.8
Propionic Acid-3- ² H	65-75	360	2.4
n-Butyric Acid-2,3,4- ² H ₇	65-75	180	3.0
Acetic Acid-2- ² H ₃	65-75	90	0.0

can be estimated by oxidizing the non-radioactive compound in a tritiated or deuterated medium and analyzing the resulting acetic acid for isotopic hydrogen.

EXPERIMENTAL

Oxidation of tritiated or deuterated fatty acids to acetic acid.

The procedure used to convert tritiated or deuterated compounds to acetic acid-2-³H or acetic acid-2-²H has been described⁽¹⁾. The tritium labeled compounds were assayed by liquid scintillation counting using the Bush Channels Ratio Method to correct for quenching⁽²⁾. The deuterium labeled compounds were analyzed by mass spectrometry using a Perkin-Elmer CH-3 instrument.

General procedure for isotopic C-methyl determination.

The number of millimoles of sodium dichromate employed is equal to the number of carbon atoms oxidized to carbon dioxide in the compound times the millimoles of compound oxidized times 1.5. The

millimoles of water used is equal to the number of carbon atoms oxidized to carbon dioxide in the compound times the millimoles of compound oxidized. Generally, the milliliters of concentrated sulfuric acid employed is equal to the number of carbon atoms oxidized to carbon dioxide in the compound times the millimoles of compound oxidized times 1.3. Sometimes the amount of sulfuric acid is varied so that the dichromate does not precipitate from solution. The number of carbon atoms oxidized to carbon dioxide is equal to the number of carbon atoms in the compound minus the number of carbon atoms in the acetic acid produced on oxidation. The purity of the acetic acid from all oxidations was established by gas chromatographic analysis. Also, in some experiments, preparative gas chromatographic techniques and fraction collection were employed to obtain acetic and other acids.

REFERENCES

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