

Determination of carbon-14 and/or tritium in the methyl group of organic compounds. Isotopic C-methyl analysis *

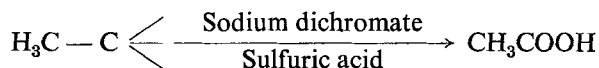
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SUMMARY

The determination of the millimolar radioactivity of C-methyl groups in selected carbon-14 and tritium labelled compounds by modification of the Kuhn-Roth method is presented. Oxidation of the radioactive sample in an open vessel using a solution of sodium dichromate and sulfuric acid allows radioassay of the C-methyl group as acetic acid. This procedure has been tested with a representative group of labelled compounds and found to be isotopically reliable.

This paper describes the development of an isotopically reliable procedure for the determination of the millimolar radioactivity of C-methyl groups in a variety of carbon-14 and tritium labelled compounds. A solution of sodium dichromate and sulfuric acid is employed to convert suitable radioactive compounds to acetic acid.



The acid produced is isolated by steam distillation and high vacuum line techniques. Then the purity of the acetic acid is established by gas chromatography before radioactive measurement.

Radioassay of the acetic acid from the oxidation of tritiated compounds gives the concentration of tritium in the methyl group directly. However, acetic acid, from the breakdown of carbon-14 labelled compounds, may be

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inactive or labelled in the 1-,2-, or the 1,2-positions. Therefore, carbon-14 labelled acetic acid samples require further degradation to determine the concentration of carbon-14 in the methyl group and in the carboxyl group. Two simple and satisfactory procedures can be used for this purpose^(1, 2). Excellent results have been obtained with propionic acid-1-¹⁴C, propionic acid-2-¹⁴C, propionic acid-3-¹⁴C, uniformly labelled palmitic acid-¹⁴C, acetic acid-2-¹⁴C, acetic acid-2-³H, propionic acid-2,3-³H, 1-hexene-³H, and 1-octene-1,8-¹⁴C.

The micro method commonly employed for the analysis of the C-methyl function is carried out in the following way. A 5-12 milligram sample of organic compound is heated with 1 ml of concentrated sulfuric acid and 4 ml of 5 N chromic acid in a sealed tube^(3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13). Next, the reaction mixture is cooled and treated with hydrazine hydrate to remove the excess of chromic acid and partially neutralized with sodium hydroxide solution. Then after the addition of phosphoric acid the acetic acid is obtained by steam distillation of the reaction mixture. Finally, the amount of acetic acid in the distillate is found by titration with 0.01 N sodium hydroxide to the phenolphthalein endpoint.

Modification of the micro procedure for work with 200-800 milligram samples of carbon-14 and/or tritium labelled compounds will now be described. The radioactive sample is heated with a solution prepared from sodium dichromate and sulfuric acid in either a 100-ml or a 200-ml round-bottomed flask sealed to a small Liebig condenser. Complete oxidation of C-methyl compounds to acetic acid by the chromic acid-sulfuric acid mixture does not take place in the open reaction vessel. After oxidation of the labelled compound and cooling, a saturated solution of sodium sulfate is added to the reaction mixture before isolating the acetic acid by steam distillation. Neutralization of the acid with sodium hydroxide followed by removal of the water furnishes sodium acetate which is converted to acetic acid by high vacuum line techniques. Then the purity of the acetic acid is determined by gas chromatographic analysis before radioassay.

By the modified procedure C-methyl compounds are oxidized to acetic acid in yields ranging from 50 to 80 % with no observable isotope effect. Presumably, over-oxidation occurs and destroys some of the acetic acid produced. However, this is not a serious problem because the millimolar radioactivity is found by radioassay of a small sample of radiochemically pure acetic acid. Results are given in Table I.

EXPERIMENTAL.

Oxidation of propionic acid-3-¹⁴C to acetic acid-2-¹⁴C.

In a 100-ml round-bottomed flask sealed to a Liebig condenser was added 22 mg (3 millimoles) of propionic acid-3-¹⁴C. Next, the oxidation

TABLE I. Oxidation of radioactive compounds to acetic acid

Starting material	Microcuries of ^{14}C or ^3H per millimole of compound	Microcuries of ^{14}C or ^3H per millimole of acetic acid	
		Calculated	Found
Propionic acid-1- ^{14}C	3.02	0.00	0.01
Propionic acid-2- ^{14}C	3.51	3.51	3.44
Propionic acid-3- ^{14}C	0.75	0.75	0.74
Acetic acid-2- ^{14}C	2.63	2.63	2.55
1-Octene-1,8- ^{14}C	0.97	0.48	0.49
Palmitic acid-UL- ^{14}C ^a	1.31	0.16	0.06
Acetic acid-2- ^3H	6.26	6.26	6.04
Propionic Acid-2,3- ^3H ^b	9.60	6.70	6.60
1-Hexene- ^3H ^c	11.80	2.95	2.20

^a The palmitic acid-UL- ^{14}C was obtained from *Chlorella* grown in $^{14}\text{CO}_2$. No explanation can be given for the lowered millimolar radioactivity in positions 15 and 16. However, degradation of the acetic acid- ^{14}C produced, showed that the 1- and 2-positions were equally labelled.

^b The propionic acid-2,3- ^3H purchased from the Radiochemical Centre, Amersham, England was prepared by catalytic tritiation of ethyl acrylate followed by hydrolysis. A sample of this acid was converted to methyl propionate which was equilibrated with sodium methoxide in methanol as described by COPE and GALE (14). By this procedure it was found that 30 % of the tritium was in the 2-position and 70 % in the 3-position of the tritiated propionic acid.

^c The 1-hexene- ^3H was prepared by isomerization of tri-*n*-hexylborane- ^3H (followed by displacement. The possibility exists that complete scattering of the tritium did not occur under the experimental conditions employed.

mixture was prepared by adding 5 ml of concentrated sulfuric acid dropwise to a cooled solution of 1.34 g (4.5 millimoles) of sodium dichromate in 3 ml of water contained in a 25-ml Erlenmeyer flask. If a solution was not obtained a minimum amount of water was added to dissolve the dichromate. Then the oxidation mixture was carefully added to the propionic acid-3- ^{14}C . After the exothermic reaction subsided, the solution was refluxed for 90 minutes. Next, the reaction mixture was cooled to room temperature and transferred to a steam distillation apparatus by washing with 30 ml of a saturated sodium sulfate solution. The steam distillation was carried out and the distillate collected in a 500-ml three-necked flask equipped with a magnetic stirring bar, a buret, and a reflux condenser. As the distillation proceeded the acid in the distillate was titrated with 0.1 N sodium hydroxide solution using phenolphthalein as the indicator. After collection of 400 ml of distillate, no more sodium hydroxide was consumed. The yield of acetic acid-2- ^{14}C based on titration was 80 %.

Conversion of sodium acetate-2-¹⁴C to acetic acid-2-¹⁴C.

The sodium acetate-2-¹⁴C solution obtained by titration was transferred to a 500-ml round-bottomed flask fitted with a 2.5 × 10 cm tip at the bottom. After attaching the flask horizontally to a rotary evaporator, the water was removed by heating *in vacuo*. Then the flask was connected to a U-trap equipped with a side-arm and the entire assembly attached to a high vacuum line. The sodium acetate-2-¹⁴C was dried at 90° C and 10⁻⁴ mmHg for 2 hours. Next, hydrogen chloride was prepared in the high vacuum line by the addition of 15 ml of concentrated sulfuric acid to 585 mg (10 millimoles) of sodium chloride. The gas was dried by distilling the material at 10⁻⁴ mmHg through a trap at -80° C to a trap cooled by liquid nitrogen. Then the hydrogen chloride was vacuum distilled into the flask containing the sodium acetate-2-¹⁴C. After the transfer was complete, the U-trap and the flask were isolated from the high vacuum line, the liquid nitrogen coolant removed from the condensed hydrogen chloride, and the reaction allowed to proceed for two hours at room temperature. The tip of the large reaction vessel was cooled with liquid nitrogen to condense the acetic acid-2-¹⁴C and hydrogen chloride before evacuating the system to 10⁻⁴ mmHg. Then the excess hydrogen chloride was removed by cooling the U-trap at -80° C, one trap on the high vacuum line with liquid nitrogen, and warming the tip of the large reaction vessel to room temperature. The hydrogen chloride was collected in the trap cooled by liquid nitrogen and the acetic acid-2-¹⁴C in the trap cooled at -80° C. Next, the U-trap and reaction flask were isolated from the high vacuum line, the U-trap warmed to 60° C, and the acetic acid-2-¹⁴C collected by cooling the tube connected to the U-trap with liquid nitrogen. Finally, the tube containing the acetic acid-2-¹⁴C was removed from the apparatus.

Establishment of the purity of the acetic acid-2-¹⁴C by gas chromatography.

The purity of the acetic acid-2-¹⁴C was determined by gas chromatography using an Aerograph Autoprep A-700 under the following conditions : column packing, 20 % Carbowax 20M-terephthalic acid on 60/80 mesh Chromosorb W coated with dimethyldichlorosilane and acid-washed; column length, 10 feet; column diameter, 3/8 inch; temperature, 190° C; carrier gas, helium; flow rate, 90 ml/minute; and thermal conductivity record, one peak at 5.5 minutes (acetic acid).

Radioactivity measurements.

The carbon-14 and tritium labelled compounds were assayed by liquid scintillation counting using the Bush Channels Ratio method ⁽¹⁵⁾ to correct for quenching.

General procedure for isotopic C-methyl determination.

For each carbon atom in the organic compound being oxidized 1.5 millimoles of sodium dichromate and 1 ml of water were employed. Different amounts of concentrated sulfuric acid were used so that the dichromate did not precipitate from the oxidizing solution. Representative examples are given in Table II. The number of carbon atoms oxidized is equal to the number of carbon atoms in the organic compound minus the number of C-methyl groups minus the number of carboxyl groups. The purity of the acetic acid obtained from all oxidations was established by gas chromatographic analysis.

TABLE II

Compound	Number of carbon atom oxidized	Millimoles of compound oxidized	Millimoles of sodium dichromate	Milliliters of water	Milliliters of concentrated sulfuric acid
Propionic acid-3- ¹⁴ C	1	3.0	4.5	3.0	5.0
Palmitic acid-UL- ¹⁴ C . . .	14	3.1	65.1	44.0	55.0
1-Hexene-UL- ³ H . . .	5	3.5	26.2	17.5	19.0
1-Octene-1,8- ¹⁴ C . . .	7	3.5	36.7	24.5	30.0
Propionic acid-2,3- ³ H	1	3.0	4.5	3.0	5.0

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