[CONTRIBUTION FROM THE RADIOISOTOPE LABORATORY AND THE VENABLE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

The Isotope Effect. III. The Reaction of Acetone-1-C^{14} with Alkaline Hypoiodite[1]

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A small but real isotope effect was found when acetone-1-C^{14} was treated with alkaline hypoiodite; the iodoform contained more than half and the acetate less than half of the activity of the original acetone. The effect is in the opposite direction from most of the other isotope effects so far reported, the C^{12}–C^{14} bond apparently being more easily broken than the C^{12}–C^{12} in this case. The magnitude of the effect is of the order of 3 to 6%.

The first report of an isotope effect involving carbon-14 was that of Yankwich and Calvin,[3] who reported that the carbon dioxide evolved in the decarboxylation of malonic-1-C^{14} acid was poorer in carbon-14 than the residual acetic acid; they reported a 12% isotope effect. This work has been repeated by Roe and Hellmann,[4] who report a 6% effect for the same reaction. Reports of several other isotope effects involving carbon-14 have appeared in the literature.[5–12] Inasmuch as carbon-14 is being extensively used as a tracer in chemical and biological work, it seems important to study the extent and size of the isotope effect, and to see if the effect is large enough to invalidate or make questionable the use of the isotope as a tracer element.

The reaction of acetone with alkaline hypoiodite is often used to degrade labeled acetone in biological tracer work; it was therefore of interest to determine if there is an isotope effect involved in this reaction. It was found that when acetone-1-C^{14} was treated with alkaline hypoiodite, the iodoform produced had more than half and the residual acetate had less than half of the activity originally present in the acetone. The results are shown in Table I. The isotope effect is expressed as a ratio of the activity of the iodoform to the activity of the acetate; thus the isotope effect in run No. 1 would be 6%. This approximates as closely as possible the method of calculation used by other workers[3,8,11] who were dealing with reactions of different types.

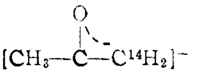
Activity assays were carried out by oxidizing the products to carbon dioxide, which was put into an ionization chamber, the current of which was measured by means of a Vibrating Reed Electrometer.

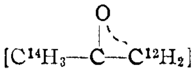
TABLE I[a]

Run	CHI_3	NaOAc	Total act.	Acetone	Isotope effect
		A. Wet combustion of samples			
1	28.5 ± 0.2	27.0 ± 0.3[b]	55.5	55.5 ± 0.7	1.06 ± 0.01
2	28.6 ± 0.1	26.9 (one[b] anal.)	55.5	55.5 ± 0.7	1.06
3	28.6 ± 0.1	27.0 ± 0.0[c]	55.6	55.5 ± 0.7	1.06 ± 0.005
4	28.4 ± 0.2	27.2 ± 0.4[c]	55.6	55.5 ± 0.7	1.04 ± 0.02
		B. Dry combustion of samples			
4	29.2 ± 0.1	....	..	57.4 ± 0.2	1.03 ± 0.01[f]
5	29.3 ± 0.1	28.4 ± 0.2[d]	57.7	57.4 ± .2	1.03 ± .01
6	28.8 ± 0.2	27.9 ± 0.3[e]	56.7	56.7 ± .3	1.03 ± .02

[a] Activities expressed as millivolts per second per millimole. [b] Acetate as benzylisothiuronium acetate. [c] Acetate as iodoform. [d] Acetate as acetone 2,4-dinitrophenylhydrazone. [e] Acetate as benzylisothiuronium acetate. [f] Calculated by assuming the acetate activity would be as far below the half-activity of the acetone as iodoform activity is above it.

All previously published reports of isotope effects, with the exception of the study of the enzymatic hydrolysis of labeled urea by Daniels and Meyerson,[6] indicate that the C^{12}–C^{14} bond has greater stability than the C^{12}–C^{12} bond. In the present study of the reaction of acetone-1-C^{14} with hypoiodite, rupture of the C^{12}–C^{14} bond occurs preferentially. Since the rate determining step in the basic iodination of acetone is the removal of a proton from the methyl group, it is concluded that the carbanion formed by proton loss from the C^{14}-methyl group (1) is more stable than that involving loss of a proton from the C^{12}-methyl group (2).


$$[CH_3{-}\overset{O}{C}{-}C^{14}H_2]^-$$
(1)


$$[C^{14}H_3{-}\overset{O}{C}{-}C^{12}H_2]^-$$
(2)

The inference is drawn that the C^{12}–C^{14} bond because of its greater stability favors the formation of carbanion 1 over carbanion 2. One would expect that the C^{14}–H bond would be more stable than the C^{12}–H bond; analogy with the carbon–carbon bonds would lead to this conclusion, although there seems to be no experimental evidence on this point. The facts disclosed by this investigation do not bear out this analogy; either the C^{14}–H bond is less stable than the C^{12}–H bond, or the greater stability of carbanion 1 over carbanion 2 is a more important factor in this reaction than the C–H bond strength. Whatever the reason, once carbanion 1 has formed, iodination will proceed and cleavage will occur resulting in iodoform of enriched activity.

The acetone used was prepared by the following series of reactions: runs were made on a 20 millimolar scale, over-all yields from methanol ranging from 60–75%.

$$C^{14}H_3OH \longrightarrow C^{14}H_3I \longrightarrow C^{14}H_3MgI \xrightarrow{CH_3CHO} C^{14}H_3{-}CHOH{-}CH_3 \xrightarrow{(O)} C^{14}H_3{-}CO{-}CH_3$$

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[2] National Research Council Fellow, 1949–1951.

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[6] F. Daniels and A. Meyerson, *Science*, **108**, 676 (1948).

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[12] D. R. Stranks and G. M. Harris, *J. Chem. Phys.*, **19**, 257 (1951).

Further studies of the reactions of acetone-1-C^{14} involving proton removal are being studied in this Laboratory in an attempt to shed more light on this matter.

Experimental

Preparation of Acetone-1-C^{14}.—Several runs were made, all on a 20-millimolar scale. Methanol-1-C^{14} was converted to methyl-C^{14} iodide by the method of Tolbert.[13] The product was distilled into a flask containing 15 ml. of ether and some phosphorus pentoxide; after standing, the ether-methyl iodide mixture was distilled into a flask containing 20 mmoles of magnesium and a magnetic stirrer. All these operations were carried out on a vacuum line. The flask was now removed from the vacuum line and attached to a condenser with a side-arm dropping funnel; the magnetic stirrer was activated, which initiated formation of the Grignard. When Grignard formation was complete, a solution of 25 mmoles acetaldehyde in 5 ml. of ether was added slowly (10 minutes). After stirring for one hour, the ether and excess acetaldehyde were removed in the vacuum line. Hydrolysis of the reaction mixture was effected by distilling 10 ml. of water into the reaction flask and stirring for 10 minutes. The mixture of water and 2-propanol-1-C^{14} was then distilled from the residual material and transferred to a 100-ml. volumetric flask. A solution of 20 ml. of 2 *M* H_2CrO_4 and 10 ml. of 2 *M* $HClO_4$ was added, together with sufficient water to bring the volume to 100 ml. The flask was then placed in a constant temperature bath at 40° for 48 hours.[14] The yield of acetone-1-C^{14} was determined by removal of an aliquot and precipitation of the acetone as the 2,4-dinitrophenylhydrazone. Over-all yields in preliminary work ranged from 60 to 75%.

In the preparation of radioactive acetone, 0.8 ml. of methanol-C^{14} (having an activity of about 240 mv./sec./mmole) produced 0.66 ml. of acetone having an activity of 240 mv./sec./mmole. This was diluted with 3.00 ml. of C.P. acetone. Activity of the samples used in the experiments is shown in Table I.

The Iodoform Reaction.—An aliquot portion of the solution containing 2–5 mmoles of the labeled acetone was removed by pipet from the oxidizing solution and distilled on the vacuum line. The distillate was then made slightly basic and redistilled. The acetone solution was transferred to a 250-ml. erlenmeyer flask containing 50 ml. of 2 *N* NaOH and 70 ml. of a solution of iodine in potassium iodide (2.5 g. of iodine, 4 g. of potassium iodide per 100 ml.) was added in 15-ml. portions with shaking. At the end of the addition the flask was shaken vigorously for several minutes and allowed to stand for 10 minutes. The iodoform was filtered, dried and weighed to determine the yield, then twice recrystallized from ethanol for activity measurements. Yields of iodoform averaged 85%.

The filtrate containing sodium acetate-2-C^{14} was evaporated to 100 ml., acidified with sulfuric acid and treated with sodium arsenite and silver sulfate to remove iodine and iodide ion. After filtration of the silver iodide, the solution was steam distilled.

Activity Analysis.—All activities were measured by combustion of the sample to carbon dioxide, transferring the gas to an ionization chamber, and measuring the activity by means of a Vibrating Reed Electrometer (Model 30, Applied Physics Corporation, Pasadena, California). An ionization chamber containing a small amount of radioactive polystyrene was used as a standard to correct for small daily variations in the reed.

The iodoform was burned either using the Van Slyke[15] wet combustion method in the apparatus of Neville,[16] or using the standard dry combustion method. Both produced consistent analyses, although the activity figures in the dry method were 2% higher than the others.

Activities of the acetone preparations were determined by wet and dry combustion of the 2,4-dinitrophenylhydrazone, recrystallized 2–4 times from 95% ethanol. Again, wet combustion figures were 2% lower than those from dry combustion, and the results were not quite so consistent.

Four methods were used for analysis of the acetate solution. In each, the distillate from the iodoform reaction, obtained as described above, was titrated with either sodium or lithium hydroxide (carbonate free).

Method A.—Aliquot portions of the standard acetate solution were evaporated to dryness in 25-ml. flasks and converted to carbon dioxide by wet oxidation. Results were inconsistent, and 3–4% lower than results from the other three methods.

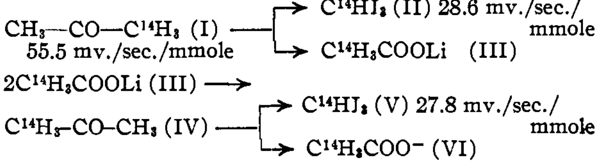
Method B.—A portion of the solution of standard acetate was evaporated to 5 ml. and treated with an equimolar amount of S-benzylthiuronium chloride in 10 ml. of 95% ethanol. The resulting acetate was purified by recrystallization from ethyl acetate; the solid was assayed by both wet and dry methods.

Method C.—The standard solution of lithium acetate was evaporated to dryness in a platinum boat and converted to acetone by pyrolysis at 490° for 20 minutes[17]; the evolved acetone carried by a stream of nitrogen was collected in a liquid air trap. The acetone was converted to the 2,4-dinitrophenylhydrazone, purified and assayed.

Method D.—The acetone produced as in method C was converted to iodoform by reaction with hypoiodite, the activity of the iodoform measured, and the activity of the original acetate calculated from this value.

The use of methods C and D requires the assumption that there is no isotope effect in the pyrolysis of lithium acetate-2-C^{14}; this assumption seems justified, and the yields of acetone were over 80%. Side reactions resulting in the conversion of the methyl carbon of lithium acetate-2-C^{14} to lithium carbonate (which would simulate an isotope effect) were shown to be negligible in this instance; only 2% of the activity originally present in the lithium acetate-2-C^{14} was found in the lithium carbonate.

Calculation of the original acetate activity by method D is illustrated by



$$\underset{55.5\ \text{mv./sec./mmole}}{CH_3{-}CO{-}C^{14}H_3\ (I)} \longrightarrow \begin{cases} C^{14}HI_3\ (II)\ 28.6\ \text{mv./sec./mmole} \\ C^{14}H_3COOLi\ \ (III) \end{cases}$$

$$2C^{14}H_3COOLi\ (III) \longrightarrow$$

$$C^{14}H_3{-}CO{-}CH_3\ (IV) \longrightarrow \begin{cases} C^{14}HJ_3\ (V)\ 27.8\ \text{mv./sec./mmole} \\ C^{14}H_3COO^-\ (VI) \end{cases}$$

The analysis of V was 27.8 mv./sec./mmole; due to enrichment in the second iodoform reaction, V could have arisen only from acetone having an activity of 54.0 mv./sec./mmole (IV). The activity of acetate (III) is then calculated as $^1/_2$ of 54 mv./sec./mmole or 27.0 mv./sec./mmole.

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