[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF OAK RIDGE NATIONAL LABORATORY]

An Isotope Effect during Ozonization[1]

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When stilbene-α-C^{14} was ozonized fractionally in the presence of 2,4-dinitrophenylhydrazine sulfate, a series of samples of radioactive benzaldehyde 2,4-dinitrophenylhydrazone was obtained representing differentially increasing stages of completion of ozonization. Assay of these showed a diminished C^{14}-content in samples obtained at the outset of ozonization and an augmented C^{14}-content in samples obtained from the latter stages. The results indicate a normal isotope effect of 4.2%, occurring during initial molozonide formation,

Since ozonization of olefinic systems enjoys widespread use in chemical and biochemical degradation studies, and further, since carbon isotope effects have not been observed during addition reactions to carbon-labeled olefinic bonds[2a,b] it seemed desirable to establish whether or not the ozonization reaction was subject to the carbon isotope effect.

Stilbene-α-C^{14} was ozonized fractionally on a semi-micro scale in alcoholic 2,4-dinitrophenylhydrazine sulfate solution. Under such conditions the ozonide is decomposed as soon as formed, resulting in benzaldehyde 2,4-dinitrophenylhydrazone. By conducting the ozonization for short periods of time, filtering the solid derivative, then continuing ozonization on the filtrate, a series of fractions was obtained representing progressive increments of ozonization of the total quantity of stilbene-α-C^{14} employed. The resulting fractions of benzaldehyde 2,4-dinitrophenylhydrazone were purified by recrystallization, then assayed for C^{14}-content. The results of these experiments are shown in Table I.

TABLE I

FRACTIONAL OZONIZATION OF STILBENE-α-C^{14}

Assay of starting stilbene-α-C^{14}: 0.977; divided by 2 0.489 mc./mole. Assay of benzaldehyde 2,4-DNPH from fully ozonized stilbene-α-C^{14}: 0.491 mc./mole.

Fraction	2,4-DNPH of benzaldehyde wt., g.	% of total	Radioactive assay, mc./mole
1	0.2424	8.26	0.4695 ± 0.0015
2	.2206	7.52	.4700
3	.2281	7.77	.4775 ± .0015
4	.2516	8.57	.4765 ± .0005
5	.2284	7.78	.4720
6	.2148	7.32	.4800 ± .0010
7	.2156	7.35	.4820 ± .0030
8	.2496	8.50	.4835 ± .0000
9	.2801	9.54	.4825 ± .0005
10	.3023	10.30	.4925 ± .0005
11	.2491	8.49	.4960 ± .0000
12	.2530	8.62	.5060 ± .0000

The gradual upward trend in the assays of the twelve fractions make it obvious that an isotope effect attends the ozonization of stilbene-α-C^{14}. The radioassay data in Table I are shown graphically in Fig. 1. Comparison of the assay of fraction 1 with the assay (0.490 mc./mole average) of starting stilbene-α-C^{14} or benzaldehyde 2,4-dinitrophenylhydrazone from fully ozonized stilbene-α-C^{14} suggests that k^{14}/k^{12} is 0.958 or that the ozonization reaction here shows a normal isotope effect of about 4%.

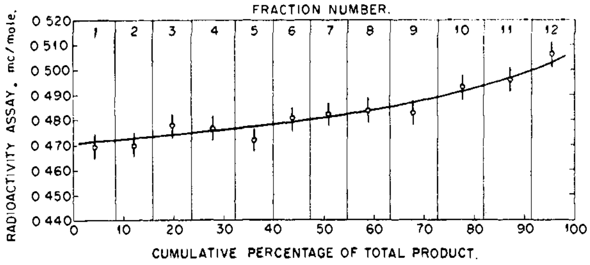
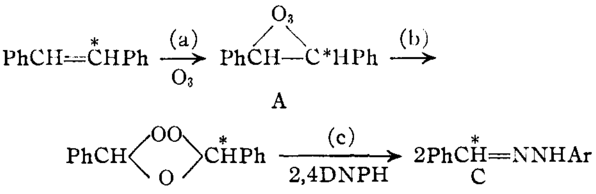


Fig. 1.—Ozonization of stilbene-α C^{14}.

Ozonization of olefins is known[3] to take place ordinarily in two steps, (a) addition of ozone to the double bond to form the molozonide and (b) rearrangement of this adduct to the isozonide. In the present reaction, the isozonide is presumably decomposed by the 2,4-dinitrophenylhydrazine reagent forming the aldehyde derivative



In principle, an isotope effect might occur in any or all of the stages (a), (b) and (c). Because of our experimental conditions, however, it is possible to state that the observed effect occurs in all probability in stage (a), the initial addition reaction forming the molozonide. Since 2,4-dinitrophenylhydrazine is present in excess, all B present at any period during reaction is converted to C, so no isotope effect involving stage (c) would be apparent. Since the ozonization was conducted in twelve discrete steps, an isotope effect due only to stage (b) would similarly not be apparent. For if A were produced without an isotope effect, interruption of the ozonization and removal of C formed *via* B, would result in a series of fractions of identical radioactive assay. Thus, if each step in the entire series of reactions is irreversible, stage (a) must be responsible for the present isotope effect.

Experimental Part

Stilbene-α-C^{14}.—This material was prepared by the Wagner rearrangement dehydration of 2,2-diphenylethanol-α-

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[1] This document is based upon work performed under Contract Number W-7405-eng-26 for the Atomic Energy Commission at the Oak Ridge National Laboratory.

[2] (a) G. A. Ropp, *Nucleonics*, **10**, 22 (1952); (b) G. A. Ropp, V. F. Raaen and A. J. Weinberger, THIS JOURNAL, in press.

[3] L. Long, *Chem. Revs.*, **27**, 437 (1940).

C^{14} in the general manner described[4] by Burr and Ciereszko. The material had m.p. 125–126° and showed a radioactive assay of 0.977 ± 0.003 mc./mole.

Ozonization of Stilbene.—Stilbene (0.200 g.) was dissolved in ethanol (8 ml.) and treated with a hot solution of 2,4-dinitrophenylhydrazine (0.7 g.) and sulfuric acid (3 ml.) in ethanol (10 ml.). The mixture was diluted with ethanol (10 ml.), cooled to room temperature ozonized in a semimicro ozonizer[5] after seeding with a crystal of benzaldehyde 2,4-dinitrophenylhydrazone. Formation of the solid derivative was almost instantaneous, though in the absence of seed precipitation did not begin for about five minutes. Ozonization was continued for 25 minutes, when the solid was filtered, rinsed with ethanol and dried, 0.603 g. (95%), m.p. 237–238°. Recrystallization of 0.20 g. of the product from 5 ml. of dioxane gave 0.16 g. of red needles, m.p. 238–239°.

To establish further that the reaction of 2,4-dinitrophenylhydrazine sulfate with stilbene ozonide was "instantaneous and quantitative," stilbene (0.3604 g.) was suspended in ethanol (15 ml.) and ozonized until dissolved (*ca.* 30 minutes). A seed of benzaldehyde 2,4-dinitrophenylhydrazone was added, followed by a solution of 2,4-dinitrophenylhydrazine (1 g.) and sulfuric acid (5 ml.) in ethanol (15 ml.). Formation of a heavy precipitate was instantaneous. This was filtered, rinsed and dried, 1.042 g. (92%). When this reaction was repeated with the above stilbene-α-C^{14}, the benzaldehyde 2,4-dinitrophenylhydrazone, recrystallized from dioxane, showed a radioactivity assay of 0.4915 ± 0.0025 mc./mole.

Fractional Ozonization of Stilbene-α-C^{14}.—The above stilbene-α-C^{14} (1.0000 g.) was dissolved in hot ethanol (35 ml.) and treated with a solution of 2,4-dinitrophenyldrazine (3.2 g., 18% excess) and sulfuric acid (10 ml.) in ethanol (40 ml.). The mixture was diluted with ethanol (15 ml.) cooled to room temperature, seeded with benzaldehyde 2,4-dinitrophenylhydrazone, and ozonized for several minutes. The ozonization was stopped and the accumulated derivative filtered on a tared sintered-glass funnel, rinsing with a little ethanol. The filtrate was returned to the ozonizer, and the process repeated. A series of successive fractions was collected in this manner until further ozonization of the filtrate produced no additional derivative. The sum of the weights of the twelve fractions collected represent a 92.4% yield. Each fraction was recrystallized from purified dioxane[6] (2.5 ml./0.1 g. derivative) to obtain an analytical sample suitable for radioactivity assay.

Assay of Radioactive Benzaldehyde 2,4-Dinitrophenylhydrazone Fractions.—The above purified fractions were assayed for C^{14}-content by a modification of the previously described wet combustion procedure.[7] For assaying large (30–40 mg.) samples of compounds such as the present ones containing a large percentage of nitrogen, it was found necessary to include a heated (175–180°) lead peroxide cartridge (1 × 5 cm.) in the combustion line to remove oxides of nitrogen. In the absence of such a lead peroxide cartridge highly erratic radioactive assays resulted and the wet combustion procedure was unreliable.

The assays of the successive fractions are given in Table I, along with the average deviation from the mean obtained on duplicate assays. The estimated over-all precision of *ca.* ±1% for the wet combustion method is taken into account in the vertical line over each of the data points in Fig. 1. An over-all radiochemical balance between our product fractions and the starting stilbene-α-C^{14} gives agreement within about 1%.

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[4] J. G. Burr, Jr., and L. S. Ciereszko, THIS JOURNAL, **74**, 5426 (1952).

[5] W. A. Bonner, *J. Chem. Educ.*, in press.

[6] L. F. Fieser, "Experiments in Organic Chemistry," 2nd Ed., D. C. Heath & Company, New York, N. Y., 1941, p. 368.

[7] O. K. Neville, THIS JOURNAL, **70**, 3501 (1948).

OAK RIDGE, TENNESSEE

[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF OAK RIDGE NATIONAL LABORATORY]

Studies[1] of Some Carbon-14 Isotope Effects in Organic Chemistry

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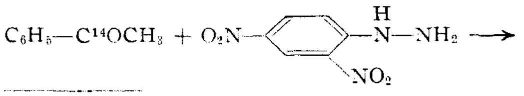
Isotope fractionation was shown to occur in the decomposition of mercurous formate-C^{14} and in some of the keto group reactions of acetophenone-α-C^{14} and benzophenone-α-C^{14}. No isotope fractionation was found in addition of bromine to the carbon-14-labeled ethylenic bonds of styrene or methyl cinnamate. No isotope effect was found in the Diels–Alder addition of 2,3-dimethylbutadiene to β-nitrostyrene-α-C^{14}. No isotope effect was found in absorption of carbon-14 dioxide in two alkaline media.

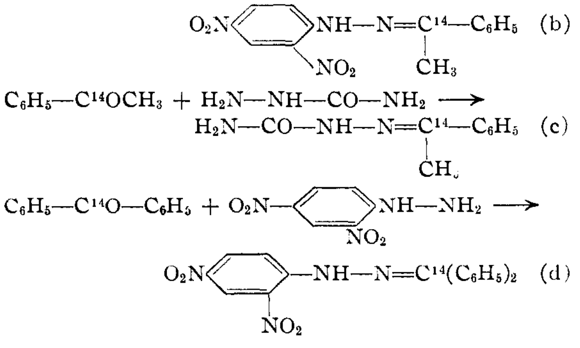
A significant number of carbon isotope effects in organic reactions have been reported recently in the literature.[2] Because such isotope fractionations are of particular interest as sources of error in quantitative tracer studies, it seems desirable to report reaction studies in which no isotope effects were found, as well as those in which isotope effects were discovered. In this paper the results of a series of examinations of organic reactions for isotope effects are reported; in a number of these no isotope fractionation was indicated.

The following is a list of reactions studied:

With isotope effects:

$$2Hg\ OOC^{14}H \xrightarrow{80°} C^{14}O_2 + 2Hg + HC^{14}OOH \quad (a)$$





$$C_6H_5-C^{14}OCH_3 + H_2N-NH-CO-NH_2 \longrightarrow H_2N-CO-NH-N{=}\underset{CH_3}{C^{14}}-C_6H_5 \quad (c)$$

Without isotope effects:

$$C_6H_5-C^{14}H{=}CH_2 + Br_2 \xrightarrow{-80°} C_6H_5-C^{14}HBr-CH_2Br \quad (e)$$

$$C_6H_5-CH{=}C^{14}H_2 + Br_2 \xrightarrow{-80°} C_6H_5-CHBr-C^{14}H_2Br \quad (f)$$

$$C_6H_5-CH{=}C^{14}H_2-COOCH_3 + Br_2 \xrightarrow{25°} C_6H_5-CHBr-C^{14}HBr-COOCH_3 \quad (g)$$

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[1] This paper is based on work performed under contract Number W-7405-eng-26 for the Atomic Energy Commission at Oak Ridge National Laboratory.

[2] For a review, see G. A. Ropp, *Nucleonics*, **10**, 22 (1952).