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

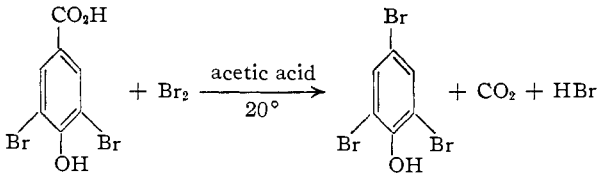
Carbon-13 Isotope Fractionation as a Criterion of the Mechanism of Bromodecarboxylation of 3,5-Dibromo-4-hydroxybenzoic Acid

BY ERLING GROVENSTEIN, JR.,[1] AND GUS A. ROPP[2]

RECEIVED NOVEMBER 19, 1955

By making special application of carbon-13 isotope fractionation as followed in the carbon dioxide product by mass spectrometric measurements, it has been found possible to verify the mechanism previously proposed for bromodecarboxylation of 3,5-dibromo-4-hydroxybenzoic acid. This application of isotope fractionation to the study of a reaction is novel in that the fractionation factor, k_{12}/k_{13}, could be caused (a) to have a value approaching that calculated from reaction rate theory or (b) to approach unity depending upon the amount of hydrogen bromide added. These variations in the measured carbon-13 isotope effect with changes in the chemical *composition* were predictable using a mechanism suggested by earlier kinetic studies.

The bromodecarboxylation of 3,5-dibromo-4-hydroxybenzoic acid proceeds quantitatively according to the equation



The kinetic studies of Grovenstein and Henderson[3] indicated that this reaction and the analogous reaction of 3,5-dibromo-2-hydroxybenzoic acid proceed by the mechanism[4a] (III)

$$ArH_2 \underset{\text{fast}}{\rightleftharpoons} ArH^- + H^+ \quad (1)$$

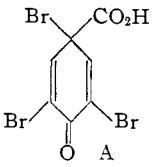
$$ArH^- + Br_2 \rightleftharpoons ArHBr + Br^- \quad (2)$$

$$ArH_2 + Br_2 \rightleftharpoons ArHBr + H^+ + Br^- \quad (3)$$

$$ArHBr \underset{\text{fast}}{\rightleftharpoons} ArBr^- + H^+ \quad (4)$$

$$ArBr^- \longrightarrow Ar'Br^- + CO_2 \quad (5)$$

where ArH_2 is the dibromohydroxybenzoic acid, $Ar'Br^-$ is the anion of tribromophenol and ArHBr is a reactive intermediate which is assumed, on general evidence, to have the structure A for 3,5-dibromo-4-hydroxybenzoic acid.



A

However another mechanism I such as

$$Br_2 + H_2O \underset{\text{fast}}{\rightleftharpoons} H_2OBr^+ + Br^- \quad (6)$$

$$ArH_2 \underset{\text{fast}}{\rightleftharpoons} Ar^{--} + 2H^+ \quad (7)$$

$$Ar^{--} + H_2OBr^+ \rightleftharpoons ArBr^- + H_2O \quad (8)$$

$$ArBr^- \longrightarrow Ar'Br^- + CO_2 \quad (9)$$

with either steps 8 or 9 rate determining could account for the kinetics of bromodecarboxylation of the *p*-hydroxy acid (but not the *ortho* acid) over much of the range of experimental conditions investigated. It seemed of interest, therefore, to seek confirmation of mechanism III for 3,5-dibromo-4-hydroxybenzoic acid.

For the carboxyl labeled[5] acid mechanism I would predict, regardless of bromide ion concentration, an isotope effect[6,7] if step 9 were rate determining and no appreciable isotope effect if step 8 were rate determining. These results are predicted since for step 9 the bonding of the labeled carboxyl group is being severed while for step 8 the bonding of this group is little affected. On the other hand, mechanism III predicts no appreciable isotope effect in the absence of added bromide ions since steps 2 and 3 will here be rate determining and essentially every molecule of intermediate I formed will go into products. At high bromide ion concentration, however, mechanism III predicts an isotope effect since under these conditions a large part of the cyclohexadienone intermediate would revert to reactants and only a relatively small fraction would decompose into products. In the limiting case, the intermediate A would be in rapid, reversible equilibrium with the reactants and the rate-controlling step would be 5 which, of course, involves the labeled center—the carboxyl group. Under these conditions, a carbon-13 isotope effect should be observable by following the change in the isotopic composition of the carbon dioxide as a function of percentage reaction. The first carbon dioxide produced should have a lower than normal carbon-13 to carbon-12 ratio. All carbon isotope effects so far observed and verified have been in a direction such that the bond involving the lighter isotope was severed more rapidly.[7] A study was undertaken therefore to see which of these results was at-

(1) School of Chemistry, Georgia Institute of Technology, Atlanta, Ga.; Research Participant at Oak Ridge National Laboratory, Summer, 1954.

(2) Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

(3) E. Grovenstein and U. V. Henderson, THIS JOURNAL, **78**, 569 (1956).

(4) (a) Steps 1 and 4 of mechanism III and 6 and 7 of mechanism I are considered always to be at equilibrium and hence the equations given for these steps refer to the stoichiometry and not necessarily to the mechanisms of the equilibria. All ions and other species are, of course, considered to be fully solvated. (b) Other forms or variations of mechanism I are considered in ref. 3; however, for the purpose of the present discussion these are so similar to mechanism I presented above as to warrant no separate discussion.

(5) Since the fractionation in the present study was sought for only by measuring the isotopic abundance in the evolved carbon dioxide, the carboxyl group may be referred to as the "labeled center" even though at the beginning of the reaction it had a normal carbon-13 content.

(6) For a general reference to the use of analogous isotope effects in the study of aromatic substitution, see C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 280, 295 and 302.

(7) G. A. Ropp and O. K. Neville, *Nucleonics*, **9**, 22 (1951); M. Dole, *Chem. Revs.*, **51**, 263 (1952); J. Bigeleisen, *J. Phys. Chem.*, **56**, 823 (1952); G. A. Ropp, *Nucleonics*, **10**, 22 (1952); J. Bigeleisen, *Ann. Rev. Nucl. Sci.*, **2**, 229 (1953); P. Yankwich, *ibid.*, **3**, 235 (1953).

tained. It was possible to study carbon-13 fractionation at the natural level of abundance because this reaction, like other decarboxylations, produces carbon dioxide which can be used directly for isotope abundance measurements in a mass spectrometer.[8]

Experimental Details and Calculations

Reagents and solvents were in general of the quality as previously described[3] except that Baker and Adamson, A. C. S., reagent grade bromine was used without further purification and a freshly opened bottle of J. T. Baker Co., C.P., "purified" grade of 34% hydrobromic acid was used. In the present work all solvents were swept with a good stream of helium gas for 1 hr. or more to remove any dissolved carbon dioxide and were subsequently stored out of contact with the atmosphere.

The all-glass apparatus used for the bromodecarboxylations consisted of a reaction vessel attached to a series of three traps. The reaction vessel, of about 1-liter capacity, was immersed in a water-bath held at 20.5 ± 0.5° and was equipped with a sintered glass bubbler such that the system could be swept with helium. Solutions of bromine in the required solvent were added to the reaction flask by means of an attached dropping funnel and the contents of the reaction vessel were kept mixed by means of an underwater magnetic stirrer. The first or Dry Ice–acetone cooled cold-trap served to remove most of the solvent vapors and bromine from the exit gases. The second trap was a U-tube filled with stannous chloride crystals for removing any remaining bromine. The third trap was cooled with liquid nitrogen and was constructed of U-shape with the side which the carbon dioxide product entered being some three times the ordinary diameter of tubing used in order to minimize entrainment. This trap could be isolated from the remainder of the system by stopcocks and lead through a three-way stopcock to a mercury manometer and to an exit tube. To the third trap were attached two or three sample collection bulbs which were ordinarily of about 50-ml. capacity. The sample collection bulbs were equipped with break-seal tubes to facilitate later introduction of the sample carbon dioxide into the mass spectrometer. The exit tube from the third trap led either to a vacuum pump or to an absorption bubbler filled with nearly saturated barium hydroxide solution. In later runs an additional cold trap (also cooled with liquid nitrogen) was included in the vacuum line between the stannous chloride tube and the terminal liquid nitrogen cooled trap and the product carbon dioxide was sublimed from this trap to the terminal cold trap for purification. This sublimation of the product carbon dioxide did not seem to affect the mass spectrometer analyses of the carbon dioxide.

The general procedure used in the collection of carbon dioxide samples will be illustrated by run 3 in presence of hydrobromic acid. The 3,5-dibromo-4-hydroxybenzoic acid (2.37 g., 8.00 mmoles) was dissolved in 900 ml. of 80.0% acetic acid–20.0% water (given here and elsewhere in parts by weight) containing enough hydrogen bromide to make the solution 0.300 *M* in this acid. This solution in the reaction vessel was swept with helium for 1.5 hr. such that no appreciable carbon dioxide was swept out during the last half-hour of sweeping. The presence of carbon dioxide in the effluent helium was tested for by an absorption bubbler filled with nearly saturated barium hydroxide solution and attached to the end of the vacuum-line assembly. Next 32 ml. of bromine solution containing 8.00 mmoles of bromine dissolved in 80.0% acetic acid (0.300 *M* in HBr) was added and the reaction vessel closed from the rest of the system and the atmosphere. After 20 hr. standing the reaction vessel was swept with helium at a rate of about 200 cc./min. for 1 hr. with the cold traps in place on the vacuum line as indicated above. The stopcock between the stannous chloride tube and the liquid nitrogen cooled trap was closed, and this trap was evacuated along with the sample bulbs. The stopcock leading to the pump was closed, the flask of liquid nitrogen was removed and replaced by a Dry Ice–alcohol-bath. The manometer read 71 mm. which corresponded to about 10% reaction. To ensure adequate mixing of the carbon dioxide, the sample was refrozen twice in the trap by alternate application of liquid nitrogen and Dry Ice–alcohol-baths and finally the bulbs of carbon dioxide (samples A) were sealed off with the Dry Ice–alcohol-bath in place. New sample bulbs were put in place and these and the terminal cold trap were evacuated at room temperature (to remove all carbon dioxide and small amounts of solvent which collected in the trap) and filled with helium in preparation for collection of the next sample. The room containing the bromination apparatus was kept in darkness except while collecting carbon dioxide samples. Twenty-eight and one-half hours after collection of the first samples, the second samples (B) were collected in a similar manner. At this point, in order to increase the rate of reaction, 8.13 mmoles of bromine in 33 ml. of solution (80.0% acetic acid, 0.300 *M* HBr) was added to the reaction vessel. The last samples (E) (from 51 to 73% reaction) were collected 165.5 hr. from the start of the bromodecarboxylation.

Bromodecarboxylation of 3,5-dibromo-4-hydroxybenzoic acid in 80.0% acetic acid in absence of any other added solutes was enormously faster than in presence of 0.300 *M* HBr. For this reason it was convenient to add an amount of bromine corresponding to the percentage of reaction desired, to wait for completion of the bromination and then to sweep out the carbon dioxide as before. At a concentration of dibromo-*p*-hydroxybenzoic acid as described above, the bromine added for 12.5% reaction seemed to react almost immediately upon addition to the solution as judged by the disappearance of the red color, while some three to four minutes were required for the next 12.5% reaction. Because of the favorable rate, runs for 100% bromodecarboxylation were accomplished in 80.0% acetic acid in presence of 50% excess bromine but without additional solutes for reaction times of 16 or more hours.

Bromodecarboxylations run in 80.0% acetic acid which was 0.300 *M* in perchloric acid were appreciably slower than in the absence of this solute but were fast enough to use the same general technique as described above for reaction in the absence of hydrogen bromide. Thus the aliquot of bromine added for the second some 10% reaction required 15 minutes for the bromine color nearly to disappear. Such reaction mixtures were, therefore, allowed to stand several hours or overnight before sweeping out the carbon dioxide.

The extent of reaction for the bromodecarboxylations was estimated on the basis of the amount of bromine added and/or the amount of carbon dioxide evolved as measured by the mercury manometer and was probably accurate within a small percentage error.

The sealed samples of carbon dioxide product were submitted for isotope ratio analysis by Consolidated Engineering Corporation, 300 N. Sierra Madre Villa, Pasadena 15, California. The samples were run alternately with samples of Southern Oxygen Co. tank carbon dioxide. Since the samples were actually submitted in two batches separated by a period of about six months, the absolute values could not be compared directly. For analysis I the eight assays of tank carbon dioxide run alternately with the samples gave an isotopic mass ratio R (mass 45/mass 44) of 0.012211 to 0.012227, a spread of ±0.07%, while for analysis II twelve such assays varied from 0.011814 to 0.011828, a spread of ±0.06%. For purposes of comparison the values of the isotope ratios (R) were divided by the corresponding values from analysis on the same day of the carbon dioxide produced from 100% decarboxylation. Since, therefore, all data were used as ratios of ratios, R/R_{100}, no attempt was made to correct the values of R for the presence of $C^{12}O^{16}O^{17}$. Such correction would have only a negligible effect on the

(8) Carbon-13 fractionation was chosen over carbon-14 fractionation in the present case because the mass spectrometer permits somewhat more sensitive measurement of isotope ratios with carbon-13 dioxide than are possible by radioassay of carbon-14 dioxide. However, the difference between the precision of the two methods is not one order of magnitude, as has sometimes been stated, but a factor of about four or five. It should also be made plain that carbon-13 fractionation measurements are rarely feasible as part of an organic reaction mechanism study unless the product containing the label is carbon dioxide or some other gas such as methane which contains a single carbon atom. No such limitation applies to carbon-14 fractionation studies. Obviously, therefore, carbon-13 at normal level of abundance can find few applications of this type while the use of carbon-14 is applicable generally. Furthermore, the fact that carbon-14 isotope effects are about twice the carbon-13 effects tends to offset the superior precision obtainable with carbon-13. The net result is that the use of carbon-13 offers little more than a two- or threefold advantage in precision over carbon-14 even in the relatively few reactions where the former can be applied.

values of $k_{C^{12}O_2}/k_{C^{13}O_2}$ (henceforth called k_{12}/k_{13}) calculated. The values of k_{12}/k_{13} were calculated from values of R/R_{100} and the measured percentages of reaction by making use of material balances and the equation derived by Stevens and Attree.[9] The available data and calculated results are presented in Table I.

TABLE I

ISOTOPIC MASS RATIOS FOR CARBON DIOXIDE FROM BROMODECARBOXYLATION OF 3,5-DIBROMO-4-HYDROXYBENZOIC ACID IN 80.0% ACETIC ACID AT 20.5 ± 0.5°

Run	Analysis	Reagent concn.	Reaction % range represented	$R = \frac{\text{mass 45}}{\text{mass 44}} \times 10^5$	R/R_{100}[a]	k_{12}/k_{13}
1	I	None	0 –100	12,221	...	...
7	II	None	0 –100	11,848	...	...
2B	I	None	12.5–25	12,226	1.000	1.000
2E	I		75 –100	12,307	1.007	1.005
8B	I	None	13 –26	12,224	1.000	1.000
8B dup.	II		13 –26	11,812	0.997	1.004
8E	II		75 –100	11,887	1.003	1.002
				Mean		1.002±0.003
3A	II	0.300 *M* HBr	0 –10.5	11,367	0.959	1.045
3B	I		10.5–23	11,812	.967	1.044
3C	II		23 –37	11,508	.971	1.044
3E	I		51 –73	12,245	1.002	...
9A	II	0.300 *M* HBr	0 –10	11,347	0.958	1.046
9B	II		10 –23.5	11,432	.965	1.045
9C	II		23.5–37	11,529	.973	1.045
9D	II		37 –47.5	11,645	.983	1.044
				Mean		1.045±0.001
4A	II	0.300 *M*	0 –9.0	11,795	0.995	1.005
4B	I	$HClO_4$	9 –20	12,144	.994	1.006
4C	II		20 –50	11,733	.990	1.012
4E	I		79 –100	12,586	1.030	1.019
				Mean		1.011±0.007

[a] R_{100} is taken as 0.012221 for analyses I and 0.011848 for analyses II.

Discussion

According to Table I, bromodecarboxylation occurs with a ratio of k_{12}/k_{13} of 1.002 ± 0.003 or essentially unity[10] in absence of additional solutes (runs 2 and 8); however, when the solvent is made 0.300 *M* in hydrogen bromide this ratio becomes 1.045. It is of importance to note that this effect of hydrogen bromide cannot be due solely to increase of the hydrogen ion concentration of the solution for 0.300 *M* perchloric acid is much less effective. Indeed in the case of perchloric acid the apparent[11] value of k_{12}/k_{13} increases with the percentage reaction in such a way as to suggest that the isotopic fractionation observed is due to the bromide ion formed during the bromodecarboxylation according to the equation

$$RCO_2H + Br_2 \longrightarrow R\text{-}Br + CO_2 + HBr$$

Hence the initial value of k_{12}/k_{13} in the case of perchloric acid seems to be essentially unity or the same as in the case without additional solutes. The present data upon isotope fractionation, therefore, provide good evidence against mechanism I since while for this mechanism step 8 might conceivably be rate determining in absence of strong mineral acid and 9 rate determining in presence of strong acid, this mechanism is unable to account for the differences observed between hydrobromic and perchloric acids.

On the other hand, the isotope effects observed are just what are demanded by mechanism III and the differences between hydrobromic and perchloric acids are attributed to the role of bromide ion in reversing steps 2 and 3. Furthermore the greater sensitivity to reversibility due to formed bromide ion in the case of 0.300 *M* perchloric acid than in the absence of additional solutes can be readily explained by mechanism III. Strong mineral acids are expected to aid the reversal of step 3 and to hinder the ionization of step 4 and, therefore, in the presence of bromide ion to promote reversion of the reactive intermediate into the initial reactants rather than into the final products. The present isotope fractionation studies, in consequence, provide good confirmation for the mechanism III proposed on the basis of kinetic investigations. Like the latter, however, the present study provides no detailed information concerning the structure of the reactive intermediate, though structure A seems most probable on the basis of general evidence summarized earlier.[3]

The present isotopic fractionation study is to the best of our knowledge unique in that we have demonstrated for a *single* reaction the three outstanding possible relations between transition-state energy-barrier heights in the separate stages of two-stage electrophilic substitutions as have been discussed by Ingold.[12] Thus in absence of hydrogen bromide the reactive intermediate passes rapidly to the products of substitution without suffering appreciable reversion to the starting compounds. In presence of 0.300 *M* perchloric acid after some 50% reaction, sufficient bromide ion has been formed for the intermediate to partition approximately equally into products and reactants. Finally in the presence of 0.300 *M* hydrobromic acid, most of the intermediate formed reverts to reactants, a relatively small portion going to the products. That the isotope fractionation factor, k_{12}/k_{13}, has probably nearly reached its maximum value under the latter conditions is confirmed by comparison of the measured value with that of about 1.04 calculated for 20° by the method of Bigeleisen[13] and others for decarboxylation reactions involving *intermolecular* isotope effects.

We hope to be able to present a more detailed study of the manner in which k_{12}/k_{13} varies with hydrogen and bromide ion concentrations in a future communication.

Acknowledgments.—E. G. wishes to acknowledge the advice of Dr. W. H. Eberhardt and Dr. L. D. Frashier in making some of the calculations.

OAK RIDGE, TENNESSEE

(9) W. H. Stevens and R. W. Attree, *Can. J. Research*, **B27**, 807 (1949).

(10) Since for the runs without additional solute at 12–26% reaction the half-life of the added bromine was about equal to the time required for mixing of bromine with dibromo-*p*-hydroxybenzoic acid, the value given for k_{12}/k_{13} may be too small. After correction for this possible effect, a conservative estimate of a maximum value for k_{12}/k_{13} is 1.004–1.010. For the corresponding range of reaction in 0.300 *M* $HClO_4$ (or HBr), the time for mixing was relatively insignificant compared to the time for reaction and thus here the value of k_{12}/k_{13} could not be appreciably affected by this factor.

(11) The equation of Stevens and Attree (ref. 9) is not strictly applicable here since it assumes that the ratio k_{12}/k_{13} is constant throughout the reaction.

(12) See ref. 6 especially p. 302; free energy rather than potential energy is intended in the present discussion.

(13) J. Bigeleisen, *J. Phys. Chem.*, **56**, 823 (1952).