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ORIGINAL PAPER

Carbon-14 kinetic isotope effects in the debromination of *p*-nitrodibromocinnamic acid

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Abstract The kinetic isotope effect (KIE) method was applied to study the mechanism of elimination of bromine from *p*-nitro-*erythro*-α,β-dibromocinnamic acid labeled at the α-carbon. This compound was obtained starting from [2-^{14}C]malonic acid via [2-^{14}C]cinnamic acid and subsequent addition of bromide. The value of ^{14}C KIE determined for α-position of side chain of *p*-nitro-*erythro*-α,β-dibromocinamic acid proves that elimination of bromine leading to formation of (*E*)-*p*-nitrocinnamic acid proceeds via E2 mechanism.

Key words bromine • carbon-14 • elimination • kinetic isotope effect • mechanism • *p*-nitrodibromocinnamic acid

Introduction

The elimination of bromine from *p*-nitro-*erythro*-α,β-dibromocinnamic acid, **1**, depending on the conditions, may lead to the formation of (*E*)-*p*-nitrocinnamic acid, **2**, or to *p*-nitro-β-bromostyrene, **3**, according to Fig. 1.

The above reactions may occur according to E1 (stepwise) or E2 (concerted) mechanisms. They both have been known for a long time [9, 16, 17], but there are several unresolved mechanistic questions, particularly with respect to the timing of the bonding changes. These questions can be resolved by determining the ^{14}C kinetic isotope effects (KIE) for each carbon atom involved in the postulated rate-determining step.

In our previous studies [3] we have measured the kinetic isotope effects (KIE) in base-promoted elimination of bromine from *erythro*-α,β-dibromocinnamic acid successively labeled with ^{14}C at 1, 2, and 3 positions of side chain. The large ^{14}C KIE for both α- and β-position proves that elimination of bromine leading to formation of (*E*)-cinnamic acid proceeds according to the concerted mechanism E2. Very small KIE was measured for the 1-position, more remote from the center of reaction, proving that there is no bonding changes between these first and second labeled carbon atoms in the side chain of cinnamic acid dibromide in the rate-determining step of the reaction [5, 6].

Elimination reactions, by their very nature involve bonding changes at least at five atomic centers and are therefore particularly susceptible to mechanism studies using successive labeling approach [6]. Such reactions are discussed in terms of E1, E2, the mechanistic spectrum [5] of which may be presented as follows in Fig. 2.

The differences in bonding between reactant and activated complex will be reflected in a particular set of isotope effects, and each type of mechanism will have a different set of isotope effects [7–10]. A qualitative analysis of the KIE's expected for the various mechanism of elimination of bromine from

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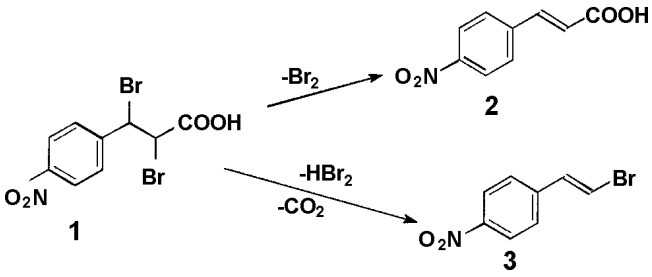


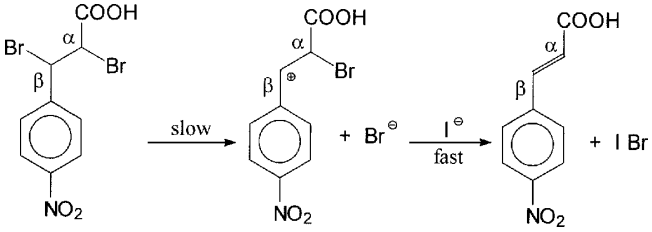
Fig. 1. Elimination of bromine and hydrogen bromide from *p*-nitrodibromocinnamic acid, **1**, leading to formation of (*E*)-*p*-nitrocinnamic acid, **2**, and *p*-nitro-β-bromistyrene, **3**, respectively.

p-nitrodibromocinnamic acid for the various mechanism is shown in Table 1.

Continuing our study of the influence of different substituents (electron donating and electron withdrawing) on the mechanism of elimination of bromine we have determined ^{14}C KIE for *p*-nitrodibromocinamic acid labeled with ^{14}C at 2-position of the side chain. Our study was directed to investigation of reaction leading to formation of (*E*)-*p*-nitrocinnamic acid, **2**, only. We are expecting that some of these mechanistic questions will be answered by determining ^{14}C KIE's for *p*-nitro-*erythro*-α,β-dibromo[2-^{14}C]cinnamic acid, a derivative with electron withdrawing group (-NO_2). No attempts were made to determine KIE for carboxylic carbon atom because earlier studies have shown [3] that this carbon is not involved in the course of elimination reaction. Some of these mechanistic questions are presented in Fig. 2.

The labeled compound needed for KIE experiments, i.e. *p*-nitro-*erythro*-α,β-dibromo-[2-^{14}C]cinnamic acid, **4**, was obtained from the commercially available [2-^{14}C]malonic acid. The labeled ^{14}C *p*-nitrocinnamic acid, **5**, was prepared by a Knovenagel condensation according to the procedures described elsewhere [1, 11, 14]. For α-labeling, commercial [2-^{14}C]malonic acid and *p*-nitrobenzaldehyde in the presence of pyridine, piperidine, and anhydrous sodium sulphate were used in the reaction in a sequence shown in Fig. 3. Bromine was easily added [2] to the double bond of *p*-nitro[2-^{14}C]cinnamic acid, **5**, to form the corresponding labeled

1. Carbonium ion mechanism, E1



2. Concerted mechanism, E2

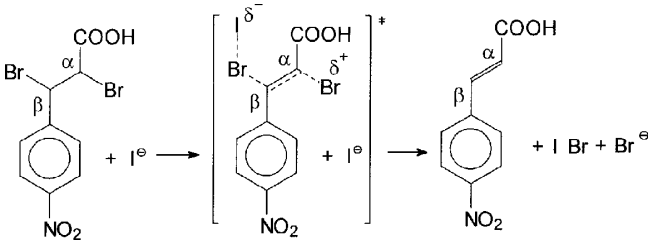


Fig. 2. Mechanism of elimination of bromine from *p*-nitrodibromocinnamic acid.

Table 1. Predicted KIE's for debromination of *p*-nitro-*erytho*-α,β-dibromocinnamic acid.

Predictions	KIE	
	$k/^{\alpha}k$	$k/^{\beta}k$
Carbonium ion mechanisms, E1	no	yes
Concerted mechanism, E2	yes	yes

erythro-dibromide, **4**. For the intermediate **5** and product **4** in the preparation of ^{14}C-labeled of *p*-nitro-*erythro*-α,β-dibromocinnamic acid the physical properities, chemical yields, and radiochemical purities, and yields were in agreement with literature values and/or expectations based on the prior experiments with unlabeled compounds. In particular, the radiochemical and chemical yields were essentially identical, showing both the high chemical and the high radiochemical purities.

Experimental

Methods

Melting points were determined on a Boëthius melting point apparatus and were uncorrected. Radioactivity of samples was measured by liquid scintillation counting on the automatic Liquid Scintillation Counter PW4700 (Raytest-Germany).

Synthesis of *p*-nitro-erythro-α,β-dibromo[2-^{14}C]cinnamic acid

Synthesis of p-nitro[2-^{14}C]cinnamic acid

This intermediate was synthesized by condensation of *p*-nitrobenzaldehyde (*p*-$NO_2C_6H_4CHO$) with [2-^{14}C]malonic acid according to the procedure reported earlier [4, 11]. Briefly, to a flask containing 0.5 g (4.8 mmol) of [2-^{14}C]malonic acid (total activity 9.25×10^6 Bq, sp. activity 1.92×10^6 Bq/mmol), 1 ml (9.9 mmol) of freshly distilled *p*-nitrobenzaldehyde, 0.5 ml pyridine, 0.03 ml of piperidine, and 105 mg of anhydrous Na_2SO_4 were added. A crude **5** obtained was recrystallized from ethanol-water affording the pure product (695 mg, 3.6 mmol), total activity 6.9×10^6 Bq, specific activity 1.91×10^6 Bq/mmol, m.p. 283°C – lit. 286°C [18].

Synthesis of p-nitro-erythro-α,β-dibromo[2-^{14}C]cinnamic acid

To 25 ml reaction flask 10 ml of CCl_4 and 300 mg (1.85 mmol) of **5** with total activity 3.54×10^6 Bq was added. The mixture was stirred and gently refluxed, and to this 0.2 ml of bromine dissolved in 5 ml of CCl_4 was added dropwise during 30 min. The reaction mixture was gently refluxed and stirred by additional 6 hours and left overnight. The precipitate was filtered and several times recrystallized from CCl_4 until the appearance of white color was observed.

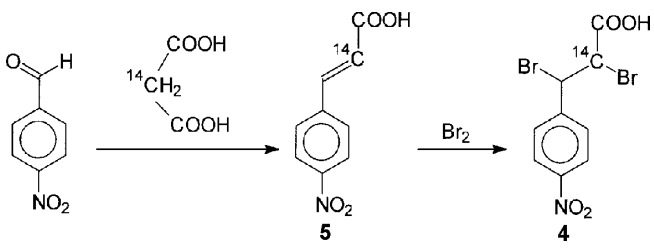


Fig. 3. Synthesis of *p*-nitro-*erythro*-dibromocinnamic acid.

Finally the product was recrystallized from ethanol-water and sublimed (100°C, 0.5 mmHg). As a result 610 mg of **4**, was collected (total activity 3.3×10^6 Bq, sp. activity 1.91×10^6 Bq/mmol, m.p. – 202–204°C, lit. – 204–206°C [18]).

KIE assays

The labeled starting material for KIE assay, i.e. **4**, was diluted with non active *p*-nitro-*erythro*-α,β-dibromocinnamic acid, purified by crystallization just prior to each set of experiments aimed for determining R_0 value. The desired fractions of reaction, *f*, were controlled by using limited amounts of potassium iodide. For each KIE experiment a sample (300 to 500 mg) of assayed ^{14}C-*p*-nitrodibromocinnamic acid with proper amount of KI was dissolved in 20 ml of 80% acetic acid and kept at 50.0°C. After a preset time (as estimated from kinetic experiments) the sample was quenched with cold water. The insoluble product, **2**, and unreacted **1** were recovered by filtration and washed with water. The solid was taken up in diethyl ether and washed with 0.1N sodium thiosulphate solution until the color coming from iodine disappeared. The ether was removed by distillation and the residue was dried in a desiccator over P_4O_{10}. Next, **1** and **2** were separated by sublimation. First, **1** was sublimed at 100°C under 0.05 mm Hg pressure and next **2** was crystallized (water--ethanol) from residue. Finally both compounds were purified by crystallization to constant molar radioactivity. The purity of samples were also checked by TLC (ethyl acetate:chloroform – 4:1 v/v) and melting point determination. These samples were used for radioactivity assay, giving the R_p and R_r values. The radioactivity of samples (about 20 mg) was measured by automatic liquid scintillation counter applying cocktail (Sigma-S4273).

Results and discussion

The KIE experiments were carried out in acetic acid at 50.0°C in the presence of potassium iodide. The nucleophilic iodide ion attacks one of the two vicinal bromine atoms donating its electron pair to the bromine atom and IBr is formed. The adjacent bromine atoms with its electron pair is pushed away from the molecule leading to formation of the double bond. Under these reaction conditions the product of bromine elimination was shown to be (*E*)-*p*-nitrocinnamic acid [17] only. The stoichiometry of the KI and **1** reaction is substantially that given below [12, 13]:

$$p\text{-}NO_2C_6H_4CHBrCHBrCOOH + KI \rightarrow$$
$$\rightarrow p\text{-}NO_2C_6H_4CH{=}CHCOOH + KBr + IBr$$

$$KI + IBr \rightarrow KBr + I_2$$

$$I_2 + KI \rightarrow KI_3$$

For each isotope effect experiment, the *f* (fraction of reaction), R_0 (molar activity of starting material), R_p (molar activity of product after partial conversion), and R_r (molar activity of the recovered starting material) were used to calculate the KIE's by applying the equations (1)–(4) of Tong and Yankwich [15]

$$\frac{k^{12}}{k^{14}} = \frac{\ln\dfrac{R_p - R_0}{R_p - R_r}}{\ln\dfrac{R_r\left(R_p - R_0\right)}{R_0\left(R_p - R_r\right)}} \tag{1}$$

$$\frac{k^{12}}{k^{14}} = \frac{\ln\dfrac{(1-f)(R_0+1)}{R_r+1}}{\ln\dfrac{R_r(1-f)(R_0+1)}{R_0\left(R_r+1\right)}} \tag{2}$$

$$\frac{k^{12}}{k^{14}} = \frac{\ln\left[1-\dfrac{f\left(R_0+1\right)}{R_p+1}\right]}{\ln\left[1-\dfrac{R_p f\left(R_0+1\right)}{R_0\left(R_p+1\right)}\right]} \tag{3}$$

$$\frac{k^{12}}{k^{14}} = \frac{\ln\left[\dfrac{1}{1-f}-\dfrac{f(R_p-R_r)}{(1-f)(R_p+1)}\right]}{\ln\left[\dfrac{1}{1-f}+\dfrac{f(R_p-R_r)}{(1-f)R_r(R_p+1)}\right]} \tag{4}$$

The results of the calculations are listed in Table 2. Agreement between the KIE's calculated in the four different ways is a measure of the adequacy of the procedures used.

As is seen from the data presented in Table 2 there is distinctive KIE for *p*-nitro-*erythro*-α,β-dibromo[2-^{14}C]-cinnamic acid (1.023). However, it is substantially lower than earlier determined by us [3] ^{14}C KIE's for α and β carbon atoms in *erythro*-dibromocinnamic acid which are equal to 1.053 and 1.048, respectively [3]. These data support the concerted (E2) mechanism elimination of bromine from *erythro*-dibromocinnamic acid. In the case of its *p*-nitroderivative the electron withdrawing action of -NO_2 group decreases the electron density on the carbon atoms at 1- and 2-positions and facilitates the leaving Br^+ from α-carbon, and therefore lowering isotope effects at α-carbon. According to supposed predictions, listed in Table 1, these facts rule out the stepwise mechanism with concerted loss of both the alpha and the beta bromine atoms and support a carbonium ion mechanism, E2 (Fig. 2). This mechanism could be fully proved by determining the ^{14}C KIE for β-carbon atom in *p*-nitroderivative. Unfortunately, up to now we encountered many difficulties in synthetic routes leading to synthesis of starting compound, i.e. *p*-nitro--*erythro*-dibromo[3-^{14}C]cinnamic acid with reasonable yield and specific activity needed (mainly connected with instability of ^{14}C-labeled intermediates).

Fraction of reaction *f*	$k/^{\alpha}k$ calculated with Eqs: (1), (2), (3) and (4), respectively			
	R_0, R_r, R_p Eq. (1)	R_0, R_r, *f* Eq. (2)	R_0, R_p, *f* Eq. (3)	R_r, R_p, *f* Eq. (4)
0.25	1.0308	1.0185	1.0216	1.0201
0.5	1.0298	1.0214	1.0253	1.0238
0.75	1.0186	1.0265	1.0205	1.0227

Table 2. ^{14}C KIE, $k/^{\alpha}k$, in the debromination of *p*-nitro--*erythro*-dibromo[2-^{14}C]cinnamic acid.

Average value: $k/^{\alpha}k$ = 1.023±0.002 (Standard average deviation has been calculated with respect to confidence level equal to 0.95.)

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References

1. Bennett GJ, Lee H-H, Das NP (1990) Biosynthesis of mangostin. Part 1: The origin of the xanthone skeleton. J Chem Soc Perkin Trans 1:2675–2685
2. Berman JD, Price CC (1958) Reaction of bromine with silver salts of some unsaturated acids. J Org Chem 23:102–103
3. Bukowski J, Kanski R, Kanska M (2002) Carbon-14 isotope effects in the debromination of dibromocinnamic acid. J Radioanal Nucl Chem 251:101–104
4. Bukowski J, Szul J, Kanski R, Kanska M (2000) Enzymatic synthesis of [3-^{14}C]cinnamic acid. J Radioanal Nucl Chem 243:635–638
5. Eubanks JRI, Sims LB, Fry A (1991) Carbon isotope effect studies of the mechanism of the Hoffman elimination reaction of para-substituted (2-phenyl-1-^{14}C)- and (2-phenyl-2-^{14}C)trimethylammonium bromides. J Am Chem Soc 113:8821–8829
6. Fry A (1964) Application of the successive labelling technique to some carbon, nitrogen and chlorine isotope effect studies of organic reaction mechanisms. Pure Appl Chem 8:409–419
7. Fry A (1970) Heavy atom isotope effects in organic reaction mechanism studies. In: Collins CJ, Bowman NS (eds) Isotope effects in chemical reactions. Van Nostrand-Reinhold, New York, pp 364–414
8. Fry A, Sims LB, Eubanks JRI *et al*. (1983) Carbon-14 isotope effect studies of mechanisms of some elimination ractions. In: Duncan WP, Susän AB (eds) Synthesis and applications of isotopically labeled compounds. Elsevier, Amsterdam, pp 133–138
9. Grovenstein E Jr, Lee DE (1953) The stereochemistry and mechanism of the transformation of cinnamic acid dibromide to β-styrene. J Am Chem Soc 75:2639–2644
10. Hasan T, Sims LB, Fry A (1991) Heavy atom isotope effect studies of elimination reaction mechanism. 1. A kinetic and carbon-14 kinetic isotope effect studies of the base-promoted dehydrochlorination of substituted 1-phenylethyl-2-^{14}C chlorides. J Am Chem Soc 105:3967–3975
11. Jemielity J, Kanska M, Kanski R (1998) Enzymatic synthesis of [1-^{13}C]-, and [1-^{14}C]-L-phenylalanine. Isotopes Environ Health Stud 34:335–339
12. Kwok WK, Mathai IM, Miller SI (1970) Debromination of meso- and DL-stilbene dibromide by lithium bromide in dimethylformamide. J Org Chem 35:3420–3423
13. Mathai IM, Miller SI (1970) Debromination of meso- and DL-stilbene dibromide by sodium iodide in dimethylformamide. J Org Chem 35:3416–3419
14. Murray A III, Williams DL (1958) Organic syntheses with isotopes. Vol. 1. Interscience Publ, New York
15. Tong JY, Yankwich PE (1957) Calculation of experimental isotope effects for pseudo first-order irreversible reactions. J Phys Chem 61:540–543
16. Trumbull ER, Finn RT, Ibne-Rasa KM, Sauers CK (1962) Kinetics of the decarboxylative elimination of cinnamic acid dibromides. J Org Chem 27:2339–2344
17. Trumbull ER, Ibne-Rasa KM (1963) Reaction of cinnamic acid dibromide with iodide ion. J Org Chem 28:1907–1909
18. Tüzün C, Malik KH (1974) Debromination of cinnamic acid dibromides by potassium iodide in 80% aqueous ethyl alcohol. Comm Sci Univ Ankara:1–12