STERIC ORIGIN OF SOME SECONDARY ISOTOPE EFFECTS

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Exclusive of special techniques, there are three general methods for determining isotope effects—kinetic, competing reactions (cumulative and differential), and product analysis.

Primary isotope effects of deuterium and tritium are large; therefore only limited care is required to minimize errors. For secondary isotope effects, however, small differences between large numbers are measured; consequently, it is necessary to be very cautious in evaluating the data. In some instances, erroneous kinetic methods are being used to investigate small isotope effects. For example, many years ago Roseveare[1] showed that in the first-order rate expression

$$k = \frac{1}{t} \ln \frac{C_0}{C} \tag{1}$$

the pairing of successive data points to calculate an average value for k from the expression

$$nk = \left(\frac{1}{t_1 - t_0}\right)\left(\ln \frac{C_0}{C_1}\right) + \left(\frac{1}{t_2 - t_1}\right)\left(\ln \frac{C_1}{C_2}\right) + \dots + \left(\frac{1}{t_n - t_{n-1}}\right)\left(\ln \frac{C_{n-1}}{C_n}\right) \tag{2}$$

is an incorrect method. The possibility for error is greater if all the time intervals are equal, so that

$$t_1 - t_0 = t_2 - t_1 = \dots = t_n - t_{n-1} = \Delta t \tag{3}$$

Then all data points between t_0 and t_n cancel; only the first and last points determine $\bar{k}$, that is,

$$\bar{k} = \left(\frac{1}{t_n - t_0}\right)\left(\ln \frac{C_0}{C_n}\right) \tag{4}$$

A second method in which the initial data point is paired with every other data point is also still in use, even though the method was shown by Guggenheim[2] to give undue weight to the initial concentration of reactant.

The method of least squares[3] is used frequently to reduce random errors. In this method the deviations in the square of the function ln $[a_0/(a_0 - x)]$ is minimized; a_0 is the number of moles of reactant at $t = 0$, and x is the

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† Operated for the Atomic Energy Commission by Union Carbide Corporation.

number of moles of product at time t. There are shortcomings to this method:

(*i*) The sum of the squares of the residuals of the function $\ln [a_0/(a_0 - x)]$ rather than of x is minimized; thereby, a serious weighting imbalance can be imparted to the calculation.

(*ii*) It is usually assumed that there is no error in a_0, when in fact the error in a_0 is often larger than the error in any observation of x. For example, the value for a_0 is usually infinite titre.

In order to show the effect of an uncertainty in a_0, the four sets of data originally reported by Guggenheim[2] were subjected to repeated least-squares calculations by use of an IBM 7090 computer. The effect of an error in a_0 on the value calculated for k (*Figure 1*) is particularly great for sets Nos. 1 and 4 of Guggenheim's data. The effect of varying a_0 only $\pm 0{\cdot}075$ per cent, for an equal weighting ($w = 1$), and next a weight equal to the magnitude of the observation ($w = x$) is, for set No. 4:

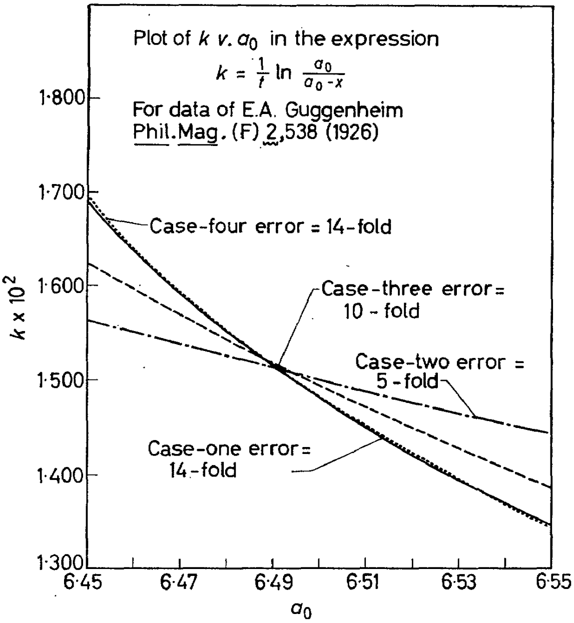


Figure 1. Variation in k with deliberate variation in a_0 for the first-order linear rate expression

For $w = 1$:

$$a_0 = 6{\cdot}488,\ 100\,k = (1{\cdot}5222 \pm 0{\cdot}0031)$$
$$a_0 = 6{\cdot}492,\ 100\,k = (1{\cdot}5084 \pm 0{\cdot}0030)$$
$$a_0 = 6{\cdot}497,\ 100\,k = (1{\cdot}4917 \pm 0{\cdot}0032)$$

For $w = x$:

$$a_0 = 6{\cdot}488,\ 100\,k = (1{\cdot}5227 \pm 0{\cdot}0014)$$
$$a_0 = 6{\cdot}492,\ 100\,k = (1{\cdot}5083 \pm 0{\cdot}0013)$$
$$a_0 = 6{\cdot}497,\ 100\,k = (1{\cdot}4908 \pm 0{\cdot}0014)$$

The estimate of the standard error, which is first of all deceivingly small, is found to change very little over a wide range of a_0 values. A variation in a_0 of only $\pm 0{\cdot}075$ per cent caused k to change by 2 per cent. The possibility of drawing erroneous conclusions from the measurement of small isotope effects is obvious. One solution is to use methods other than the kinetic method for isotope rate studies.

If the method of competing reactions is used instead of the kinetic method, either the cumulative or the differential technique can be chosen. The cumulative technique requires the isolation of all accumulated product or all remaining reactant after a known fraction of reaction. In the differential technique, small increments of accumulated product are isolated at known fractions of reaction. The differential technique is best suited to quantitative reactions that yield gases or precipitates. Downes[4] first derived the equation

$$\ln x = \left(\frac{k^*}{k} - 1\right) [\ln (1 - f)] + \ln \frac{k^*}{k} \tag{5}$$

which relates the change in x to the fraction of reaction (f). In equation (5) $x = A/A_0$, where A is the molar radioactivity of the product at f, A_0 is the initial molar radioactivity of the reactant, and k^*/k is the isotope effect as it is usually defined. Collins and Lietzke[5] rewrote the Downes equation, programmed it for a computer, and then—assuming arbitrary values of k^*/k—calculated values of A/A_0 for given fractions of reaction. Their plots of f v. x (*Figures 2* and *3*) strikingly illustrate the fact that the less reactive

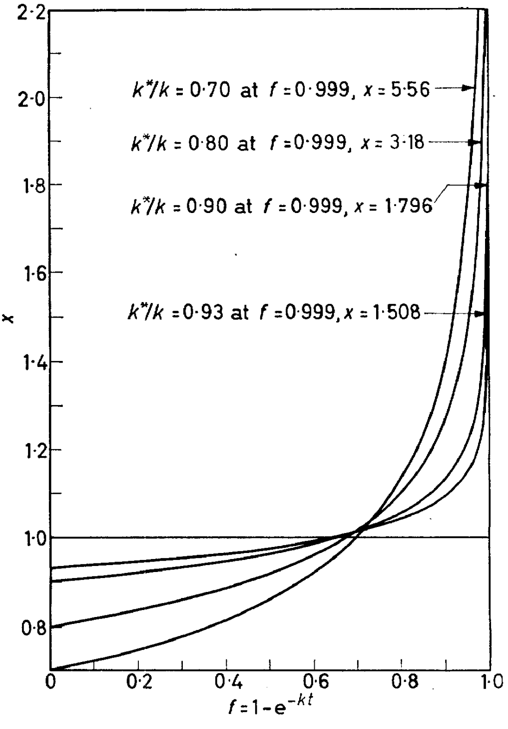


Figure 2. Change in isotopic content of differentially produced product in the isotope effect (k^*/k) range from 0·70 to 0·93

(From C. J. Collins in *Advances in Physical Organic Chemistry* (Ed. V. Gold), Vol. 2, Academic Press, London and New York (1964); with permission) isotope becomes more and more concentrated as the reaction approaches completion. From these plots it can be seen that great risks attend the use of low-yield tracer experiments with deuterium and tritium.

It seemed advantageous to apply the differential method to the study of small secondary isotope effects. To be suitable for such a study, a reaction should afford an essentially quantitative yield of a product that is insoluble in the reaction medium. The reaction can be carried out in such a way as to allow removal of small aliquots (1 to 2 per cent) of reaction product at known fractions (f) of completion. The molar radioactivity of each small purified aliquot divided by the original molar radioactivity of the reactant then provides values for x in equation (5). The reaction chosen for such a study was the formation, at 0°, of a number of 2,4-dinitrophenylhydrazones; the reactants studied first were acetophenone-β-^{14}C and acetophenone-*phenyl*-1-^{14}C. The isotope effects (k^*/k) observed for these compounds were 1·0085 ± 0·0005 and 1·0038 ± 0·0003, respectively. The data used to determine the smaller of these values are plotted in *Figure 4*. The plot demonstrates the usefulness of the differential method for determining very small isotope effects.

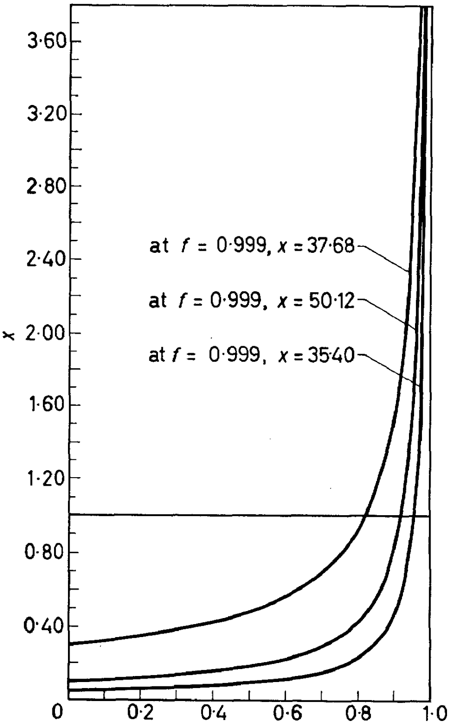


Figure 3. Change in isotopic content of differentially produced product in the isotope effect (k^*/k) range from 0·05 to 0·30

(From C. J. Collins in *Advances in Physical Organic Chemistry* (Ed. V. Gold), Vol. 2, Academic Press, London and New York (1964); with permission)

The differential method was then used to determine secondary deuterium isotope effects in the acid-catalysed formation of the 2,4-dinitrophenylhydrazones of acetophenone-*methyl*-d_3 as well as other deuterated ketones and aldehydes (*Table 1*). Known mixtures of deuterated and undeuterated ketones were prepared. Either the deuterated or undeuterated species was labelled with carbon-14. Because carbon-14 can be determined very accurately, it was possible, by following the rate of change of the carbon-14 content, to make a very sensitive determination of the rate acceleration or deceleration caused by substitution of deuterium. However, unless the carbon-14 label is far removed from the reaction site, this method requires that the isotope effects contributed by carbon-14 be known. These were

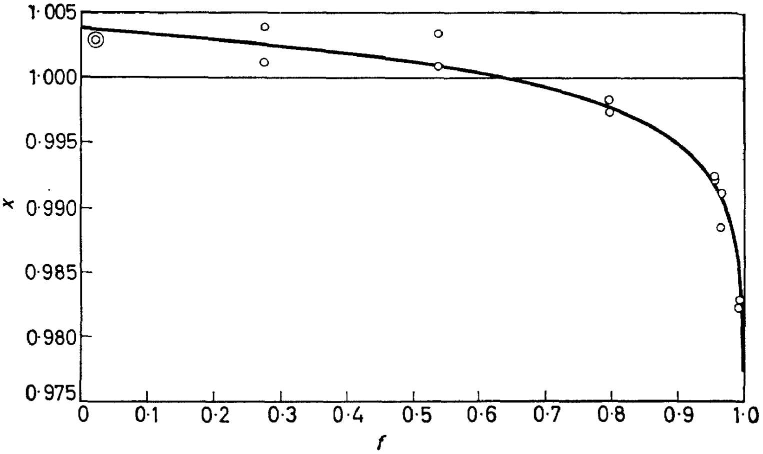
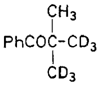
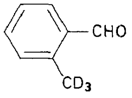


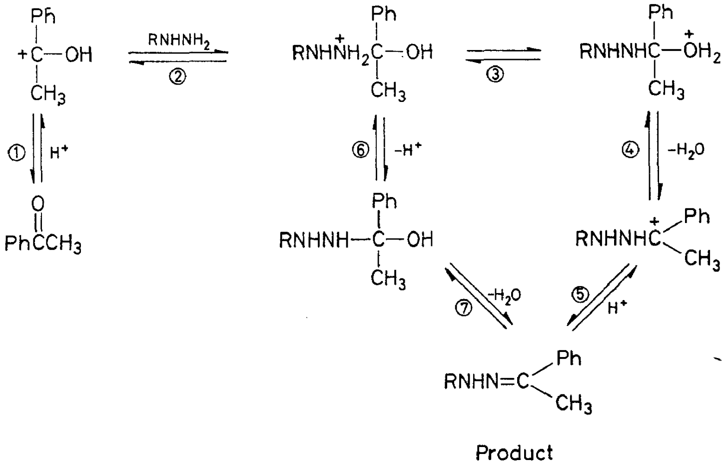
Figure 4. Experimental data, together with a plot of equation (5), for an isotope effect (k^*/k) of 1·0038 ± 0·0003

Table 1. Secondary deuterium isotope effects observed in 2,4-dinitrophenylhydrazone formation

Competing species	k_D/k_H
$PhCOCD_3$ (I)	1·119 ± 0·005
$PhCOCD_2CH_3$ (II)	1·124 ± 0·005
$PhCOCH_2CD_3$ (III)	0·9952 ± 0·0006
 $\mathrm{PhCO\overset{CH_3}{\underset{CD_3}{C}}{-}CD_3}$ (IV)	1·046 ± 0·0015
 (V)	1·0072 ± 0·0004

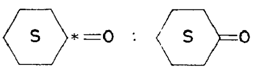
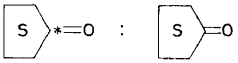
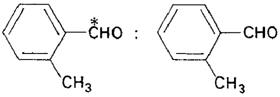
determined in separate experiments. *Table 2* illustrates the constancy of the primary carbon-14 isotope effect for a wide variety of carbonyl-labelled compounds. The substitution in the benzene ring of groups that have a pronounced tendency to withdraw electrons from, or supply electrons to, the reaction site apparently modifies this isotope effect[6]; steric interactions, however, appear to be negligible. On the other hand, secondary isotope effects of deuterium-labelled ketones and aldehydes reflect greater dependence on the size and conformation of substituents attached to the carbonyl group. Steric factors also appear to be important in determining the comparative reaction rates of the non-labelled compounds. For the secompounds the relative rates are: acetophenone > propiophenone ≫ phenyl t-butyl ketone. Electron-withdrawing or electron-donating groups, when present on the phenyl group of acetophenone, produce a much smaller effect on the rate of 2,4-dinitrophenylhydrazone formation.

Possible paths for the reaction of a typical ketone are shown in the reaction scheme



Although steps 5 and 7 are shown as reversible, it has been found that, under the usual reaction conditions, exchange does not occur between carbon-14-labelled acetophenone and unlabelled acetophenone-2,4-dinitrophenylhydrazone. The reactions were not run at constant pH; the sulphuric acid content varied from 0·05 M as the first aliquot of reagent was added to about 0·5 M when all the carbonyl compound had reacted. Deliberate change of acid concentration at 60 to 70 per cent reaction caused no apparent shift in the errors found for individual data points. Simon and Palm[7] have likewise found that the isotope effect which occurs in the formation of the *p*-nitrophenylhydrazone of β-naphthaldehyde(t) depends little or not at all on pH. Jencks[8] observed that for oxime and semicarbazone formation the rate-limiting step changes from rate-limiting acid-catalysed dehydration in near-neutral solution to rate-limiting attack by the free base at low pH values. The data of *Table 1* are consistent with a mechanism for which the rate-determining step is the attack by 2,4-dinitrophenylhydrazine on the conjugate acid of the ketone or aldehyde. The assumption is made that all the compounds of *Table 1* form 2,4-dinitrophenylhydrazones *via* the same reaction path. The increased rate caused by deuterium substitution (compounds I, II, IV, and V) is then explained as a result of decreased steric crowding in the transition state for step 2 of equation (6). Compound IV cannot possibly assume a conformation in which a methyl group does not interfere with attack by the free base. However, compound III can assume two conformations that interfere no more than does acetophenone itself; hence the small isotope effect.

Table 2. Carbon-14 isotope effects in the conversion of aldehydes and ketones to their 2,4-dinitrophenylhydrazones

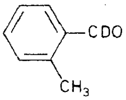
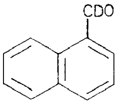
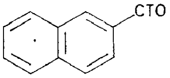
Competing species	k^*/k[a]
$Ph\overset{*}{C}OCH_2CH_3$: $PhCOCH_2CH_3$	0·9485 ± 0·0040
$Ph\overset{*}{C}OCH(CH_3)_2$: $PhCOCH(CH_3)_2$	0·9522 ± 0·0022
$Ph\overset{*}{C}OC(CH_3)_3$: $PhCOC(CH_3)_3$	0·9512 ± 0·0009
$Ph\overset{*}{C}OCH_3$: $PhCOCH_3$	0·9489 ± 0·0020
	0·9596 ± 0·0032
	0·9566 ± 0·0067
$Ph\overset{*}{C}DO$: $PhCDO$	0·9542 ± 0·0007
$Ph\overset{*}{C}HO$: $PhCHO$	0·9498 ± 0·0007
	0·9488 ± 0·0009
Average	0·9522
Average deviation	± 0·0026

[a] Ratio of specific rate constants for carbon-14-labelled (k^*) and unlabelled (k) species

Table 3 lists a number of deuterated and tritiated aldehydes that were converted to substituted hydrazones. It is not possible at this time to explain the isotope effects shown in *Table 3*. Possibly the change in direction of the isotope effect results from the operation of two different mechanisms. The absence of an isotope effect for deuterated α-naphthaldehyde could be the result of a fortuitous combination of two competitive rate-determining steps.

A further conclusion from the above experiments is worthy of mention. In a commonly used procedure for determining isotope effects, the first few per cent of reaction product is accumulated. In this so-called "low-conversion approximation", the isotopic content of the accumulated product is compared with that of the initial reactant. In the use of the differential technique, it has been observed that—even for carefully prepared reagents—the molar radioactivity of the first few per cent of reaction product often is far larger or far smaller than the value obtained by extrapolating the data taken during the remaining course of the reaction. *Figure 5* illustrates one

Table 3. Secondary isotope effects in the conversion of α-deuterated and α-tritiated aldehydes to substituted hydrazones

Aldehyde	k^*/k
PhCDO	$0{\cdot}943 \pm 0{\cdot}005^{a}$
	$0{\cdot}941 \pm 0{\cdot}004^{a}$
	$1{\cdot}000 \pm 0{\cdot}005^{a}$
	$1{\cdot}28 \pm 0{\cdot}02^{b,d}$
$PhCH_2CH_2CTO$	$0{\cdot}89 \pm 0{\cdot}02^{c,d}$

[a] 2,4-Dinitrophenylhydrazones [b] *p*-Nitrophenylhydrazone

[c] Phenylhydrazone [d] From Simon and Palm[7]

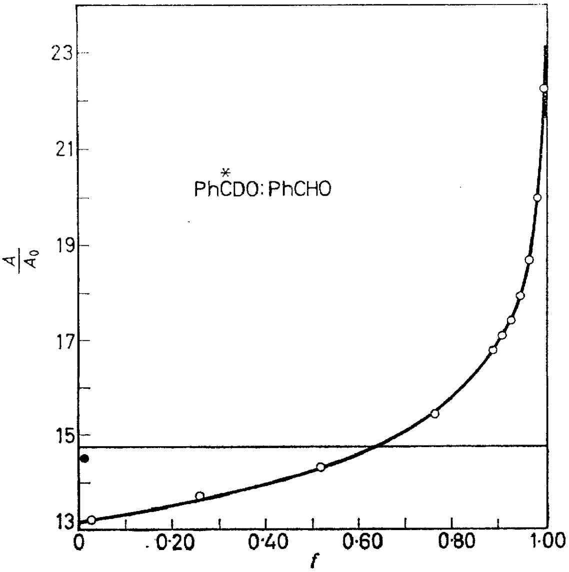


Figure 5. Example of a source of error in the "low-conversion approximation"

instance in which the molar radioactivity of the first 2 per cent of reaction product is about the same as the initial molar radioactivity of the reactant. Such errors—often duplicable—can be misleading with respect to both the magnitude and direction of the isotope effect.

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