Use of Isotopes for Studying Reaction Mechanisms

1. Isotopes as Markers

Uday Maitra and J Chandrasekhar

Isotopes can be used as markers to keep track of positions of atoms during a chemical transformation. This strategy of determining reaction mechanisms is illustrated in the article with several examples.

Uday Maitra and J Chandrasekhar are members of the Organic Chemistry faculty in Indian Institute of Science at Bangalore.

Introduction

When a reaction is carried out, the primary effort goes towards the identification of the product(s) of the reaction. A more time consuming endeavour, however, is the elucidation of the reaction mechanism. The study of reaction mechanisms is intellectually challenging, and at the same time provides opportunities to excercise greater control over the reaction. For example, a detailed knowledge of the intermediate formed in a reaction would help us optimize reaction conditions to effect higher yields, minimize reaction times, etc.

The transformation of a reactant A to product B can in principle take place via a number of pathways. To find experimental evidence for one of these pathways detailed physical studies are needed. The techniques normally used for studying reaction mechanisms include kinetic analysis, trapping of a reactive intermediate, etc. One of the most powerful techniques is the use of isotopes as labels in a reaction. An atom in the reactant is selectively replaced by one of its isotopes. The reaction is carried out as usual, and the location (and/or distribution) of the isotopic label in the product(s) is determined (see *Box 1*). The probable mechanism can then be inferred. In this approach, the focus is only on the identification of product(s), and not on the effect of the isotope on the reaction rate. In the following

Box 1

Isotopes are generally distinguished by three analytical means. The first of them makes use of radioactive isotopes, such as tritium (^3H), ^{14}C, ^{32}P etc. This is a highly sensitive technique, but special facilities are required to handle radioactive material. Mass spectroscopy can also be used to detect isotopes. This is also a highly sensitive technique. When the fragmentation pattern of a compound is known, mass spectral data provide a wealth of information. The third, and at present the most frequently used technique is nuclear magnetic resonance. This technique requires an NMR active nucleus such as ^2H, ^{13}C, ^{17}O etc. and is relatively less sensitive. But the ease of operation more than compensates for its limitations.

sections several specific examples are provided in which this strategy has been utilized.

The Case of an *Identity* Reaction

Let us consider a simple sigmatropic reaction shown in *Figure 1a*. It is easy to see that the reactant and the product are the same (it is called an identity reaction, or a degenerate rearrangement)! How does one then find out that the reaction is indeed taking place? The answer is to label one of the hydrogen atoms in the substrate with its isotope. If this is done, as shown in *Figure 1b*, we find that the deuterium in the reactant which was attached to an alkene (sp^2) carbon ends up on an alkane (sp^3) carbon in the product. These two isotopomers can be distinguished by NMR spectroscopy.

The Classic Case of the Benzyne Mechanism

One of the most important applications of isotopes as labels has been in the determination of the mechanism of nucleophilic aromatic substitution. The reaction of bromobenzene with $NaNH_2$ yields aniline. In principle, the mechanism might be a direct displacement of Br^- by NH_2^-, or an addition-elimination pathway. J D Roberts proposed a third possiblility, shown in *Figure 2*. This involves an unusual intermediate, namely, 1,2-dehydrobenzene (benzyne). How did he prove this mechanism? A simplified version of his approach is as follows. Assume that we can make bromobenzene with an isotopic carbon (^{13}C or ^{14}C, represented with an asterisk) at C-1. If the mechanism is a direct displacement, or an addition-elimination process, only aniline A, with the label attached to the amino group, would be produced. On the other hand, a benzyne formed from this reactant would be attacked by NH_2^- (almost) equally at the two carbon atoms of the formal triple bond. The attack on C-1 will produce aniline A, whereas attack on C-2 will produce aniline B. Compounds A and B differ only in the location of the isotopic carbon. This reaction was indeed found to produce almost equal quantities of

A and B, thereby confirming the existence of a symmetrical benzyne intermediate.

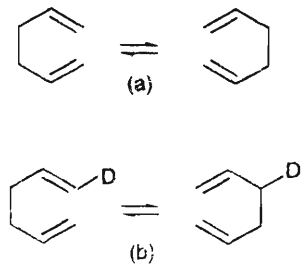

Figure 1 Labelling allows one to investigate a degenerate rearrangement such as the Cope reaction shown here.

Carbonium Ion? What Kind?

A reaction of great importance in organic chemistry is aliphatic nucleophilic substitution. The two categories of this reaction are S_N1 and S_N2. An S_N2 reaction is a single step process (*Figure 3a*), in which inversion of configuration occurs at the carbon atom undergoing the substitution. The S_N1 reaction, on the other hand, is a two step process, in which a carbonium ion is formed as an intermediate. Since carbocations are planar, the reaction will be accompanied by racemization. The problem with this simplistic view is that there are many reactions known which lead to *partial* racemization. A variety of studies have shown that the S_N1 reaction pathway is much more complex, since it does not always involve the formation of a free carbocation as illustrated in *Figure 3a*. It has been demonstrated by Winstein and others that the reaction proceeds through more than one intermediate, such as a contact ion pair and a solvent-separated ion pair.

Isotopic labelling is the only way to study degenerate rearrangements.

A carefully designed experiment done by Goering using isotopic labelling clearly provides further evidence for these intermediates. A substrate in which one of the oxygen atoms of the ester was differentiated by labelling with ^{18}O was used (*Figure 3b*). When the C-O bond just cleaves, a contact ion pair forms. Since the anionic part of this ion pair is a carboxylate

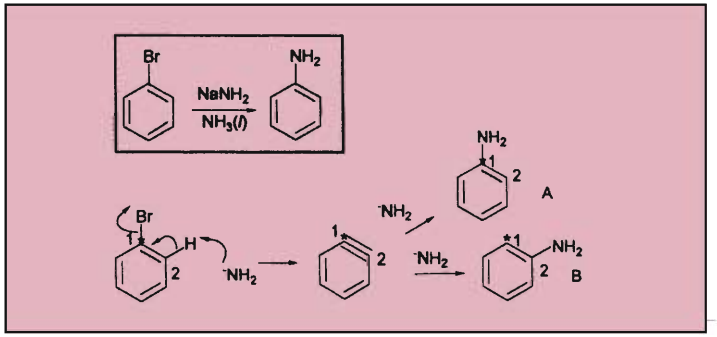

Figure 2 The benzyne mechanism.

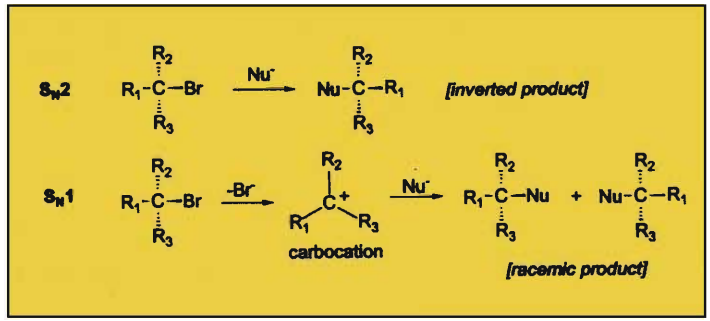

Figure 3a Stereochemical outcome of idealized S_N1 and S_N2 reactions.

species, both oxygen atoms should become equivalent. If this ion pair goes back to the reactant, the ^{18}O label will be randomized (*i.e.*, the labelled oxygen will be present at both locations). Additionally, since the reactant is chiral, the rate at which it loses optical activity (rate of racemization of the reactant) can also be independently measured. It was observed by Goering that the rate of isotope scrambling was twice the rate of racemization (under certain defined experimental conditions). Interestingly, when NaN_3 (N_3^- is a good nucleophile) was added to the reaction, the rate of isotope exchange was *unaltered*, but the rate of racemization (of the reactant) became *zero*. This suggested that N_3^- was intercepting an intermediate which is associated with racemization, and not an intermediate which leads to isotope exchange. The simplest interpretation of these experimental data is that the species which causes randomization of the label is the contact ion pair (unaffected by azide ion), and the species which is intercepted by N_3^- is the solvent separated ion pair.

The S_N1 process is much more complex than is commonly believed. A free carbocation is not always the only intermediate.

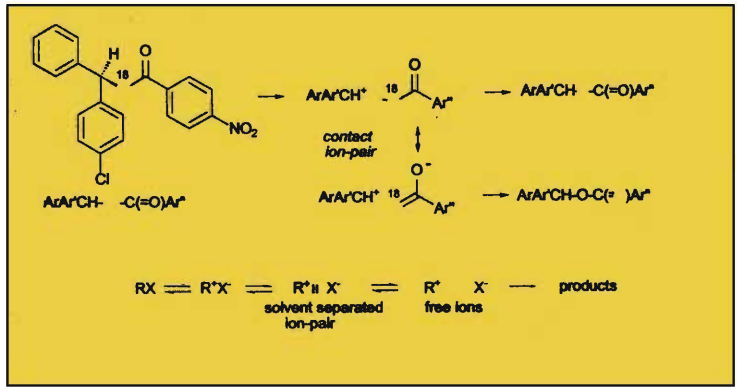

Figure 3b Goering's labelling experiment.

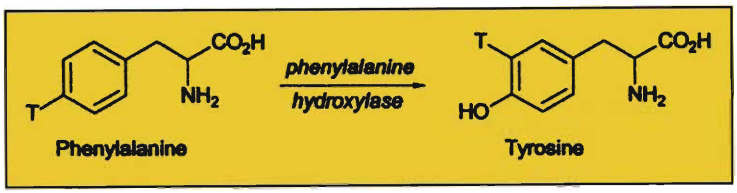

Figure 4 The "NIH" shift reaction.

The NIH Shift

In biological reactions, isotopes have been extensively used as markers to understand reaction mechanisms. One simple example is shown in *Figure 4*. The oxidation of phenylalanine to tyrosine is catalyzed by an enzyme called phenylalanine hydroxylase. The overall reaction is the replacement of the 4-H by an OH group. The reaction mechanism, however, is not as simple! It was shown by labelling the 4-position with a tritium that this atom ends up at the position indicated in the figure! This particular shift of the hydrogen is known as *NIH shift*, since it was discovered by scientists at the National Institute of Health (USA). This type of shift has subsequently been discovered in many biological oxidation processes.

In biological reactions, isotopes have been extensively used as markers to understand reaction mechanisms.

Biosynthesis of Cholesterol

We will conclude this part with another classical study of a biological reaction, the conversion of acetate to cholesterol. The acetate ion can be labelled at either carbon atom with an isotope. Let us assume that we start with acetate which is labelled on the methyl carbon, designated with an asterisk. The conversion of acetate to squalene epoxide takes places through a number of steps, and produces the labelling as shown in *Figure 5a*. It can be easily seen that in squalene epoxide, all the methyl carbons (which are labelled) are linked to unlabelled carbon atoms only. However, upon oxidation of the lanosterol (cholesterol) it can be determined that acetic acid produced from the indicated neighbouring sites contains labels at both the carbons! This suggests that the conversion of squalene to cholesterol must

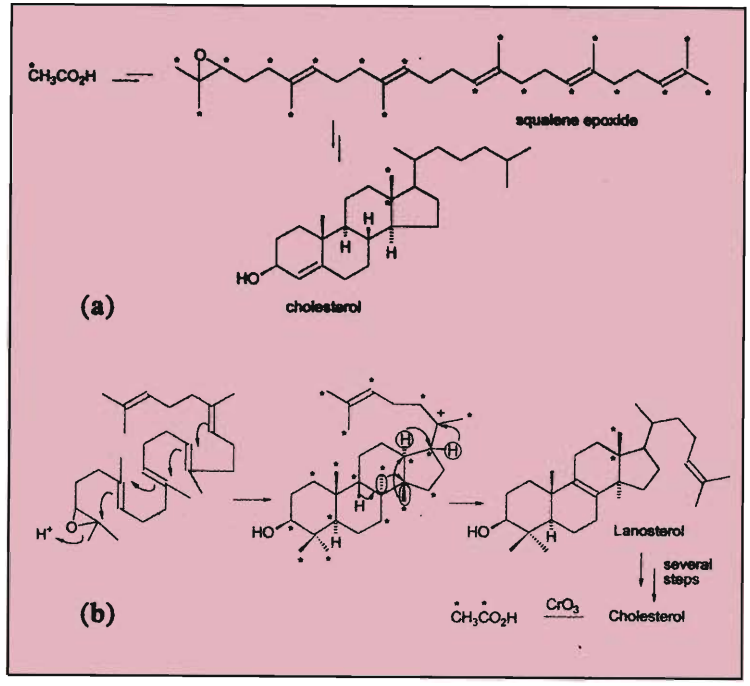

Figure 5 (a) Labelling pattern of squalene epoxide derived from labelled acetic acid. (b) Conversion of squalene epoxide to Lanosterol.

Suggested Reading

- **B K Carpenter.** ***Determination of Organic Reaction Mechanisms.*** **John Wiley. New York, 1984.**
- **F A Carey and R J Sundberg.** ***Advanced Organic Chemistry.*** **Part A. 3rd Ed. Plenum Press. New York, 1990.**

Address for Correspondence
Uday Maitra and
J Chandrashekhar
Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012 India

involve rearrangements in which methyl groups move around. The 'arrow-pushing' which fits all the observed facts is shown in the *Figure 5b.*

We can therefore see that isotopes provide a powerful way of monitoring reaction processes. Even reactions which are otherwise impossible to 'see' can be studied through isotopic labelling. In principle, some of these reaction mechanisms could have been probed using a suitable substituent. However, there is always the possibility that the substituent would alter the mechanism. Therefore, isotopic labelling is a preferred way of studying reaction mechanisms.

There is a more subtle isotope effect on reactions. The reaction rates are altered by small but significant magnitudes through isotopes. These changes, when quantified, shed light on the details of the reaction, especially about the transition state. In the next two articles of this series we will focus our attention on these effects.