

REVIEW

Tritium labelling of compounds by exchange in strongly basic media

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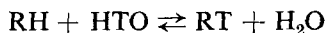
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ABSTRACT

A large body of organic compounds containing carbon-hydrogen bonds have very feeble acidic properties. In order that these compounds be made to ionise it is necessary to employ highly basic media. Recent developments now result in the use of several such systems, some of which can serve as a convenient source of labelling. The possibilities of the method are outlined together with the limitations, and a number of specific examples illustrating how compounds can be labelled are given.

Recently two monographs have appeared which concern themselves with tritium and its compounds^(1,2). Detailed accounts of the various methods for tritium labelling are given, including that of isotopic exchange which is amongst the simplest and, not surprisingly, one of the most widely used. There are a number of variants to this technique, amongst which the Wilzbach gas exposure method⁽³⁾ and the catalytic exchange⁽⁴⁾ using PtO₂ are perhaps the best known. There is in addition the simple hydrogen exchange reaction, which may or may not require a catalyst and which can be generally described by :

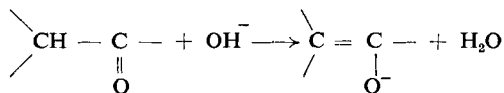


This kind of reaction has in the past tended to attract research workers interested in two different aspects of the reaction. Firstly, it concerned those whose main interest was in obtaining a labelled compound which may have been required as an intermediate in a more complex synthesis. Secondly, there were those who were interested in the mechanism of the reaction and

consequently the dependence of rate on such factors as the effect of catalyst, solvent composition and temperature. As a result of this separation of interests it often happens that the rate or mechanism of tritiation of a particular compound has been studied in some considerable detail and yet workers interested in the preparation of the same compound have resorted to what appears to be a needlessly complicated synthesis. The present article is an attempt to bridge this gap and at the same time outline the opportunities of labelling compounds by employing highly basic media.

Partly because of its ready availability, and partly because of the wide use of water as a solvent, the source of tritium is usually tritiated water. When a compound which contains hydrogen atoms attached to nitrogen, oxygen or sulfur is dissolved in this medium exchange occurs almost instantaneously and by removing the solvent either by distillation or by taking it up as a hydrated salt, the labelled compound is obtained. As the exchange is reversible it follows that by dissolving the compound in a large excess of water all the tritium will be lost. It is this behaviour which is the main disadvantage of the method and which has served to curtail its use as a method for labelling compounds. Clearly there can be only a limited use for such compounds as tracers.

For a much larger group of compounds the hydrogen atoms are attached to carbon and it is with this kind of compound that the present work will be concerned. If the C-H group is present in a compound such as benzene it has little tendency to ionise and it is not possible, for example, to prepare tritiated benzene by exchange in a sodium hydroxide-tritiated water medium. However, frequently the C-H group is adjacent to a —C=O , $\text{—CO}_2\text{R}$, or —NO_2 group, which confers a much higher acidity on the proton. Consequently, the ionisation of e.g. a ketone in basic media can readily occur :



The group of compounds such as β -diketones and β -keto esters are even stronger acids. It therefore follows that a large number of compounds can be labelled by taking advantage of the weakly acidic properties conferred upon them by the presence of activating groups. The label in such compounds will in general be more stable than in the case of —OH , —COOH , or —NH_2 containing compounds but considerable caution is necessary in their use as tracers, especially where acidic or basic media are employed.

For those compounds having no neighbouring groups to confer weakly acidic properties on the C-H groups, it has been customary to prepare the labelled form by synthetic routes. In these cases the rates of ionisation, (and therefore the rate of tritiation, if one neglects an isotope effect,) are extremely slow, and are in general related to the acid strength of the compound. In order

that the rate be increased it is necessary to increase the "efficiency" of proton abstraction and there are in general four methods whereby this can be achieved, depending on the concentration, the strength, the environment, and the nature, of the base.

(1) *Concentration* :

The rates of ionisation are invariably proportional to the concentration of base employed, so that a tenfold increase in the latter will result in a similar gain in the rate. As high concentrations of base, (e.g. 5 molar NaOH) are readily obtainable this serves as a useful means of increasing the rate.

(2) *Strength* :

The function of the base is to abstract the hydrogen atom, so that the anion formed can take up the tritium from the solvent. The more readily the first step is achieved then the more effective or stronger the base. In general the rate of ionisation increases with the strength of the base e.g. hydroxide ions are approximately 10^6 fold more effective than acetate ions. For practical purposes all the alkali hydroxides are strong bases, although the order is $\text{LiOH} < \text{NaOH} < \text{KOH}$. The alkoxides are still more powerful bases and a considerable gain in rate of ionisation can be obtained by employing them, the rate increasing in the order OMe^- in $\text{MeOH} < \text{OEt}^-$ in $\text{EtOH} < \text{OBu}^-$ -t in t-BuOH. The relative ratios (for a concentration of .01 m base) for the detritiation of acetophenone⁵, taking a value of unity for OH^- in H_2O were 3 : 20 : 10^3 .

(3) *Environment* :

For base catalysis in solution there is a growing amount of literature⁶ which points to the fact that the catalyst is heavily hydrated, $\text{B}^-(\text{H}_2\text{O})_n$, where n is an integer. In the case of hydroxide ions n has a probable value of 3; in other media its value is governed mainly by the size and charge of B, tending to decrease with increasing size. Clearly the fact that each hydroxide ion is surrounded by water molecules must inhibit its catalytic ability and this can be seen very clearly in media to which a certain amount of a polar aprotic solvent is added. The term "aprotic" indicates that these solvents have no protons that can form hydrogen bonds, and included in this category are such compounds as dimethyl sulfoxide, dimethylformamide and tetramethylene sulfone. The addition of, say, dimethyl sulfoxide to a OH^- - H_2O medium will result in the hydroxide ion becoming less solvated; in other words its activity will increase. In a manner entirely analogous to the corresponding strongly acidic solutions for which a H_0 function was introduced, a function H_- defined by $\text{H}_- = \text{pK}_{\text{HA}} + \log[\text{A}^-]/[\text{HA}]$ where HA is an indicator acid and A^- its anion, now measures the ability of the base to abstract

a proton. For those reactions in which the removal of a proton is the rate-determining step it can be shown⁷ that a plot of $\log k_{\text{ionis}}$ vs H_- should be linear with a slope usually in the region 0 to +1 depending upon how closely the activity coefficient of the anion of the indicator used in setting up the H_- scale resembles the activity coefficient of the transition state of the reaction. Basic media in which values of H_- as high as 26 can be obtained, as for example in the case of a solution of 0.01M OH^- in 99.5 % dimethyl sulfoxide⁸. This value can be compared with one of 12 for an aqueous solution containing the same concentration of OH^- . If the $\log k$ vs. H_- plot had a slope of unity, it would result in the rate of tritiation being faster by a factor of 10^{14} as compared to the rate in aqueous media, and even in those cases where the slope is less than unity a considerable gain in the rate of ionisation can be expected, making compounds with feebly acidic properties capable of ionisation and therefore of being labelled. These compounds can subsequently be employed as tracers in a wide variety of media, with little or no risk of the tritium label being lost. It should be noted that in such highly basic media the polar aprotic solvent introduced in order to achieve this high basicity will itself have ionisable protons and may therefore be preferentially labelled. This possibility will depend on the acid strengths of the substrate and the solvent. Such a situation arises in the $\text{DMSO} - \text{OH}^- - \text{H}_2\text{O}$ medium⁽⁹⁾ because although DMSO is an extremely weak acid ($\text{pK}_a \sim 32$), and would therefore only ionise at a low rate in aqueous base ($\sim 10^{-9} \text{ l}^{-1} \text{m sec}^{-1}$) in these highly basic media we find that it can be readily tritiated in a 10 % HTO-90 % DMSO solution containing .01M OH^- , with equilibrium being reached within one hour. In view of this factor an upper limit to the acid strength of a compound which can be labelled in a $\text{DMSO}-\text{H}_2\text{O}$ medium is set, corresponding to a pK_a of ~ 30 . For still weaker acids the difficulty can be overcome by using tritiated DMSO as the source of the tritium, and by employing a strong base such as methoxide, or better still, *t*-butoxide. Such an approach would also hold in other cases of highly basic media resulting from the desolvation of the base, although as yet no experimental details are available. The great advantage of this approach as opposed to the use of concentrated acidic media lies in the low concentration of base necessary thereby reducing the scope for producing labelled impurities; the presence of the latter will depend upon such properties as, for example, the oxidizing ability of the aprotic solvent.

(4) Nature

Strongly basic media can be obtained by a careful consideration of the acid strengths of a large number of solvents. One of the most frequently used is liquid ammonia¹⁰, which has a pK_a of ~ 35 , so that the NH_2^- ion has a high tendency to abstract a proton from a weak acid. In fact such is this affinity that even hydrocarbons react with potassium amide in liquid ammonia, and consequently the medium has been extensively used for studies

of deuteration of a large number of compounds. The exceedingly high catalytic activity can be seen from the observation ⁽¹¹⁾ that the reaction between hydrogen and KND_2 in ND_3 is faster by a factor of 10^{14} than that between hydrogen and KOD in D_2O , the catalyst concentration in each case being the same (1N). In some cases methylamine is preferred to ammonia in view of its greater basicity, and another system that has been used is cyclohexylamine, with either lithium or cesium cyclohexylamide being the catalyst¹². In these media it is found that a very wide range of compounds are soluble. On the other hand the amides are frequently very sensitive to traces of moisture and oxygen, and as the solvents usually have low dielectric constants the catalytic activity is somewhat reduced by the formation of ion pairs. As a result of these factors with the consequent need for a vacuum system, the highly basic media produced through the introduction of polar aprotic solvents are in general to be preferred.

Of the various systems studied the one which is likely to be most amenable to the labelling of a large variety of weakly acidic properties is that containing DMSO with either hydroxide or alkoxide ions acting as catalyst. It would be expected that such a powerfully basic system would be subject to at least some of the disadvantages of the other systems mentioned, and the following factors should be borne in mind:

- (1) DMSO is a potential health hazard¹³ in view of its ability to confer greater penetrative power into tissue for many organic compounds, with the result that greater care is required in handling solutions of compounds in DMSO than would be expected on the basis of the individual behaviour of the compounds in other media.
- (2) DMSO can behave as an oxidizing agent¹⁴.
- (3) There is a possibility of free radicals being produced by interaction with suitable substrates¹⁵.

ADVANTAGES OF THE METHOD

The following are some of the factors which commend the method as one which is suitable for the labelling of compounds having weakly acidic C-H groups :

- (1) DMSO as well as being able to produce highly basic media is a very good solvent for a large number of organic compounds, and is stable over long periods at room temperature.
- (2) The labelled material is invariably obtained in a pure form or at worst needs little radiochemical purification.
- (3) A satisfactory rate of incorporation is usually achieved at room temperature, so that compounds which tend to be unstable at high temperatures can be labelled.

- (4) Unsaturated compounds are not hydrogenated.
- (5) The mechanism of the labelling process is usually well understood and can lead to kinetic studies.
- (6) Depending upon the specific activity of the solvent (either tritiated water or tritiated DMSO), and the equilibrium constant of the reaction, the degree of tritium incorporation can be estimated. The role of DMSO in tending to move the position of equilibrium in favour of the products will of course increase the degree of incorporation.
- (7) The method is able to produce specifically labelled compounds or generally labelled compounds, depending upon the number and position of the ionisable protons in the molecule.

In view of the fact that little work on labelling of compounds in strongly basic media has been undertaken, the data in Table I refers not only to the tritiation of compounds in such media, but also to the results for detritiation, deuteration and dedeuteriation, as by suitable refinement such methods are capable of producing the tritium labelled compound. In addition to those compounds given in Table I the paper by Shatenshtein⁽¹⁰⁾ contains information on the deuteration of some aliphatic olefins and some hydrocarbons of the alicyclic and diene series. These have not been included as there is some uncertainty as to whether DMSO-containing media would lead to satisfactorily labelled products.

In order to illustrate the application of the method 3 examples of the preparation of labelled compounds are given. The acidity of these compounds, as given by the pK_a values, varies from 27 (triphenylmethane) to an approximate value of 40 for toluene, with dimethyl sulfoxide being intermediate (32). For this reason it would be necessary to use solutions whose basicity was lowest for triphenylmethane and highest for toluene. The following details illustrate this point.

Triphenylmethane $[(C_6H_5)_3T]$.

Dissolve 1 g. of the compound in purified dioxan and add in turn 1 ml. of tritiated water of the necessary activity and 4 ml of DMSO. Add some solid tetramethylammonium hydroxide so that the concentration of hydroxide is approximately 0.1 N and allow to stand for 24 hrs. Neutralise the solution with acid and add the contents to approximately 300 ml. of water when the labelled compound is precipitated. Separate off, recrystallise and dry.

Dimethylsulfoxide CH_3SOCH_2T .

To 5 ml. of DMSO add 0.1 ml. of tritiated water together with 0.1 ml of 0.1N NaOH and allow to stand for 3-4 hrs.

Neutralise the solution with acid, shake the solution with 20 ml. methanol in order to form MeOT which can be readily removed by distillation. The DMSO is then distilled under vacuum and the first portion, which contains some water, rejected.

TABLE I. — Examples of tritium labelling of compounds by exchange in strongly basic solutions.

| Substrate | Conditions | Labelled compounds | Ref. |
|---|--|---|------|
| Dimethyl sulfoxide | Exchange in OD ⁻ -D ₂ O at 25° C. | CD ₃ SOCD ₃ | 9 |
| Toluene | Tritiated DMSO containing KOBu-t(0.5 m); from 23 to 80° C | C ₆ H ₅ CH ₂ T | 16 |
| <i>o</i> , <i>m</i> , and <i>p</i> -xylene, trimethyl benzenes, tetramethyl benzenes, penta and hexamethylbenzene, cumene. | as for toluene; only hydrogens α to the aromatic ring undergo exchange. | ArCH ₂ T | 17 |
| 1- and 2-methylnaphthalene 3- and 9-methylphenanthrene 2-methylanthracene 1-, 2-, and 4-methylpyrene 3-, and 8-, fluoranthene | dedeuteration using lithium cyclohexylamide in cyclohexylamine at 50° C | ArCH ₂ T | 18 |
| <i>o</i> -, <i>m</i> -, and <i>p</i> -fluorotoluene <i>m</i> - and <i>p</i> -methoxytoluene <i>m</i> - and <i>p</i> -trifluoromethyltoluene | detrutiation and dedeuteriation using lithium cyclohexylamide in cyclohexylamine at 50° C | ArCH ₂ T | 19 |
| Benzene Naphthalene | detrutiation and dedeuteriation using lithium cyclohexylamide in cyclohexylamine; rates are 100 fold slower than for substituted toluenes. | C ₆ H ₅ T C ₁₀ H ₇ T | 20 |
| polycyclic aromatic hydrocarbons | as for benzene; generally labelled compounds produced. | C ₆ H ₅ T | 21 |
| Triphenylmethane diphenylmethane | deuteriation in ND ₃ containing KND ₂ at room temperature. | (C ₆ H ₅) ₃ CT (C ₆ H ₅) ₂ CHT | 10 |
| Heptane Cyclopentane Cyclohexane | as above but at a temperature of 120° C. | C _n H _{2n} T | 10 |

Toluene C₆H₅CH₂T.

To 5 ml. of toluene add 3 ml of tritium labelled DMSO followed by sufficient solid potassium *t*-butoxide as to make the solution 0.1 N. Allow to stand for 24 hrs before neutralising. The labelled toluene can then be obtained either by distillation or by pouring the contents into 300 ml. of water whereupon the toluene can be separated.

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