

## The Prospects for Stable Isotopes. Applications Involving Nuclear Magnetic Resonance Spectrometry

J. R. JONES

Chemistry Dept., University of Surrey, Guildford, Surrey.

Received April 20 1969.

### ABSTRACT

*Recent developments particularly in the field of nuclear magnetic resonance spectrometry suggest that there is a growing demand for compounds labelled with stable isotopes. In particular the isotopes  $H^2$ ,  $C^{13}$ ,  $N^{15}$  and  $O^{17}$  offer in some circumstances several advantages over the comparable radioactive isotopes and in this article we show how some workers have grasped the opportunities afforded. The availability of such compounds together with the problems associated with labelling and detection are discussed.*

Although a number of applications of the use of stable isotopes, particularly deuterium, had been recorded prior to 1940 the advent of the second world war brought with it considerable changes and amongst these the availability of a large number of radioactive isotopes was for the chemist one of the more important, and it was one of the reasons which led to the rapid expansion of scientific research both in industry and the universities. The inherent sensitivity of the radio-tracer technique together with (in most cases) a simple method of detection made the use of radioactive isotopes an extremely attractive one. It did however mean that interest in the field of stable isotopes and their applications was overshadowed for sometime by the new developments.

The opportunities created were quickly taken advantage of by the formation of what is now known as The Radiochemical Centre which had started out in 1940 as a small company mainly concerned with making luminous paints for aircraft and naval instruments <sup>(1)</sup>. In 1946 it was taken over by the government and some measure of the expansion which has occurred can be seen in the fact that it now has a turnover approaching £3 m. Of the four production departments, Organic, Inorganic, Natural Radioelements and Isotope Production it is the former with its concentration on tritium ( $H^3$ )

and carbon-14 which has shown the most dramatic increase in production mainly because of the wide use being made of such compounds in the investigation of biochemical problems and the development over the last ten years of liquid scintillation spectrometers able to detect the weak  $\beta$  radiation emitted by these two isotopes.

Since around 1960 there has been a reawakening of interest in the use of stable isotopes and this change has been brought about by a number of factors the most important of which has been the development of nuclear magnetic resonance spectrometry. It is interesting to note that the three elements to which high resolution NMR is particularly adaptable are hydrogen, phosphorus and fluorine since for each an isotope exhibiting a nuclear spin of  $\frac{1}{2}$  is available and these nuclei have large magnetic moments. Equally important is the fact that each isotope is present at or near 100 % natural abundance. At the same time there are amongst elements of low mass a number of stable isotopes e.g.  $C^{13}$ ,  $N^{15}$ ,  $O^{17}$  having spin properties which make them particularly useful for NMR studies provided the concentration of the isotope is sufficiently high. Although the sensitivity of the method employed in detecting a stable isotope will not in general approach that of the radiotracer technique (possible exceptions involve the use of activation analysis) improvements in performance can be expected (e.g. the development of magnets with superconducting coils) which will decrease the minimum detectable concentrations of isotope.

A better understanding of the factors involved in the radiochemical application of isotopes has come about with the passage of time and account must now be taken of the fact that in some cases undesirable side effects can be introduced if the concentration of radioisotope is very high. Thus data has been gathered on the radiation damage<sup>(2)</sup> induced in tritium labelled compounds of high specific activity on storage whereas the Wilzbach method<sup>(3)</sup> of labelling compounds which initially seemed so promising has fallen into disrepute because of the wide range of side products formed and the difficulty of purification. Similarly there are problems such as the investigation of the mechanism of a reaction within a mass spectrometer where it is not advisable to use radioactive isotopes.

Finally it is frequently the case that the radioisotopes of the elements of lower mass either emit a very weak radiation or have a very short half life which in many cases preclude their use as tracers. Thus the radioactive isotopes of nitrogen,  $N^{12}$ ,  $N^{13}$ ,  $N^{16}$ ,  $N^{17}$  all have half lives of less than 10 sec whilst a similar situation exists for the oxygen isotopes of mass number 13, 14, 15, 19 and 20.

#### HYDROGEN-2 (DEUTERIUM).

Although the increase in the use of deuterium labelled compounds has probably been less than that for  $C^{13}$  or  $O^{17}$  this can partly be explained in

terms of the greater interest which was shown in it at an earlier stage. This interest stemmed mainly from the central position which hydrogen holds in chemistry and the role played by it in proton transfer reactions. The very large mass difference between hydrogen and deuterium has at least two consequences : (1) it opened up the possibility of observing very large kinetic hydrogen isotope effects in reaction kinetics which should in turn lead to a better understanding of some of the factors which are not at present particularly clear e.g. what factors determine the potential energy surface in proton transfer reactions and what is the nature of the activated state; (2) inexpensive methods of deuterium analysis based on differences in physical properties between the ordinary and deuterium labelled compounds. Thus the falling drop method <sup>(4)</sup> shows that the densities of H<sub>2</sub>O and D<sub>2</sub>O are sufficiently different as to constitute a reliable means of measuring the percentage deuterium present in a sample. Likewise isotopic shifts in the infra-red spectra can also be employed <sup>(5)</sup>.

Of the many methods of preparing deuterium labelled molecules the simplest and in many ways the most interesting is that of exchange in alkaline media. This possibility arises from the fact that many organic compounds have feebly acidic properties and in such media can be made to ionise with the result that the anion can take up deuterium from the solvent. The range of compounds that can be prepared in this way has recently been extended as a result of the tremendous rate enhancing influence dipolar aprotic solvents such as dimethyl sulfoxide can have on rates of ionisation <sup>(6)</sup>. Thus acids as weak as triphenylmethane and toluene can be prepared in this way. In addition some of the methods used for tritium syntheses <sup>(2)</sup> can be successfully adapted for deuterium.

Analytically the falling drop, infra-red and NMR methods can all estimate the degree of deuterium incorporation whilst the latter method together with that of mass spectrometry provide a powerful tool in the elucidation of molecular structures <sup>(7)</sup>. In the case of NMR the interpretation is frequently simplified by employing deuterated solvents many of which can be readily prepared by well known synthetic routes. These include C<sub>6</sub>D<sub>6</sub>, CD<sub>3</sub>SOCD<sub>3</sub>, CDCl<sub>3</sub> and C<sub>6</sub>D<sub>5</sub>N.

### CARBON-13.

This isotope is present to the extent of approximately 1% in natural carbon although the exact percentage varies with the nature of the source. In principle C<sup>13</sup> like other nuclides with odd mass numbers can be determined by NMR although its sensitivity is less than might be desired. Thus at constant field the signal for C<sup>13</sup> is  $1.76 \times 10^{-4}$  relative to 1 for H<sup>1</sup>. On the other hand since its nuclear spin is  $\frac{1}{2}$  the C<sup>13</sup> nucleus has no nuclear quadrupole moment and sharp signals can be expected if spin couplings with other nuclei can be resolved and the concentration of C<sup>13</sup> enriched <sup>(8)</sup>. The advantages which result from this can be seen from the following example. In acetylene where

the hydrogen nuclei are all chemically equivalent the NMR spectrum consists of a single line and it would seem that no information could be obtained concerning the magnitudes of the different inter-proton coupling constants which are in fact of considerable interest. However by preparing  $C^{13}$  labelled acetylene starting from  $BaC^{13}O_3$  it is possible, as shown by Lynden-Bell and Sheppard<sup>(9)</sup> to calculate these parameters by analysis of the proton spectra obtained from mixtures of the normal hydrocarbon and those containing one or two magnetic  $C^{13}$  nuclei. The magnitudes of the ( $C^{13}$ , H) and ( $C^{13}$ ,  $C^{13}$ ) coupling constants are also obtainable and the couplings across electron pair bonds are of particular interest in relation to the electronic structure of the molecule. In particular the coupling constants can be related to the amount of *s* character of C-H bonds whereas the degree of hybridization are of special interest in determining the acidic character of the protons.

Carbon-13 enrichment can be achieved by chemical exchange reactions that involve either cyanide<sup>(10)</sup> or carbon dioxide<sup>(11)</sup> so that  $C^{13}$  concentrations in excess of 60 atom % can now be purchased. In other cases it is possible to adapt some of the well known methods of  $C^{14}$  labelling<sup>(12, 13)</sup>. Thermal diffusion of various compounds or distillation (carbon monoxide, for example) are also frequently employed.

Although infra-red techniques can be employed to determine  $C^{13}/C^{12}$  ratios it is generally assumed to be less accurate than mass spectrometry. In the latter the material is usually degraded to carbon dioxide and in some cases it will be necessary to take into account that during this process isotope fractionation may occur. The accuracy of the assay is very good (frequently better than  $\pm 0.05\%$ ) and the precision of the analysis together with the low enrichment required makes  $C^{13}$  an attractive isotope for many studies.

#### NITROGEN-15.

Considering its importance in agriculture little work has been done using labelled nitrogen mainly because there is only a single isotope ( $N^{15}$ ) suitable for tracer studies. The natural abundance of  $N^{15}$  is 0.38 % although it can be obtained in concentrations in excess of 60 atom % by using a thermal diffusion column with atomic nitrogen being produced by an electric discharge or by chemical exchange. As the minimum detectable concentration of  $N_2^{15}$  is approximately 0.004 atom % a possible dilution factor of  $1.5 \times 10^4$  is available which although large still compares rather unfavourably with factors of up to  $10^{10}$  which are associated with the radioisotope technique.

As with deuterium and oxygen-17 isotopic exchange reactions are available for synthesizing  $N^{15}$  labelled compounds e.g.



although these tend to be more restricted and hence greater emphasis is placed on synthetic routes frequently starting with labelled nitrate or nitrite.

Nitrogen itself is an ideal gas for  $N^{15}$  analysis. In the case of organic compounds the nitrogen is readily obtained in the form of gas after a Kjeldahl digestion and subsequent oxidation (in vacuo) of the ammonia with hypobromite. The precision of analysis by mass spectrometry can be as high as  $\pm 0.003$  atom % but equally satisfactory results may be obtained using a  $N^{15}$  analyzer the operation of which depends on the wavelength separation of the emission bands of the molecules  $N_2^{14}$ ,  $N_2^{15}$  and  $N^{14}N^{15}$ . The band heads are to be found in the range from 2977 to 9990 Å and correspond to the 2-0 transition. The intensities of the band heads are measured and their ratio computed. The method offers a rapid and precise means of  $N^{15}$  analysis when only small quantities of material are available.

Since  $N^{14}$  has a quadrupole moment a species with a nitrogen atom in an asymmetric electron distribution has a broad NMR signal which is so great in some compounds as to make the signal essentially unobservable and any fine structure is wiped out. This difficulty may be eliminated by using  $N^{15}$  which has no quadrupole moment and which resonates at 4.315 Mc in a 10,000 gauss field. Further applications of  $N^{15}$  NMR, for example studies of cyano and amine complexes of various metals can be anticipated but as yet the relatively few investigations reported refer to structural problems <sup>(14)</sup>.

#### OXYGEN-17.

Of the three stable isotopes of oxygen ( $O^{16}$ ,  $O^{17}$ ,  $O^{18}$ ) only  $O^{17}$  has a magnetic nucleus. It has a spin quantum number of  $5/2$  and a gyromagnetic ratio of 577 c/sec/gauss but is only present in natural abundance to the extent of 0.037 atom %. This again accounts for the relatively little work which has been done using labelled oxygen although in view of the wide occurrence of oxygen in compounds its importance should be comparable to that of hydrogen and carbon.

In order that NMR signals from compounds containing  $O^{17}$  in natural abundance be obtained it is necessary to have an extremely sensitive and noise free spectrometer and only when concentrations in excess of 10 gram atoms per litre are achieved can such signals be usefully employed. Fortunately water containing up to 30 atom % of  $O^{17}$  has been commercially available for several years and recently enrichment up to 90 atom % has been achieved by the thermal diffusion of oxygen gas. In view of the fact that oxygen exchange reactions are numerous <sup>(15, 16)</sup> and frequently proceed rapidly a number of compounds of the required enrichment can be obtained by exchange with  $H_2O^{17}$ . In this respect  $O^{17}$  is superior to  $O^{18}$  as a label (even though the cost favours the latter) as the kinetics of fast reactions can be studied using NMR whereas  $O^{18}$  work would require the separation and purification of the reacting species prior to conversion into a gas suitable for mass spectrometer analysis. Advantage of this has been taken by Meiboom <sup>(17)</sup> in his studies of proton transfer reactions in aqueous solutions. From the pH dependence of the proton line width values of  $10.6 \times 10^9$  and  $3.8 \times 10^9$   $l\ m^{-1}\ sec^{-1}$

were obtained for the reactions  $\text{H}_2\text{O} + \text{H}_3\text{O}^+ \rightarrow \text{H}_3\text{O}^+ + \text{H}_2\text{O}$  and  $\text{H}_2\text{O} + \text{OH}^- \rightarrow \text{OH}^- + \text{H}_2\text{O}$ , respectively. Another problem to which the  $\text{O}^{17}$  nucleus provides a unique probe is that of hydration of ions in solution and in particular solvation numbers and the lifetime of water molecules in the solvation shell. More details can be found in a recent review article <sup>(18)</sup>.

#### AVAILABILITY AND COST.

Although a number of manufacturers of chemical compounds include a few deuterated compounds in their catalogues they have in general refrained from entering the field of isotopic synthesis. Similarly the Radiochemical Centre continues to produce radioactive compounds only and it has been left to companies such as Merck Dohme and Sharpe in Canada to provide most of the market needs although a few smaller concerns in Britain are now able to provide  $\text{C}^{13}$ ,  $\text{N}^{15}$  and  $\text{O}^{17}$  compounds as well as provide a synthesis of a desired compound.

One of the reasons for the somewhat limited use which has been made of compounds labelled with stable isotopes has been that of cost and although this is no longer true for most deuterated compounds it can still be a major factor in deciding whether or not to start a project which requires  $\text{C}^{13}$ ,  $\text{N}^{15}$  or  $\text{O}^{17}$ . Fortunately it is frequently sufficient to obtain the required material at a low enrichment of say 10 % or alternatively a synthesis of the required material can be made by starting with one of the less costly compounds e.g.  $\text{H}_2\text{O}^{17}$ . In this way most of the difficulties can be overcome and with the ability of NMR techniques to give a continuous record of the changes occurring the amount of labelled compound required is considerably less than if the material had to be separated purified and converted into a suitable form for injection into a mass-spectrometer.

With the expanding use being made of labelled isotopes and the continuous improvements being made in synthetic methods there has been a slow but consistent decrease in costs and although statistics for a large body of compounds are difficult to obtain it is known that in the case of deuterated water of 98 % enrichment the cost was approximately 5 shillings/gramme in 1958 whereas it is now approximately 2 shillings/gramme. The actual price of course varies with the particular requirement e.g. a 25 gramme ampoule of the same enrichment costs 3/9 per gramme whereas the cost of a kilo container works out at 1/2 per gramme. During the same time interval the sales of  $\text{D}_2\text{O}$  have increased by a factor of between 4 and 5.

In conclusion then it can be safely stated that the need for compounds labelled with the stable isotopes  $\text{H}^2$ ,  $\text{C}^{13}$ ,  $\text{N}^{15}$  and  $\text{O}^{17}$  will continue to grow and that in combination with nuclear magnetic resonance spectrometry they offer a very sensitive means of attacking various outstanding chemical problems.

The author thanks Mr. J. M. Devereux for details of the cost of deuterium oxide.

## REFERENCES

1. Chem. in Britain, 1967. 3, 390.
2. EVANS, E. A. — Tritium and its Compounds, London : Butterworths, 1966
3. WILZBACH, K. E. — *J. Am. Chem. Soc.*, **79** : 1013 (1957).
4. SWAIN, C. G. and LABES, M. M. — *J. Am. Chem. Soc.*, **79** : 1084 (1957).
5. STEVENS, W. H. and THURSTON, W. — Atomic Energy of Canada Ltd. Rept. no. AECL-295.
6. JONES, J. R. — *J. Labelled Compds.*, **4** : 197 (1968).
7. WILLIAMS, D. H. — *Chem. in Britain*, **4** : 5 (1968).
8. STOTHERS, J. B. — *Q. Rev. Chem. Soc.*, **19** : 144 (1965).
9. LYNDEN-BELL, R. M. and SHEPPARD, N. — *Proc. R. Soc. A.*, **269** : 385 (1962).
10. UREY, H. C. and GRIEFF, L. J. — *J. Am. Chem. Soc.*, **57** : 521 (1935).
11. HUTCHINSON, C. A., STEWART, D. W. and UREY, H. C. — *J. Chem. Phys.*, **8** : 532 (1940).
12. CATCH, J. R. — Carbon-14 Compounds, London : Butterworths, 1961.
13. RAEEN, V. A., ROPP, G. A. and RAEEN, H. P. — Carbon-14, New York : Mc Graw-Hill 1968.
14. ROBERTS, B. W., LAMBERT, J. B. and ROBERTS, J. D. — *J. Am. Chem. Soc.*, **87** : 5439 (1965).
15. SAMUEL, D. and SILVER, B. L. — Advances in Physical Organic Chemistry, Volume 3, V. Gold(ed) London : Academic Press 1965.
16. DOSTROVSKY, I. and SAMUEL, D. — Inorganic Isotopic Synthesis, New York : W. Benjamin, 1962.
17. MEIBOOM, S. — *J. Chem. Phys.*, **34** : 375 (1961).
18. LUZ, Z. and SILVER, B. L. — *Q. Rev. Chem. Soc.*, **21** : 458 (1967).