Tetrahedron Letters, Vol.22, No.36, pp 3525 - 3528, 1981
Printed in Great Britain

0040-4039/81/363525-04$02.00/0

DEUTERIUM LABELLING AT THE NATURAL ABUNDANCE LEVEL AS STUDIED BY HIGH FIELD QUANTITATIVE ^{2}H NMR

G.J. Martin* and M.L. Martin
Laboratoire de Chimie Organique Physique, Université de Nantes, CNRS-ERA 315
44072 Nantes Cedex

Summary : It is shown that very large differences exist in the internal distribution of natural deuterium and the ^{2}H quantitative NMR method is proposed as an efficient tool for characterizing the chemical and geographical origin of a molecule and for investigating chemical and biochemical mechanisms.

Deuterium enrichment of molecular sites has been used for a long time in order to study chemical and biochemical mechanisms (1). Although deuterium labelling does not present the same difficulties as other isotopic substitution reactions using ^{3}H, ^{14}C, ^{17}O, ^{13}C... the necessity of synthetizing deuterated derivatives is a somewhat constraining condition in the investigation of a reaction mechanism. In addition, it is well known that extensive replacement of hydrogen with deuterium may alter the chemical mechanism, and the change is particularly drastic for biochemical processes. Thus working with molecules at the natural abondance level of the heavy isotope should be very gratifying with respect to the time saved and to the quality of the experimental conditions. In fact, it has been shown that many organic compounds contain an overall deuterium percentage which is slightly different from that of the Standard Mean Ocean Water (SMOW) in which % D ≈ 0.015 (2). Mass spectrometry has been extensively used to measure the overall molecular change in % D, expressed as $\delta D = \left(\frac{D/H(sample)}{D/H(SMOW)} - 1\right) 1000$ (3) but this technique is usually not able to make a discrimination between the relative deuterium contents of the different chemical sites of a molecule. We wish to present here preliminary results showing that high field quantitative deuterium NMR can provide a new method for determining both the δ D values and the *relative deuterium contents* of a molecule. Indeed this method offers a simple and effective way of studying a reaction path or the origin of a product by following the deuterium spectrum of specific organic groups *labelled at the natural abundance level.*

We have first chosen the ethyl group as the organic probe, but other groups present analogous properties. If we define the parameters R(I) = 3 I(CHD)/I(CH_2D) or R(S) = 3S(CHD)/S(CH_2D) where I and S represent the intensities or the integrated area of the deuterium resonance lines of the methylene and methyl groups, table 1 shows that these parameters greatly change in a series of ethyl derivatives. From these results it is revealed that *very high relative deuterium*

contents can be found in the methylene groups since the proportion of ^2H in the methylene with respect to the methyl group reaches a value of about 2.6/3 in $C_6H_5C_2H_5$ for example instead of the ratio 2/3 which would correspond to the statistical repartition. It is also worth noting that a deuterium depletion in the methylene with respect to the methyl site is likely to occur when the ethyl group is substituted on a nitrogen atom.

Moreover, it is also easily practicable to measure the overall deuterium content of the ethyl group -and that of the whole molecule as well- by comparing the CH_2D signal to that of a given reference, contained in a coaxial cell. Since the deuterium content of the reference can be measured with respect to the international standard SMOW, it is then possible to determine by NMR the δD value previously defined. The results are consistent with those of mass spectrometry as far as only overall δD values are concerned, and the NMR method offers a more rapid and more versatile way of measuring total natural abundance deuterium contents.

Obviously the proton spectra of the compounds shown in table I display integral curves for the ethyl group which always lead to an intensity ratio very close to 2/3 i.e to an R(I) value $3I(CH_2)/I(CH_3) = 2$. It is clear that proton spectroscopy is unable to detect very small changes in the ^2H content of the C_2H_5-groups.

The values of the parameters R(I) given in table I should not be considered as absolute numbers. In fact for a given molecule the calculated R(I) value depends on the experimental conditions which have been selected and especially on the value of the time constant of the exponential multiplication which is applied to the free induction decay. In order to determine the actual ratios of deuterium contents it is preferable, in principle, to use the surface parameters R(S) derived from the integrals. However these parameters often suffer from a poorer precision than those derived from intensity measurements and both values are reported in table 1. Work is now in progress to improve the experimental procedures and to define the accessible accuracy on a statistical basis but it is from now on clear that *the quantitative ^2H NMR method offers a good means of determining the geographical origin and discussing the chemical evolution of the compounds.* In this respect it can be noticed that two samples of ethylacetate for example, purchased from two different dealers in Europe and in America display R(I) values, 2.48 ($\pm$0.03) and 2.55 ($\pm$0.02), which are significantly different. The chemical history of the compound studied is evidently a determining factor and the relative deuterium contents reflect, in particular, the importance of the H/D exchange reactions in acidic or basic media. Interestingly we observe that the phenyl group of ethylbenzene is characterized by a very high degree of natural enrichment with respect to the ethyl since $R(I) = 3I\ (C_6H_4D)/I(CH_2D)$ reaches a value 8.31 ($\pm$ 0.10) for the compound obtained in a Friedel-Crafts reaction with benzene and 7.28 ($\pm$ 0.08) for a commercial sample, instead of the factor 5 which would correspond to a statistical repartition. This large difference between the deuterium contents of the aromatic and ethyl groups reflects the different origins of these fragments since the aromatic structures which result directly from sugars are known to possess a higher deuterium content than the aliphatic structures which are formed through a more complex biosynthetic pathway involving the acetylcoenzyme A and characterized by isotopic fractionation (3).

C_2H_5-X	R (I)	R (S)	C_2H_5- X	R (I)	R (S)
C_2H_5O-C_2H_5	2.246	2.260	C_2H_5-C≡N	2.018	2.013
C_2H_5O-CH=CH_2	2.203	2.04	C_2H_5-C_6H_5	2.626	2.594
$(C_2H_5O)_3CH$	2.450	2.440	C_2H_5-Br	2.572	2.558
C_2H_5O-$COCH_3$	2.467	2.462	C_2H_5-I	2.550	2.538
$(C_2H_5O)_2CO$	2.322	2.190	$C_2H_5NH_2$	1.988	1.934
$(C_2H_5O)_2SO_2$	2.240	2.236	$(C_2H_5)_2NH$	1.758	1.732
$(C_2H_5O)_3PO$	2.067	2.149	$(C_2H_5)_3N$	1.854	1.819
C_2H_5-SC_2H_5	2.299	2.136	$(C_2H_5)_2N$-CHO	(a)	2.008
C_2H_5-CHO	2.292	2.217	$(C_2H_5)_2N$-CH_2COOEt	1.516	1.695
C_2H_5-$COCH_3$	2.325	2.476	C_2H_5-N=C=O	1.892	1.814
C_2H_5-COOH	2.431	2.547	$C_2H_5NO_2$	2.223	2.219

TABLE 1

Intramolecular deuterium distribution in ethyl derivatives. Due in particular to unequal line widths, the R(I)values slightly differ from the R(S) values. (a) non equivalence of diastereotopic groups.

The ^2H spectra were obtained in the ^1H decoupled mode (broad band, high power) with a 5s pulse repetition time and a 90° flip angle. The sweep width was 1200Hz and usually 500 to 2500 transients were stored, using a 15 mm OD sample tube and a Brüker WM 250 spectrometer (ν_o (^2H) = 38.897 MHz). The reproducibility of the experiment is expressed as the standard deviation of 10 different spectra σ = 0.01 to 0.04 (line broadening 2s). The R(I) and R(S) values are fairly well correlated : R(I) (±0.09) = 0.115 + 0.955 (±0.07) R(S), R = 0.95, N = 21.

We therefore demonstrate that the quantitative ^{2}H NMR method at the natural abundance level may be a powerful analytical method for the study of chemical mechanisms and is expected to be highly interesting for determining the origin and following the fate of the species in the biosynthesis of natural products.

Acknowledgements : We thank Mrs MABON and Mrs MICHON for an efficient technical assistance.

REFERENCES

1. C.J. Collins, Isotope and organic reaction mechanisms in Adv. Phys. Org. Chem., Ed. V. Gold, Academic Press, Vol. 2, 1964, p. 3.

2. M.J. Garson and J. Staunton, Chem. Soc. Rev., 8,539, (1979).

3. H. Craig, Science, 133, 1833, (2961).

4. J. Bricout, Rev. Cytol. Biol. Veget. Bot., 1, 133, (1978).

(Received in France 22 July 1981)