

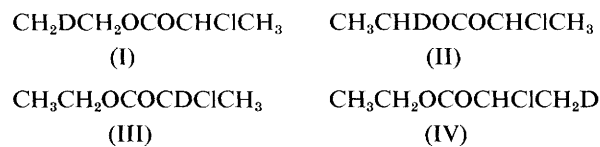
## Natural Selective $^2\text{H}$ Labelling applied to the Study of Chemical Mechanisms; Labelling without Enrichment

Gerard J. Martin,\* Maryvonne L. Martin, Françoise Mabon, and Marie-Jo Michon

*Laboratoire de Chimie Organique Physique, C.N.R.S., ERA 315, Université de Nantes, 44072 Nantes Cedex, France*

It is shown that the internal distribution of deuterium may considerably differ from the statistical distribution and that this property is exploitable, by quantitative  $^2\text{H}$  n.m.r. measurements, for investigating reaction mechanisms using isotopic labelling without the need for selective enrichment.

Deuterium labelling is one of the most useful ways of studying reaction mechanisms and it has been extensively employed since the discovery of the hydrogen isotope<sup>1</sup> in 1933. However, the statistical or selective replacement of hydrogen atoms by deuterium often requires difficult or tedious synthetic preparation. We have recently shown that non-enriched organic molecules may have a molecular deuterium distribution which differs considerably from the statistical one.<sup>2</sup> We now demonstrate that the determination of the internal  $^2\text{H}$  contents, by quantitative  $^2\text{H}$  n.m.r. spectroscopy at the natural-abundance level, offers an original and promising method for identifying the origin of a given chemical species and following its fate during chemical and biochemical reactions.



In order to illustrate the capability of the method we have considered the case of  $\alpha$ -chloroethyl propionate  $\text{CH}_3\text{CH}_2\text{OCOCHClCH}_3$  obtained from acetaldehyde and ethanol. Owing to the low natural abundance of  $^2\text{H}$  the probability of

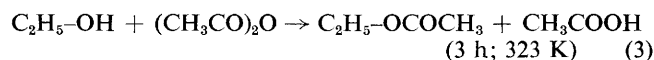
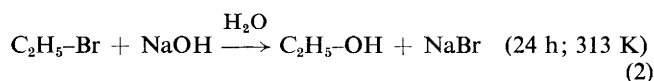
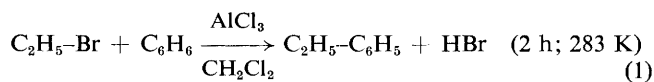
having two deuterium atoms in the same molecule is very small (1 in *ca.*  $10^8$ ) and the  $^2\text{H}$  n.m.r. spectrum corresponds in fact to that of the four monodeuteriated species (I)—(IV). Using a Bruker WM 250 spectrometer and selecting suitable experimental conditions<sup>3</sup> we have obtained sufficiently high signal-to-noise ratios to ensure a reproducibility of  $\pm 1\%$  in the intensity measurements for neat liquids in 15 mm outside diameter tubes. The molar fraction  $f(i)$  of each monodeuteriated species can be directly derived from the  $^2\text{H}$  spectrum;  $f(i) = I(i)/\sum I(i)$  [with  $i = (\text{I})$  to (IV) in the present case] where  $I(i)$  denotes the area of signal  $i$  (with identical line-widths the heights of the signals, which may be determined with a better accuracy than the integrals, can also be used, and this was done in the present experiments). The results in Table 1 show that the molar fractions  $f(i)$  differ significantly from those which would correspond to a statistical distribution of  $^2\text{H}$  between the four sites of  $\alpha$ -chloroethyl propionate. [In terms of precision the last digit of the  $f(i)$  values is significant.]

These results prove that the methyl group of the ethyl fragment is strongly depleted in  $^2\text{H}$  as compared to the methylene. Moreover the ethyl moiety has an overall deuterium content lower than that of the acyl fragment. This behaviour is understandable if we consider that natural ethyl alcohol is usually depleted<sup>4</sup> in  $^2\text{H}$  (deuterium deficit of *ca.* 200‰ with respect to

Table 1

i	(I)	(II)	(III)	(IV)
$f(i)$ { Statistical	0.33	0.22	0.11	0.33
Experimental	0.26	0.22	0.14	0.38

the Vienna Standard Mean Ocean Water<sup>5</sup>) and that acetaldehyde synthesized from oil and natural gas should have a higher deuterium content. We dispose therefore of a method which is potentially very useful for the study of chemical mechanisms. In fact when investigating chemical reactions it must be kept in mind that protolytic processes may induce deuterium-hydrogen exchanges which could modify the deuterium distribution.<sup>6,7</sup> In order to specify further the capabilities of the method, we have investigated the three well known reactions (1)–(3) which follow different mechanisms.



Reaction (1) is an electrophilic substitution and reactions (2) and (3) are nucleophilic substitutions in basic or acid media respectively. If the attention is focused on the ethyl fragments of the ethyl derivatives, it is convenient to define the normalized ratio  $R = 3 I(\text{CH}_2)/I(\text{CH}_3)$ . Since a statistical distribution of  $^2\text{H}$  would correspond to a probability factor  $R = 2$ , [ $I(\text{CH}_2)/I(\text{CH}_3) = 2/3$ ], values of  $R$  greater than 2 indicate a relative  $^2\text{H}$  enrichment in the methylene site with respect to the methyl site (or a relative depletion in the methyl as compared to the methylene). We have determined the deuterium distribution factor,  $R$ , of the ethyl group in the starting materials of reactions (1)–(3) and have checked it in the reaction products (Table 2). In the case of reaction (3) the esterification was performed with two ethanol A and B from different origins: A, which was extracted from sugar-beet, is characterized by a much higher internal ratio,  $R$ , than B which was derived from corn, and this property was checked for several samples. Similarly in the case of reactions (1) and (2)

Table 2

Reaction	Ethyl derivative	$R$ in starting material	$R$ in product
(1)	{ A	$2.56 \pm 0.02$	$2.58 \pm 0.02$
	{ B	$2.22 \pm 0.03$	$2.28 \pm 0.01$
(2)	{ A	$2.56 \pm 0.02$	$2.66 \pm 0.04$
	{ B	$2.22 \pm 0.03$	$2.29 \pm 0.03$
(3)	{ A	$2.72 \pm 0.01$	$2.71 \pm 0.02$
	{ B	$2.22 \pm 0.01$	$2.19 \pm 0.02$

two different samples of  $\text{C}_2\text{H}_5\text{Br}$  [Prolabo (A) and Aldrich (B)] were used.

The results prove that the natural labelling is not modified significantly in these reactions. [Owing to some differences in the linewidths the occurrence of small systematic errors ( $< \pm 0.05$ ) cannot be disregarded at the present state of the technique]. Interestingly we show that the differences between the labelling ratios of the two starting products A and B are maintained in the final derivatives for all three reactions. The fact that the esterification of ethanol for example occurs with complete preservation of the ethyl group is confirmed with good precision. The method offers therefore a very efficient tool for following the fate of molecular fragments in chemical transformations and consequently it provides a new way of investigating reaction mechanisms.

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