A Simple Method for Selective Isotopic Hydrogen Labelling of Amino Acids and of RCH_2COOH and Related Alcohols

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Summary DCl and TCl catalyse the exchange between isotopic water and the α-hydrogens of RCH_2COOH and amino acids; reduction of the resulting acids with borohydride yields the corresponding selectively labelled alcohols.

THE recent publication[1] of a simple and inexpensive method for the preparation of RCD_2COOH and RCD_2OH prompts us to report an even simpler and more general procedure for the specific isotopic hydrogen labelling of organic acids, including amino acids, and their related alcohols. The

present work demonstrates that there is a limitation to the conditions under which compounds labelled by the preceding method[1] can be used in tracer studies.

Our procedure depends upon an observation made during the exchange of aromatic compounds with D_2O in the presence of a homogeneous platinum(II) catalyst[2] that the solvent, acetic acid, in these reactions deuteriated slowly in the methyl group in the presence of DCl. Shatenshtein[3] has previously commented on the slow randomisation of hydrogens in acetic acid at 120° but has not developed the experimental conditions for use as a labelling technique. The use of OD^- to promote exchange in organic acids has also been reported.[4]

We have now examined the feasibility of using the acid catalysed reaction as a general isotopic labelling tool and have found that the rate of exchange is pH dependent, relatively rapid isotope incorporation being obtained at

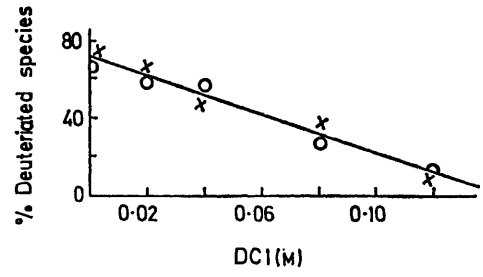

FIGURE. *Acid catalysed exchange in phenylalanine.* DL-Phenylalanine (0·05 g) with 2 ml. MeCOOD:D_2O (2:1) and DCl; reacted 4 h at 120°. (○ distribution in m.s. fragment $H_2\overset{+}{N}=CH\cdot CH_2Ph$). (× distribution in m.s. fragment $H_2\overset{+}{N}=CH\cdot COOH$).

TABLE

Hydrogen isotope exchange in carboxylic acids[a]

Compound	System[b]	D (%) (in active positions)	Deuterium distribution D_0	D_1	D_2	D_3
MeCOOH	DAc	19·0	71·5	5·7	17·1	5·7
	DCl	26·9	53·1	21·3	17·5	8·1
$PhCH_2COOH$	DAc	5·5	90·3	8·5	1·2	
	DCl	64·6	11·5	41·1	44·0	3·4
Ph_2CH COOH	DAc	20·1	77·8	20·1	2·1	
	DCl	72·0	21·5	71·0	6·5	
PhCH = CHCOOH	DAc	3·0	97·0	3·0		
	DCl	25·1	74·9	25·1		
H_2NCH_2COOH	DAc	45·0	34·6	40·9	24·5	
	DCl	26·7	56·6	33·1	10·1	
$PhCH_2CHNH_2COOH$	DAc	68·2	31·8	68·2		
	DCl	22·2	77·8	22·2		
$PhCHNH_2COOH$	[c]	91·7	6·4	91·7	1·9	

[a] Samples contained organic acid (0·15 g) and exchange media (1·0 ml) (see b); reacted at 120° (4 h); deuterium in carboxyl and amine groups back exchanged before analysis. Orientation of isotope confirmed by n.m.r. Deuterium distribution by low voltage m.s.[2] [b] DAc = MeCOOD (2 mols) in D_2O (1 mol); DCl = MeCOOD + DCl (2M). [c] $PhCHNH_2COOH$ (0·06 g) in acid solutions (2 ml) containing MeCOOD (2 mol), D_2O (1 mol) and DCl (3×10^{-3} mol); reacted at 80° (80 h).

120 °C in a range of simple carboxylic acids with 2M DCl in acetic acid (Table). Exchange occurs specifically in the α position as shown by n.m.r. and low voltage mass spectroscopy.[2] The procedure also yields an excellent source of selectively deuteriated and/or tritiated acids. When extended to the α-amino acids, rate of exchange is found to depend on the structure of the side chain. Because of the presence of the NH_2 group, rate of exchange in these latter compounds in the presence of acetic acid is inversely proportional to the hydrochloric acid concentration (Figure).

Acids labelled by this method should be used with care in tracer studies, since an isotope in the α position may be labile in certain pH ranges.

The versatility of the present technique may be extended by reducing the esters of the specifically labelled acids with borohydride to give the corresponding labelled alcohols. By contrast with the labelled acids, an isotope in the corresponding labelled alcohols is not labile under similar pH conditions.

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