Tritium Labelling of Optically Active Amino-acids by the Wilzbach Procedure

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THE labelling of optically active amino-acids by the Wilzbach[1] technique by exposure of the compound to tritium gas is important to both biological and fundamental radiation chemistry studies. Previous Wilzbach labelling experiments[2] with other organic compounds have shown that a preference for retention of configuration occurs in the tritiation of crystalline solids, whereas racemization predominates with liquids. However, preliminary results[3,4] with the amino-acids (methionine, proline, tryptophan, and glutamic acid) show that significant racemization can occur even in the solid phase.

The amino-acids are unique in these studies since these compounds contain NH bonds which are known to be sensitive to rupture by ionizing radiation. In particular, loss of hydrogen to give an imine intermediate [eq. (1), R = R^1 = H] has been suggested as a predominant radiolysis pathway.[5] If, however, the hydrogen atoms on the

$$\begin{matrix} MeCH\cdot CO_2H \\ | \\ R^1NR \end{matrix} \rightarrow \begin{matrix} MeC\cdot CO_2H \\ \| \\ NR \end{matrix} + R^1H \qquad (1)$$

nitrogen are replaced by substituents, then the disubstituted derivative cannot give this reaction. Thus, if this mechanism is correct, tritiation of optically active alanine (R = R^1 = H) and benzoylalanine (R = H, R^1 = Bz) should proceed with appreciable racemization whereas predominant retention of configuration should be observed with phthaloylglutamic acid (I).

In the present experiments, two different methods of separation and analysis were used. With L-benzoylalanine and the phthaloylglutamic acids, the compounds, after tritium exposure, were diluted with racemic carrier, the two enantiomorphs were chemically separated,[6] and each enantiomorph was recrystallized from aqueous methanol to constant specific activity, then counted by the ion-chamber–vibrating reed method.[7] With the D- and L-alanines after exchange, carrier DL-alanine was added, then the labile tritium was removed and the enantimorphs were separated by gas chromatography.[8] Their specific activity was determined by a flow-type isotope detection system with a combustion furnace, and also by scintillation counting of the g.l.c. eluant.

The specific activities of the products (Table) have been calculated and normalized after considering the appropriate dilution factors due to the added carrier. For example, with L-alanine, the crude product (31 mg. aliquot) after irradiation, was diluted with carrier DL-alanine (58 mg.), the mixture was resolved, and the two enantiomorphs were separated into their radiochemically pure forms. In the Table, the term "parent compound" refers to the originally irradiated enantiomorph after resolution and radiochemical purification. Thus, for L-alanine, the activity in the "parent compound" is 3·42 μc (per mmole), whereas the activity in the corresponding carrier enantiomorph is 0·890 μc (per mmole of irradiated L-alanine).

Tritiation of optically active amino-acids[a]

Acid	Specific activity (μc/mole irradiated acid)[b] Parent compound[c]	Carrier enantiomorph
L-Alanine	3·42	0·890
D-Alanine	0·262	0·0385
L-Benzoylalanine	2·48	0·394[d]
L-Phthaloylglutamic acid ..	0·527	0·022[e]
D-Phthaloylglutamic acid ..	0·527	0·007[e]

[a] All compounds (0·200 g.) except D-alanine exposed to one curie of tritium gas for 14 days at room temperature. D-alanine exposed to 0·1 c for same time.
[b] These specific activities have been normalized using the appropriate dilution factors (see text).
[c] Refers to resolved, radiochemically purified enantiomorph which was originally irradiated (see text).
[d] Constant (±2%) after four recrystallizations.
[e] Still dropping significantly after four recrystallizations.

The results (Table) support the suggested pathway (eqs. 1 and 2) for the incorporation of tritium at the asymmetric centre of the simple amino-acids. The data also show that certain amino-acids can be labelled predominantly with the

$$\begin{matrix} MeC\cdot CO_2H \\ \| \\ NR \end{matrix} + T_2 \rightarrow \begin{matrix} T \\ | \\ Me\text{–}C\text{–}CO_2H \\ | \\ TNR \end{matrix}$$

retention of configuration in the solid state. The tritium in the scavenging enantiomorphs of the irradiated D- and L-phthalyl glutamic acids is probably not significant, since the counts are close to background and the specific activity in the scavenger was still dropping appreciably at the fourth recrystallization. This result contrasts markedly with the labelling of glutamic acid itself, where approximately 12% of the total activity was found in the opposite enantiomorph after tritiation of the L-isomer.[4]

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