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Note

Quantitative determination of D ≠ L mixtures of optical enantiomers by gas chromatography

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Recently, in connection with a number of other studies, we have been concerned with quantitative aspects of the gross degradation (excluding racemization) of amino acids by various physical processes such as photolysis[1], radiolysis[2–4] and electron bombardment[5]. In order to estimate the percent degradation achieved in such experiments, a gas chromatographic (GC) technique was developed[6] which permitted the determination of the weight of residual undegraded amino acid (optically active or racemic) in the presence of unspecified quantities of extraneous degradation products. This technique[6] involved adding a known weight of the corresponding amino acid enantiomer (or its racemate) to the crude degradation product as an internal standard ("enantiomeric marker"), then converting the mixture to a suitable volatile derivative, and finally determining the enantiomeric composition of the mixture by quantitative GC analysis[7,8]. If an L-amino acid had been partially degraded, for example, and a weight W_D of the D-enantiomer were added as the marker, it was shown that the weight, X_L, of residual L-amino acid in the degradation mixture was given by $X_L = W_D F_L / F_D$, where F_L and F_D were the fraction of each enantiomer as determined by GC. Analogous equations were developed for D- and DL-amino acids. The advantage of using an "enantiomeric marker" as internal standard in the above analytical procedure, as opposed to a non-enantiomeric internal standard, lies in the resulting possibility of applying enantiomeric GC phases for the analysis of such mixtures, to improve analytical precision and eliminate ambiguities[8].

In more recent experiments we have found[9–13] that the undecomposed residual amino acids from the partial γ-radiolysis of optically active amino acids were significantly racemized (radioracemization). As pointed out earlier[6], racemization from any source must introduce an error into the calculation of percent decomposition by the above enantiomeric marker technique. Clearly it would be advantageous te develop alternative equations which would eliminate racemization as a source of error in determining percent degradation by the enantiomeric marker technique. This can be accomplished as follows.

DISCUSSION

In a sample containing an unknown amount of a mixture of D and L components the fraction of L-isomer is given by

$$F_L = X_L/(X_D + X_L) \quad (1)$$

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whence

$$X_L = F_L(X_D + X_L) \quad (2)$$

where X_D and X_L are the unknown weights of the two enantiomers in the mixture. If now a known weight W_D of the D-enantiomer (sufficient to give a suitably sized GC peak) is added as an internal standard, then the fraction of the L-enantiomer becomes

$$F_L' = X_L/(X_D + X_L + W_D) \quad (3)$$

whence

$$X_L = F_L'(X_D + X_L + W_D) \quad (4)$$

From eqns. 2 and 4 it follows that the total weight of the original mixture of enantiomers is given by

$$X_D + X_L = F_L' W_D/(F_L - F_L') \quad (5)$$

Similarly, if the *L*-antipode is used as marker, then the original weight is given by

$$X_D + X_L = F_D' W_L/(F_D - F_D') \quad (6)$$

Finally, in case the enatiomeric marker should be unavailable, the DL racemate may be used as a marker in most situations (except when $X_D = X_L$). In this situation the weight of the original mixture is given by

$$X_D + X_L = W_{DL}(F_L' - 0.5)/(F_L - F_L') = W_{DL}(F_D' - 0.5)/(F_D - F_D') \quad (7)$$

For eqns. 5, 6 or 7 F_D or F_L are obtained from the GC analysis of the original "unmarked" sample, while F_D' or F_L' are obtained from the GC analysis after the enantiomeric marker has been added. In practice it is thus necessary to divide the sample in question quantitatively into two portions, one of which is derivatized and analyzed directly to determine F_D and F_L and the other of which is treated with the enantiomeric marker and then derivatized, after which F_D' or F_L' are determined. If no racemization accompanies the degradation in question, *i.e.* $X_D = 0$ for example, then eqns. 5, 6 and 7 reduce to the simpler equations previously derived[6].

Finally, eqns. 5, 6 or 7 have proved useful in another, unrelated application. Recently we have been concerned with the measurement of enantiomer ratio changes as a function of extents of partial hydrolysis of polyleucine oligomers derived from leucine monomers where D ≠ L (ref. 14). Here the hydrolysate is divided, part is derivatized and analyzed directly to determine F_D and F_L, and the remainder is treated with W_D or W_L of leucine marker, then derivatized and analyzed for F_D' and F_L'. The above equations again provide $X_D + X_L$, now the total weight of leucine recovered on partial hydrolysis of the oligomer, and the percent hydrolysis follows directly. Clearly these equations are in principle applicable to the determination of the weight of any D ≠ L mixture of enantiomers arising from any source whatsoever. Except for weighing or GC integration errors, the principal potential source of error would appear to be an optically inactive contaminant fortuitously co-eluting at the

same GC retention time as one of the enantiomers. Such a situation should be recognizable and correctable by alternately employing GC columns coated with enantiomeric stationary phases[8].

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