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Note

Facile hydrolytic cleavage of N,O-diheptafluorobutyryl derivatives of thyroidal amino acids

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The present United States Pharmacopeia assay method for sodium levothyroxine (T_4Na, sodium salt of 3,3′,5,5′-tetraiodothyronine), sodium liothyronine

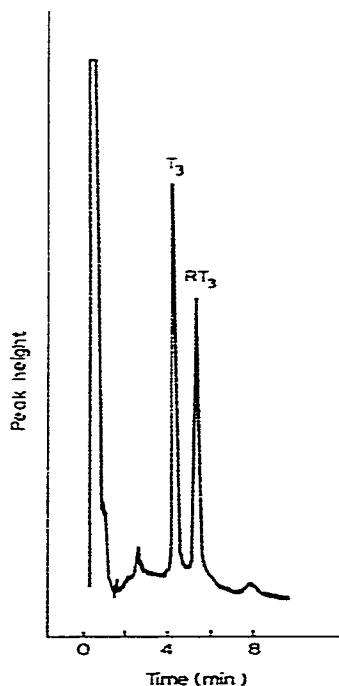

Fig. 1. A Hewlett-Packard 5830A gas-chromatograph, equipped with a Model 18803A Hewlett-Packard electron capture detector (^{63}Ni is the radioactive source) and a Model 18850A Hewlett-Packard GC terminal was used. GLC chromatogram obtained by processing a 5 μg sodium liothyronine tablet. The chromatography was performed on a glass column (4 ft. × 0.4 mm I.D.), packed with 2% OV-101 on 100–120 mesh Chromosorb W HP. A temperature programming was utilized for the separation of the derivatized T_3 and RT_3, with the initial temperature set at 255°C for 0.5 min and the temperature was increased to 275°C at the rate of 15°/min, to reach 275°C and kept at that temperature for 8 min. The retention times were 5.3 and 7.9 min. respectively for the derivatives of T_3 and RT_3; injection temperature 300°C, oven max 325°C, chart speed 0.5 cm/min. The carrier used was 5% methane and 95% argon and the flow-rate was 25 ml/min.

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(T_3Na, sodium salt of 3,3′,5-triiodothyronine) tablets and some other thyroid dosage forms[1] is a non-specific, iodometric assay. Recently specific and sensitive high-performance liquid chromatographic (HPLC) methods for analyzing these compounds from tablet formulations were reported[2–5]. However, the reported HPLC procedures were not sensitive enough for conducting either dissolution studies on small dosage forms of T_3Na or T_4Na (smallest dosage form of T_3Na is 5 μg) or to determine plasma levels of these hormones in bioavailability studies.

A highly sensitive gas–liquid chromatographic (GLC) procedure for the determination of T_3 and T_4 from plasma was reported by Petersen and co-workers[6,7], which consists of isolation of the amino acids from plasma and derivatization to the N,O-diheptafluorobutyryl amino acid methyl esters and detection by electron capture. (See Fig. 1 for conditions). We attempted to adapt the above procedure for the analysis of tablets. Basically the procedure consisted of the addition of RT_3 (3,3′,5′-triiodothyronine) as the internal standard, isolation of the amino acids by acidic butanolic extraction[2], followed by derivatization to N,O-diheptafluorobutyryl amino acid methyl ester and GLC determination as described by Petersen *et al.*[6]. As low as 20 pg injected on the column could be quantitated using this procedure, thus establishing that the method would have the required sensitivity. The resultant peaks had good chromatographic characteristics (Fig. 1). The optimum conditions described for the separation of the peaks were chosen after a preliminary investigation of different columns and chromatography conditions.

The method was reported to exhibit good precision in the analysis of plasma samples[6,7]. However, when we attempted this procedure using pure amino acids, unacceptable coefficients of variation (up to 30%) resulted. Further kinetic studies revealed that these derivatives are not stable and are sensitive to moisture. The problem was not eliminated even by using freshly distilled dry solvents. The ease of decomposition of the N,O-diheptafluorobutyryl-amino acid methyl ester was $T_3 > T_4 \approx RT_3$.

Similar decomposition phenomena were reported earlier of N,O-ditrifluoroacetyl-T_3 and T_4-O-Me esters by Docter and Hennemann[8]. This is understandable as the trifluoroacetyl group is highly electronegative, thus enhancing the ease of hydrolytic cleavage (Fig. 2). In comparison to the trifluoroacetyl group, the heptafluorobutyryl moeity is more electronegative and is expected to be extremely moisture sensitive. Presently it is not known whether it is the heptafluoro derivative of the amino or phenolic group or both that are cleaved and the reason for the much more facile cleavage of the derivative of T_3 as compared to RT_3 and T_4. It is likely that cleavage occurs more easily at the 4′ (phenolic position), as it is more similar to

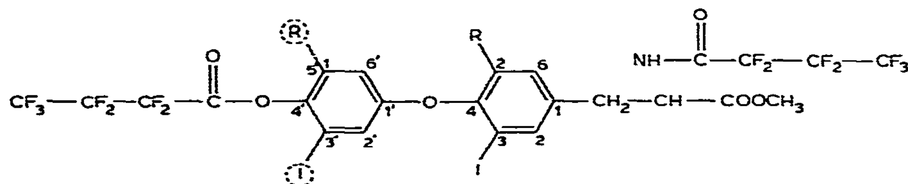

Fig. 2. Chemical structure of basic iodothyronines derivatized as the N,O-diheptafluorobutyryl-amino acid-methyl ester. R_1 = I, R_2 = I is thyroxine (T_4); R_1 = I, R_2 = H is reverse T_3 (RT_3); R_1 = H, R_2 = I is triiodothyronine (T_3 or liothyronine).

an activated ester in structure than at the amide linkage. If indeed the cleavage occured primarily at the phenolic position of the heptafluoro group, it could partially be explained by the presence of steric and hydrophogic interactions. For the derivatized T_3, there is considerable strain due to steric interaction and crowding between the iodine atom and the heptafluoro group. This steric strain would be released by the hydrolytic cleavage of the heptafluoro group. In the case of RT_3 and T_4, the heptafluoro group at the phenolic 4′ position is surrounded by iodine at the 3′ and 5′ positions, with the resultant increase in steric strain. However, hydrophobic interaction between the bulky iodines or between the iodine atoms and the lipophilic heptafluoro moiety is likely. This would result in a hydrophobic interaction, which excludes water, and thus makes hydrolytic cleavage of the heptafluorobutyryl moiety more difficult. This explanation is in agreement with our experimental results that the derivative of T_3 is more prone to decomposition than the derivatives of T_4 and RT_3.

In conclusion, although a procedure was developed for the isolation, derivatization and quantification of thyroidal amino acids using GLC, the procedure was not pursued further due to the relative instability of the derivative N,O-diheptafluorobutyryl amino acid methyl ester. The reason for the instability is explained on the basis of steric interactions and electronegativity.

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