THE SYNTHESIS OF 3-METHYL-2-[4-^{14}C]THIOHYDANTOIN A PHARMACOLOGICALLY ACTIVE METABOLITE OF THE ANTITHYROID DRUG METHIMAZOLE

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SUMMARY

Under basic conditions [^{14}C]glycine reacts with methyl isothiocyanate producing 5-methylthiohydantoic acid which cyclises at acidic pH to 3-methyl-2-[4-^{14}C]thiohydantoin.

Key Words: 3-methyl-2-[4-^{14}C]thiohydantoin, metabolite, antithyroid compound, synthesis.

INTRODUCTION

Recently the *in vivo* metabolism in the rat of [^{14}C]methimazole (1-methyl-2-thioimidazole), a thiocarbamide used for the treatment of hyperthyroidism has been partially elucidated (1). Four of the metabolites, one of which is 3-methyl-2-thiohydantoin still retain the sulphur atom. This metabolite has also been shown to be present in the blood, thyroid tissue and urine of patients receiving methimazole (2). Because 3-methyl-2-thiohydantoin is a more effective inhibitor of the iodination of monoiodotyrosine by sheep thyroid peroxidase (3) than methimazole, and because it has a biological half-life three times longer than methimazole in man (4), it may also contribute to the inhibition of thyroid hormone biosynthesis.

Since 3-methyl-2-thiohydantoin is a primary metabolite of methimazole, and is still pharmacologically active, it is of considerable interest to know its metabolic fate. This paper describes the synthesis of 3-methyl-2-[4-^{14}C]thiohydantoin, which has not previously been reported. The method used was based on that described by Edman (5), for the synthesis of the thiohydantoins of amino acids. The synthesis proceeds in two stages. Initially a base catalysed addition between glycine (I) and methyl isothiocyanate (II) occurs under basic conditions yielding 5-methylthiohydantoic acid (III) which at pH 1 cyclises to 3-methyl-2-thiohydantoin (IV, Scheme 1).

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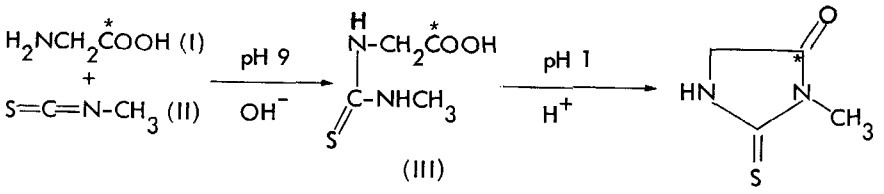

Scheme 1.

EXPERIMENTAL

Chemicals and Instrumentation

[1-^{14}C]Glycine (0.0369 mmoles), specific activity 54.2 mCi/mmol was obtained from Amersham International (Amersham, UK), glycine from B.D.H. Chemicals Ltd., (Poole, UK),and methyl isothiocyanate from Sigma Chemical Co.,(Kingston-on-Thames, Surrey, UK). All solvents were of AnalaR grade and were glass-distilled before use unless stated otherwise.

A Packard Tri-Carb 460C Liquid Scintillation Counter was used for the measurement of radioactivity: The efficiency of counting was determined using an external standard. Pre-coated silica gel sheets (Polygram Sil G/UV$_{254}$, 0.25mm Cam Lab, Cambridge, UK) were used for thin-layer chromatography (TLC). The developing solvent was either ethyl acetate or chloroform-methanol-water(140:60:25, by vol.). The R_f values for 3-methyl-2-thiohydantoin in these solvents were 0.45 and 0.64 respectively.

A Thin-layer Radiochromatogram Scanner (Berthold Thin-Layer Scanner II) was used to locate radioactivity on the TLC plates.

Radiochemical purity was determined after TLC, by cutting the silica gel sheet in sections and counting them using the Liquid Scintillation Counter and by reverse radioisotope dilution analysis.

The ultraviolet spectrum of the synthesised product was recorded on a Cary 210 spectrophotometer and its high-resolution mass spectrum with an AEI MS9 instrument.

Synthesis

An aqueous solution of [1-^{14}C]glycine (10ml, 0.0369 mmoles) was added to an aqueous solution of glycine (10ml, 0.733 mmoles) in a 50ml round-bottom flask, after which methyl isothiocyanate (170µl, 2.485 mmoles) was added from a microsyringe. The mixture was heated to 40°C with stirring. Dilute sodium hydroxide solution (3ml, 0.5M) was added slowly over a period of 5 hours, whilst the reaction mixture returned to ambient temperature. Dilute hydrochloric acid solution (3ml, 1M) was then added and the reaction mixture left at room temperature for at least 16 hours. The mixture was then refluxed for 2.5 hours, after which the water was removed with the aid of a rotary film evaporator. The residue was extracted with chloroform (ethanol free) (3 x 10ml). The combined extracts were filtered into a 100ml round flat-bottomed flask. This step removed sodium chloride from the product. The chloroform was removed with a rotary film evaporator, and 3-methyl-2-[4-^{14}C]thiohydantoin was crystallised from toluene (3 x 2ml). Yield 83.8mg (83.6% of theoretical). However the product was not radiochemically pure as determined by TLC.

The product was further purified by placing it on a silica gel column (25 x 1cm) which had been slurry packed with silica gel (60-120 mesh) in ethyl acetate. The column was eluted with ethyl acetate (100ml), and 5ml fractions were collected, which were analysed by TLC. The product from the fractions containing radiochemically pure 3-methyl-2-[4-^{14}C]thiohydantoin was crystallised from toluene (3 x 2ml). Yield 51.7mg (51.6% of theoretical), melting point 164-166°C (uncorrected), literature 163-164° (6). The specific activity of the product was 19.86 ± 0.36µCi/mg with a greater than 98% radiochemical purity (95% confidence limits) as determined by reverse isotope dilution analysis. Ultraviolet absorption maximum (λ_{max} ethanol) 263nm, literature λ_{max} ethanol, 263nm (7).

The mass spectrum of the product gave a M^+ m/z 130.020 (100%) with ions at m/z 102.025 (10.5%) and 98.047 (2.6%).

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