LABELLING OF A MUCOLYTIC AGENT WITH CARBON-14 SYNTHESIS OF N-[2-(N'-CYCLOHEXYL-N'-METHYL-N'-METHYLENE)--4,6-DIBROMO-PHENYL]-3-METHOXY-4-ACETOXY-BENZAMIDE, HYDROCHLORIDE

Received December 7, 1976

Key words : brovanexine-^{14}C.

INTRODUCTION

N-[2-(N'-Cyclohexyl-N'-methyl-N'-methylene)-4,6-dibromo-phenyl]--3-methoxy-4-acetoxy-benzamide, hydrochloride (I.N.N.-brovanexine HCl) has been found to be an active mucolytic agent in animals and man (1-5).

In order to know its metabolic pathways and to carry out its pharmacokinetics, it was of interest to synthesize the compound labelled with ^{14}C at the amido grup. The possibility of any cleavage in the gastrointestinal tract was investigated with cold brovanexine. This study evidenced that the product is absorbed as unchanged drug and so, that the chosen position for the labelling is suitable for the metabolic studies.

The 3-methoxy-4-hydroxy-benzoic acid (Carboxyl-^{14}C) is commercially available. For this reason, it was chosen as the starting material for a three step-synthesis of brovanexine-^{14}C (Scheme 1).

This work describes this synthesis.

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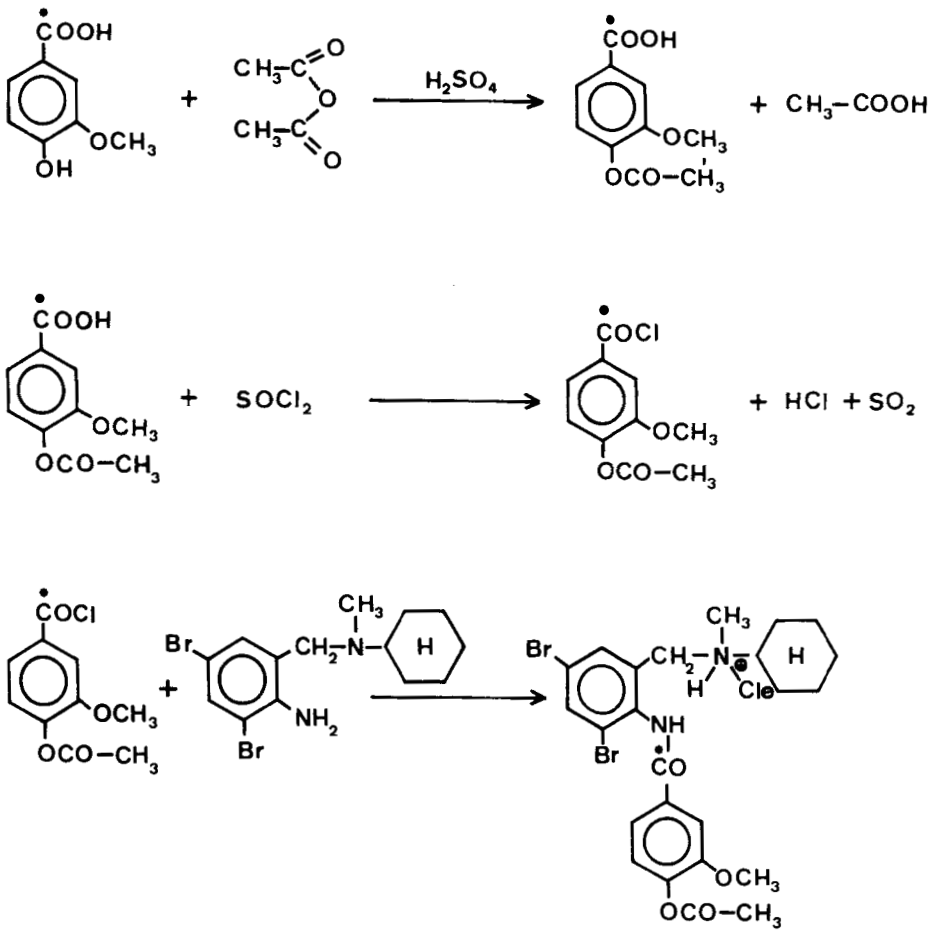

Scheme 1

EXPERIMENTAL

1.- Analytical methods.-

Thin layer chromatography (TLC) was conducted with precoated silica gel glass plates (E. Merck) and solvent systems :

a) Chloroform-dioxane (9:5)

b) Propanol-ammonium hydroxide (7:3)

Radiolabelled zones were visualized by autoradiography with Kodak X-ray film. The radiochemical purity of ^{14}C-brovanexine was estimated by liquid scintillation counting of the silica scraped from the plates. The chemical purity was tested by U.V. spectrophotometry in ethanol (λ_1 = 288 nm ; λ_2 = 220 nm) and by IR absorption. Melting points are uncorrected.

2.- Synthesis.-

2.1.- 3-methoxy-4-acetoxy benzoic acid (Carboxyl-^{14}C)

4 mM of 3-methoxy-4-hydroxy-benzoic acid (Carboxyl-^{14}C) were treated with 0,75 ml of acetic anhydride and 10 μl of H_2SO_4 for 45 min. at 50°-60°C in a centrifugation tube. Agitation was needed at the beginning of the reaction. The crude product solidified on cooling and it was washed with cold water. The recovered product was dissolved in absolute ethanol and the solution treated with charcoal, which was separated by centrifugation. The alcoholic solution was then poured into 20 ml of boiling water with rapid stirring. After slow cooling, centrifugation and drying, 0,45 g (yield 52%) of the acetyl derivative were obtained as a white, crystalline solid m.p. 148°-149°C.

2.2.- 3-methoxy-4-acetoxy-benzoylchloride-^{14}C

The former product was refluxed in a centrifugation tube for 2 hours with 1 ml of freshly distilled thionyl chloride. The excess of thionyl chloride was eliminated in vacuo and the

residue rinsed with 60°-80°C petroleum ether. An extremely white crystalline solid was obtained. The yield was 0,46 g (93%) m.p. 59°-60°C.

2.3.- ^{14}C-brovanexine hydrochloride

The above obtained acyl chloride was dissolved in 10 ml of anhydrous benzene and 0,75 g of 3,5-dibromo-N^{α}-cyclohexyl--N^{α}-methyltoluene-α,2-diamine in 10 ml of anhydrous benzene were added dropwise. Rapid stirring and reflux were maintained during the addition. The mixture was warmed 3 hours more and the stirring was continued overnight. The suspended ^{14}C-brovanexine was recovered by centrifugation and recrystallized from absolute ethanol yielding 0,45 g (37%), m.p. 210°-215°C. U.V. and IR spectra corresponded to the standard brovanexine. TLC with the solvent systems a) and b) and liquid scintillation counting indicated the radiochemical purity was superior to 99,5% (a) Rf = 0,76 b) Rf = 0,81 which are identical to those of the non labelled sample). The specific activity was 0,7 mCi/mM, representing an overall radiochemical yield of 26%.

ACKNOWLEDGEMENT

The author is grateful to Drs. A. Marin and J. Bachs for their helpful advice and to Miss M. Roca for her careful technical assistance.

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