^{3}H-LABELLED BILIRUBIN AND BILIVERDIN

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Summary

A simple method is described for the preparation of [^{3}H]bilirubin IX-α by the reduction of biliverdin IX-α using sodium boro[^{3}H]hydride. The [^{3}H]bilirubin is obtained with a high specific radioactivity and the tritium is located at the methylene bridge (C-10). Oxidation of the [^{3}H]bilirubin with chloranil results in some loss of tritium from C-10 and [^{3}H]-biliverdin is obtained.

Key Words

[^{3}H]bilirubin, [^{3}H]biliverdin, reduction with sodium boro[^{3}H]hydride

Introduction

Several methods have been published for the synthesis of isotopically labelled bilirubin XI-α (1-7). However, these methods are often difficult to carry out experimentally and give low yields of product with a low specific radioactivity. We now report a simple method which can be carried out in a few hours for the preparation of [^{3}H]bilirubin of high specific radioactivity. If biliverdin IX-α is reduced with sodium boro[^{3}H]hydride, [^{3}H]bilirubin is obtained with over 98% of the tritium located on the methylene bridge (C-10). If the [^{3}H]bilirubin is oxidised with chloranil (8), [^{3}H]biliverdin is obtained as some tritium is lost from C-10 during the oxidation .

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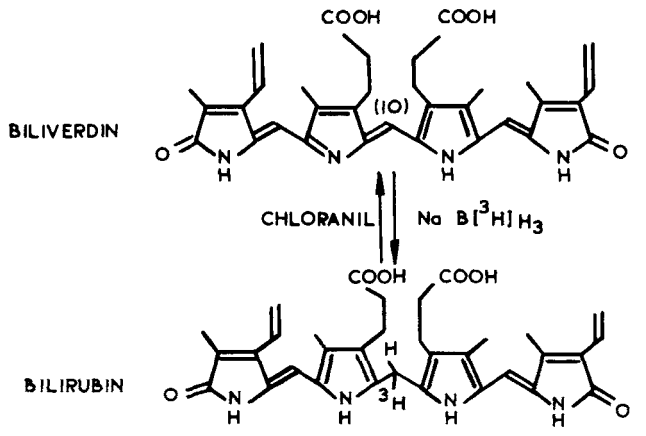

Materials and Methods

Biliverdin IX-α and bilirubin IX-α were obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. The biliverdin was used without further purification but the bilirubin was purified by the method of Fog (9) before use. Sodium boro[^{3}H] hydride, obtained from The Radiochemical Centre, Amersham, U.K., was diluted with unlabelled material before use to a specific radioactivity of 625 μCi/mg (23.5 mCi/mmol).

Preparation of [^{3}H]bilirubin - Sodium boro[^{3}H]hydride (4 mg) was added to biliverdin IX-α (5 mg) in ethanol (9 ml) and water (1 ml). Additional, unlabelled sodium borohydride (45 mg) was added immediately and the reaction was allowed to proceed with frequent mixing in the dark at room temperature for 7 min. Glycine-HCl buffer (0.4 M, pH 2.75, 10 ml) was added and the aqueous mixture extracted with chloroform (10 ml). The chloroform extract was concentrated to a small volume and applied to a silica gel t.l.c. plate (20 x 20 cm) which was developed with chloroform/methanol/water (40/9/1, v/v). The yellow band R_F 0.75 was excised and the bilirubin eluted from the silica with chloroform. The chloroform

was removed under reduced pressure and water (5 ml) followed by a few drops of NH_4OH (sp. gr. 0.88) added. The solution was immediately acidified with 10 mM HCl and extracted with chloroform (4 x 5 ml). The chloroform solution was dried with a little anhydrous sodium sulphate and rechromatographed on silica t.l.c. plates as described above. Excision of the bilirubin-containing band followed by elution of the bilirubin with chloroform gave bilirubin (0.7 mg, 14%) with a light absorption maximum at 454 nm (ε = 58 000 l $mol^{-1}cm^{-1}$). The specific radioactivity of the [^{3}H]bilirubin after correction for colour quenching was 2.7 μCi/μmol. Diazotisation of the [^{3}H]bilirubin (10) gave red diazopigments (λ_{max} 530 nm) with a specific radioactivity of 4.6 x 10^{-2} μCi/μmol.

Oxidation of [^{3}H]bilirubin - To [^{3}H]bilirubin (0.5 mg) obtained as above was added unlabelled bilirubin (30 mg) to give material with a specific radioactivity of 4.4 x 10^{-2} μCi/μmol. This was oxidised by the method of Manitto and Monti (8) to give [^{3}H]biliverdin (2 mg, 7%) with a specific radioactivity of 1.4 x 10^{-2} μCi/μmol.

Discussion

Our method for the reduction of biliverdin IX-α using sodium boro[^{3}H]hydride gives [^{3}H]bilirubin of high specific radioactivity. Provided the reaction time is kept short, few side products are formed and the reduction takes place almost exclusively at C-10 of the biliverdin (> 98% as demonstrated by the very low specific radioactivity of the diazopigments derived from our [^{3}H]bilirubin). For convenience we diluted our commercial sample of sodium boro-[^{3}H]hydride (sp. act. 10 Ci/mmol) with unlabelled material. Therefore, if undiluted sodium boro[^{3}H]hydride were to be used in our preparation [^{3}H]bilirubin of very high specific radioactivity could be obtained and hence may be of use in following metabolic processes in the liver. Oxidation of our [^{3}H]bilirubin (specific activity

4.4 x 10^{-2} μCi/μmol) with chloranil (8) leads to some loss of tritium from C-10 of the bilirubin and [^{3}H]biliverdin (specific activity 1.4 x 10^{-2} μCi/μmol) is obtained, i.e. 69% of the tritium activity has been lost. Hence stereospecific tritiation and/or dehydrogenation must have occurred.

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