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Synthesis of tritium-labeled bilirubin

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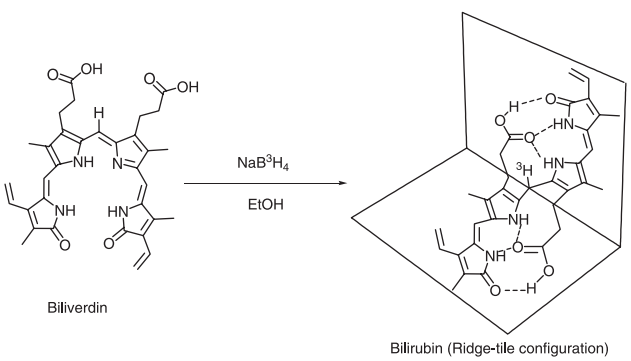
Tritium-labeled bilirubin with specific activities ranging from 6.8 to 8.8 Ci/mmol was prepared in a single step by reducing biliverdin dihydrochloride with sodium borotritide in ethanol.

Keywords: bilirubin; biliverdin; tritium; radiosynthesis

Introduction

Bilirubin is the major metabolite of heme degradation catalyzed by heme oxygenase. Initially the breakdown of the heme ring in old red blood cells produces iron, carbon monoxide, and biliverdin. Biliverdin is then readily reduced by biliverdin reductase to bilirubin. Bilirubin has four pyroles and two carboxylic acids and is bent about the central sp^3-carbon into a ridge-tile shape,[1,2] see Scheme 1. Intramolecular hydrogen bonding renders this molecule highly lipophilic and thus metabolically un-excretable. The free, unconjugated bilirubin is carried by albumin to the liver, where it is converted or conjugated and made water soluble and then excreted to the urine. Glucuronyl transferase is necessary for the conjugation of bilirubin. The lack of this enzyme, or the presence of drugs that interfere with glucuronyl transferase, impairs the liver's ability to conjugate bilirubin. Thus, the presence of relative amounts of bilirubin is an indication of pathologic and metabolic disorders. The neonatal jaundice, hyperbelirubinemia, for example, is caused by high concentrations of bilirubin, which may cross the blood brain barrier and cause neurological damage. Patients are usually irradiated with blue or white lights.[3,4] This treatment may cause the double bonds in the molecule to isomerize and the molecule adapts a new configuration away from the ridge tile and thus becomes more soluble or easily conjugated to human serum albumin or glucuronides. On the positive side, bilirubin in low nanomolar concentrations has been reported to protect neurons against oxidative stress injury.[5].



Biliverdin

Bilirubin (Ridge-tile configuration)

Scheme 1

Results and Discussions

Commercial bilirubin is an orange powder that contains bilirubin ix α (93%), bilirubin iii α (3%), and bilirubin xiii α (4%) see Figure 1.[3,4] Tritium-labeled bilirubin has been previously prepared biosynthetically or by pyrolysis of a bilirubin derivative. In the biosynthetic procedure, beta-aminolevulin acid-2,3-^{3}H with a specific activity of 7 Ci/mmol was injected intravenously in 25 mCi doses to dogs and the bile was then collected in the dark every 4 h to give tritium-labeled bilirubin with specific activities ranging from 175 to 230 μCi/mg.[6] In the pyrolysis procedure, a bilirubin derivative was prepared by acid-catalyzed addition of thioacetic acid to bilirubin in chloroform, and then it was heated to 280°C for 45 min under vacuum (6×10^{-4} mmHg). The derivative undergoes a thioacetic acid elimination giving bilirubin ix α in a very low yield. Only 11 mg of product was isolated with a specific activity 41.21 μCi/mmol from 500 mg of bilirubin and 80 mL of tritiated water (6.4 mCi/mL).[7,8]

These rather exhaustive and low yielding methods are not practical. To our surprise, some institutions are still preparing labeled bilirubin biosynthetically. Here, we report the synthesis of tritium-labeled bilirubin by reducing the commercially available biliverdin dihydrochloride with sodium borotritide in ethanol. Usually, one equivalent of sodium borotritide is used. However, due to exchanges with acidic protons, we found that an excess of sodium borohydride was necessary to reduce biliverdin to bilirubin, which was indicated by the change of color of the reaction from deep green to yellow. Reduction of

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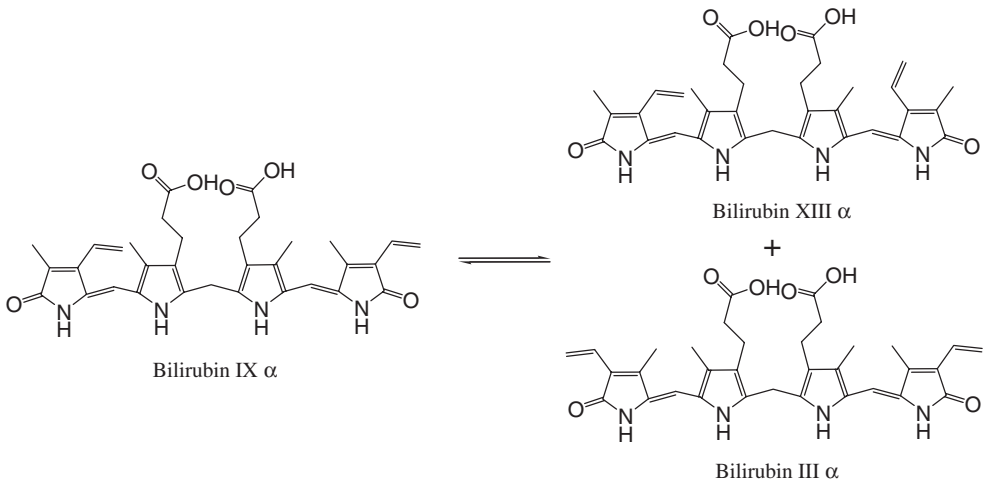

Figure 1. Bilirubin isomers.

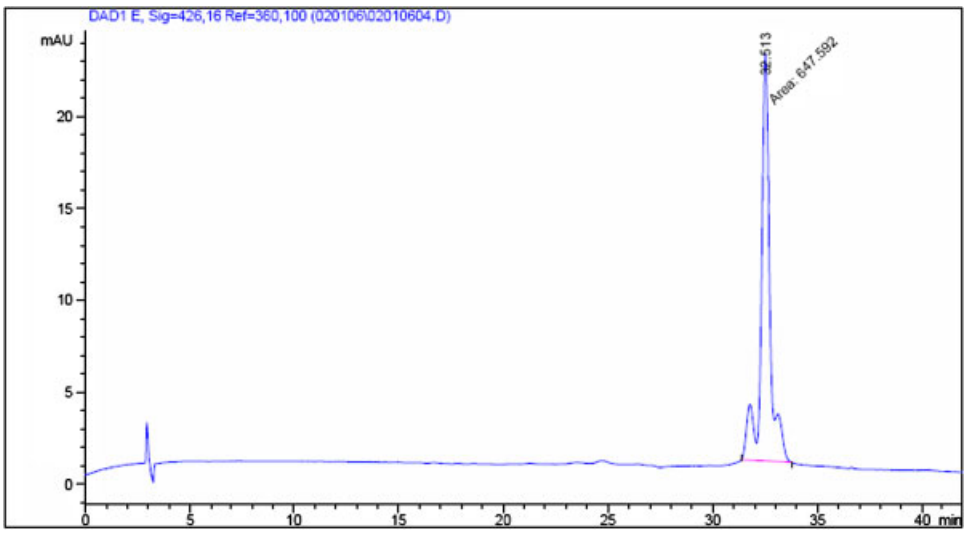

Figure 2. UV chromatogram of tritium labeled bilirubin.

biliverdin with sodium borohydride has been previously described.[9,10]

Experimental Procedures

Materials and Methods

Biliverdin dihydrochloride was purchased from MP Biomedicals (Solon, OH). Sodium borotritide (1.0 Ci with a specific activity of 70–100 Ci/mmol) was purchased from American Radiolabeled Chemicals (Saint Louis, MO). Semi-preparative high-performance liquid chromatography (HPLC) purification was carried out on Waters Breeze using Xterra 4.6 × 250 mm C18 column and a gradient of 50–88% MeOH in water (10 mM sodium acetate) over 40 min, UV detection at 426 nm. Analytical HPLC was carried out on an Agilent 1100 equipped with a IN/US β-RAM® Model 3 Radiochromatography detector using Xterra 4.6 × 150 mm C18 column and a gradient of 50–88% MeOH/water (10 mM sodium acetate) over 40 min, UV detection at 426 nm Figure 2, Figure 3.

Synthesis of [^{3}H]-3-2-3-(2-Carboxy-ethyl)-4-methyl-5-[4-methyl-5-oxo-3-vinyl-1,5-dihydro-pyrrol-(2E)-ylidenemethyl]-1*H*-pyrrol-2-ylmethyl-4-methyl-5-[3-methyl-5-oxo-4-vinyl-1,5-dihydro-pyrrol-(2Z)-ylidenemethyl]-1*H*-pyrrol-3-yl-propionic acid ([^{3}H]-Bilirubin)

A deep green solution of biliverdin dihydrochloride (8.2 mg, 12.5 μmole) in ethanol (3 mL) and aqueous NaOH (1 N, 0.05 mL) were degassed three times before NaB^3H_4 (1 Ci, SA = 80 Ci/mmol) was added in one portion under nitrogen. After stirring at room temperature for 12 h in the dark, unlabeled $NaBH_4$ (38 mg, 1 mmol) was added and the mixture was further stirred for 12 h. The resulting orange solution was treated with water (6 mL) and stirred for 10 min. Acetic acid (80 μL) was added dropwise until no gas evolution was noticeable. The aqueous was then extracted with chloroform (4 × 5 mL) and the combined extracts were concentrated under a stream of nitrogen to half its volume. The solution was dried over Na_2SO_4, filtered, and the solvent was evaporated under a stream of nitrogen until 11 mL. An aliquot was taken to measure the

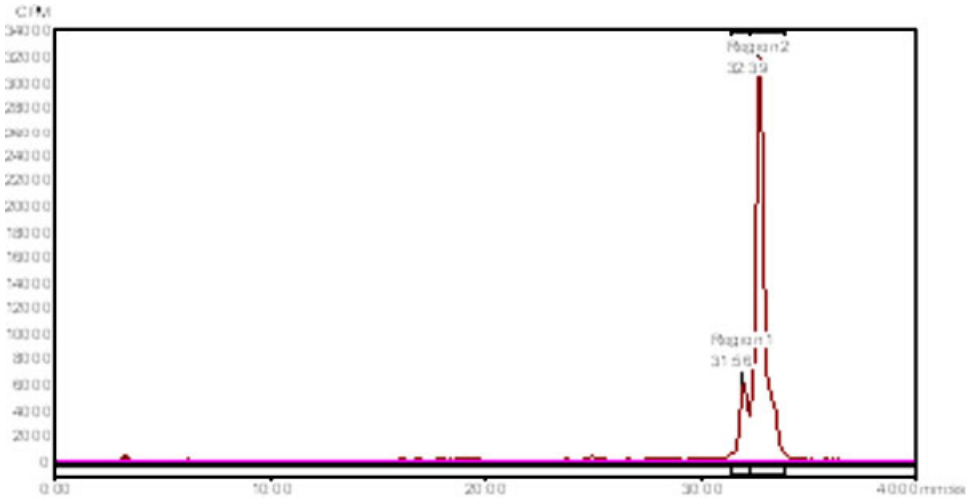

Figure 3. Radioactive chromatogram of tritium labeled bilirubin.

radioactivity and the stock solution was kept frozen under nitrogen at −80°C until purification. A total of 115 mCi was obtained in 11.5% radiochemical yield of the crude product. An aliquot of 25.6 mCi of this crude [^{3}H]-bilirubin was purified by preparative HPLC under dim light to give 1.962 mCi of material with a specific activity of 8.8 Ci/mmol and a radiopurity of 95%. The major impurity eluted with a retention time similar to biliverdin. This may be due to the fact that bilirubin is unstable in air and under light and can get oxidized back to biliverdin. The HPLC chromatogram of the labeled bilirubin was identical to unlabeled bilirubin purchased from Aldrich.

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