Large Scale Synthesis of Bilirubin-XIIIα from IXα

M. Reisinger, F. R. Trull and D. A. Lightner*

Department of Chemistry, University of Nevada,
Reno, Nevada 89557-0020

Received March 1, 1985

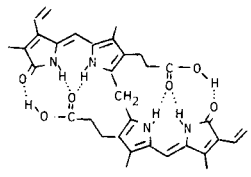
A convenient large-scale preparation of bilirubin-XIIIα from IXα *via* acid-catalyzed isomerization is described.

J. Heterocyclic Chem., **22**, 1221 (1985).

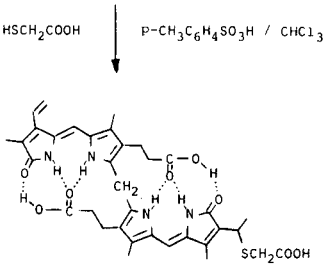
The most direct partial synthesis of bilurubin-XIIIα involves acid-catalyzed isomerization of the commercially available natural product, bilirubin-IXα, to a mixture of bilirubins-IXα, XIIIα and IIIα [1]; however their separation is achieved only with difficulty and on a small scale by chromatographic methods [2,3]. To remove the attendant serious chromatographic difficulties, the IXα isomer is derivatized as its *exo*-vinyl adduct with mercaptoacetic acid before acid-catalyzed isomerization [4]. The isomerization mixture thus contains the XIIIα isomer, in addition to the derivatized IXα and IIIα isomers. The last two, which are tri and tetracarboxylic acids, have very different solubility and chromatographic properties than the (underivatized) XIIIα isomer [5,6]. We previously showed how the isomer mixture can be separated and how the derivatized IXα and IIIα isomers can be reconverted back to the parent bilirubins [5]. In the present, note we describe a carefully developed, simple procedure for a large-scale preparation and isolation of hundreds of milligrams of >99% pure bilirubin-XIIIα.

The method consists of two synthetic steps followed by an isolation step. The first two involve (1) regiospecific addition of mercaptoacetic acid to the *exo* vinyl group of bilirubin-IXα, then (2) acid-catalyzed isomerization of the adduct. Unless these steps are carefully controlled, artifacts may be produced, and these artifacts prove to be difficult to remove from the desired final product, bilirubin-XIIIα.

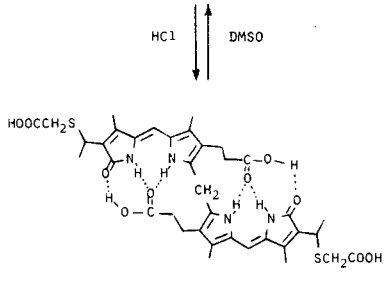
In step (1) artifact formation must be avoided (as described below), and the adduct must be separated from adventitious reaction by-products. In order to achieve smooth, complete conversion of bilirubin-IXα to its mercaptoacetic acid adduct, it is essential that (a) the chloroform be ethanol-free and, in the last purification step, distilled from phosphorus pentoxide, (b) the mercaptoacetic acid be freshly distilled and (c) the reaction time be short (<20 hours). If the reaction time is lengthened, by-product formation increases, a by-product which we believe to be an intramolecular anhydride. If the chloroform is not distilled from phosphorus pentoxide as indicated, the addition reaction is slowed significantly, allowing side reactions to produce the same by-product, inter alia. This by-product has, interestingly, the same acid-base solubility properties as bilirubins-IXα, XIIIα or IIIα, *e.g.* it is insoluble in aq. sodium bicarbonate but soluble in aqueous sodium carbonate. If it is not separated from the mercaptoacetic acid adduct of bilirubin-IXα before acid-catalyzed isomerization, it appears in the isomerized mixture, and subsequently is carried along as a contaminant of the desired bilirubin-XIIIα. Consequently, in order to avoid



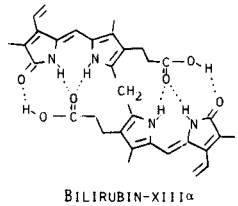
BILIRUBIN-IXα



18-DEVINYL-18(1-CARBOXYMETHYLTHIO)ETHYL-BILIRUBIN-IXα



+



BILIRUBIN-XIIIα

end product contamination problems, the mercaptoacetic acid adduct of IXα must be freed of reaction by-products. This is achieved by extracting the pure adduct from chloroform into 1% aqueous sodium bicarbonate-- a procedure that leaves reaction by-products in the chloroform layer.

Artifact formation in step (2) must also be avoided, and this can be achieved if a sufficiently dilute solution of bilirubin-IXα adduct in dimethylsulfoxide is treated with 12 *N* hydrochloric acid. At higher solution concentrations, small amounts of one by-product of undetermined structure are produced. This by-product has similar solubility properties to that of bilirubin-XIIIα and is thus carried along with it in the isolation step. It can be removed, albeit with some loss of XIIIα, by treatment with activated charcoal or by selective extraction from chloroform into a (3:1 volume/volume) 1% aqueous sodium bicarbonate-1% aqueous sodium carbonate wash.

EXPERIMENTAL

Bilirubin-IXα and mercaptoacetic acid were obtained from Sigma; the former was >95% of the IXα isomer. Chloroform, methanol, acetic acid and *p*-toluenesulfonic acid were reagent grade from MCB, phosphorus pentoxide was reagent grade from Baker. The basic alumina used was Woelm activity super I; the 4Å molecular sieves were from Linde. Thin layer chromatography (tlc) was carried out using Baker Silica gel 7G on analytical (125 μ) plates.

Purification and Treatment of Chloroform Used for the Addition Reaction.

Twenty-four milliliters of distilled hexane were added to 3.8 liters of freshly distilled chloroform, and the solution was stirred for 5 minutes. To remove traces of ethanol, the solvent mixture was first passed through a column packed with a slurry of 150 g of basic alumina (Alumina Woelm B - Super I) in chloroform, 2.5 cm diameter × 50 cm high. Then it was passed through the same size column filled with 100 g of Linde 4Å molecular sieves. Finally 50 g of phosphorus pentoxide was added, and the chloroform was distilled at atmospheric pressure to the *very last drop.*

The Addition Reaction to give 18-Devinyl-18-(1-carboxymethylthio)ethyl-bilirubin-IXα.

It is recommended that a small scale test reaction be carried out in order to determine whether the chloroform is of sufficient acidity to ensure a completely successful addition reaction. Mercaptoacetic acid (0.1 ml, freshly distilled) is added to a solution of 10-20 mg of bilirubin-IXα in 15-30 ml of chloroform containing 2 mg of *p*-toluenesulfonic acid. After 16-20 hours, at 40°, this reaction is checked by tlc (chloroform-methanol-acetic acid, 97:2:1, vol/vol/vol) on silica gel to determine that no bilirubin-IXα remains. If unreacted bilirubin-IXα is detected, the chloroform must be redistilled from 50 g of phosphorus pentoxide. Ten milliliters of freshly distilled mercaptoacetic acid (84-86°/2mm) and 200 mg of *p*-toluenesulfonic acid were added to a solution of bilirubin-IXα (2.00 g, 3.42 mmoles) in 2.9 liters of chloroform, and the mixture was stirred for 16-20 hours at 40°. Prolonged reaction times or higher temperatures lead to an increasing formation of impurities. After cooling to room temperature, the chloroform solution was divided into two 1.45 liter portions, which were both worked up in the same way. The chloroform solution was extracted with 0.36 *M* aqueous sodium bicarbonate (300 ml) then with 0.12 *M* aqueous sodium bicarbonate (150-200 ml). The combined aqueous solutions were washed with chloroform (2 × 100 ml) and acidified with acetic acid to precipate the adduct, which was collected by centrifugation, washed with water and dried to give 1.78 g (77%) of >95% pure product, purity determined by hplc [7] and tlc.

Isomerization of 18-Devinyl-18-(1-carboxymethylthio)ethyl-bilirubin-IXα.

The adduct from above (1.34 g , 1.98 mmoles) was dissolved in 120-150 ml of dimethylsulfoxide that had been purged previously with a slow stream of nitrogen for 0.5 hours. Concentrated hydrochloric acid (14.7 ml) was added at once with stirring. Stirring was continued for about 5 minutes at room temperature, then the reaction mixture was poured onto ice (100 g)/water (150 ml) and stirred for 15-30 minutes. The resulting greenish precipitate was collected by centrifugation and washed with distilled water to neutral *p*H. After final decantation, the still wet mixture was dissolved in 0.12 *M* aqueous sodium bicarbonate (**A**, 400 ml) and extracted with chloroform (8 × 100 ml). The combined chloroform extracts were washed first with a mixture of 0.12 *M* aqueous sodium bicarbonate (75 ml) and 0.094 *M* sodium carbonate (25 ml) then with distilled water (150 ml), dried over anhydrous sodium sulfate and evaporated to dryness (rotary evaporator). The residue was digested with a mixture of methanol (24 ml) and water (16 ml), centrifuged and the supernatant discarded. The digestion procedure was repeated three times. After the final digestion/washing, the residue was dried over phosphorus pentoxide at room temperature to constant weight. The product was 213.3 mg of pure bilirubin-XIIIα of Å96% purity as determined by hplc [7]. Additional product could be recovered from the 0.12 *M* aqueous bicarbonate wash (**A**, above) by vigorous stirring overnight with a fresh 800 ml portion of chloroform. Separation of the chloroform and treatment as above yielded an additional 167.2 mg of bilirubin-XIIIα of >96% purity, as determined by hplc [7]. The total combined yield was 380.5 mg (25%, based on starting bilirubin-IXα carried through the two steps).

The mercaptoacetic acid adducts of bilirubin-IXα (mono-adduct) and bilirubin-IIIα (bis-adduct) could be recovered from the 0.12 *M* aqueous sodium bicarbonate wash (**A**, above) first by acidification with acetic acid (20 ml) then stirring for one hour, then addition of distilled water (300 ml). The precipitate was collected by centrifugation, washed with distilled water to neutral *p*H, then dried over phosphorus pentoxide at room temperature to constant weight to afford 450 mg of combined recovered mono and bis-adducts.

Acknowledgement.

We thank the National Institutes of Health (HD-17779) for generous support of this work.

REFERENCES AND NOTES

[1] For leading references, see, A. F. MaDonagh, "Bile Pigments: Bilatrienes and 5,15-Biladienes" in "The Porphyrins" vol VI, D. Dolphin, ed, Academic Press, New York, 1979, chapter 6. See also R. Bonnett, D. G. Buckley, D. Hamzetash and A. F. McDonagh, *Israel J. Chem.*, **23**, 173 (1983).

[2] J. L. Dicesare and F. L. Vandemark, *Chromatography Newsletter, Perkin-Elmer*, **9**, 7 (1981).

[3] A low pressure liquid chromatography separation was described by R. Defoin-Straatmann, A. Defoin, H.-J. Kuhn and K. Schaffner, *Ann. Chem.*, 1760 (1982).

[4] Regioselective acid catalyzed addition of -SH and -OH groups to the *exo*-vinyl of bilirubin-IXα has been reported earlier by P. Manitto and D. Monti, *Experientia*, **29**, 137 (1973). See also references [5] and [6].

[5] D. Monti and P. Manitto, *Synth. Commun.*, **11**, 311 (1981).

[6] J. S. Ma and D. A. Lightner, *J. Heterocyclic Chem.*, **21**, 1005 (1984).

[7] The hplc system is described in A. F. McDonagh, L. A. Palma, F. R. Trull, and D. A. Lightner, *J. Am. Chem. Soc.*, **104**, 6865 (1982). We thank Dr. A. F. McDonagh, University of California, San Francisco, for analyzing the purity of our bilirubin-XIIIα and 18-devinyl-18-(1-carboxymethylthio)ethylbilirubin-IXα.