

Synthesis of Conjugated Bile Acids. Bile Acids and Steroids 5

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Bile acids conjugated with glycine or taurine were first prepared with Curtius' azide method by Bondi and Müller^{1,2}. Cortese and Bauman³⁻⁵ have used the formylated chloride of the bile acid in a Schotten-Baumann procedure but Cortese's modification⁶ of the azide method seems the best method published.

For work with labelled bile acids we needed a method more suitable for synthesis of conjugates on a small scale. Of the numerous peptide syntheses that have been published in recent years we have found the method using carbonic-carboxylic acid anhydrides introduced independently by Wieland and Bernhard⁷, Boissonnas⁸, and Vaughan *et al.*^{9,10} very suitable for this purpose. We have used the conditions worked out by Boissonnas with some minor modifications described below in the experimental section.

One of the advantages is that bile acids with free hydroxyl groups can be treated directly with ethyl chlorocarbonate for the preparation of the anhydride. The anhydride is then directly mixed with an aqueous solution of the sodium salt of glycine or taurine. Starting from cholic acid and glycine the crystalline glycocholic acid can be prepared in less than an hour. When the synthesis is made on a micro scale the product is conveniently purified by partition chromatography^{11,12}.

A report on the preparation and properties of the taurine and glycine conjugates of all the common bile acids will be published shortly.

EXPERIMENTAL

Glycocholic acid: 1.021 g of cholic acid (2.5 mmoles) was dissolved in 5 ml of dioxane containing 0.59 ml of tri-*n*-butylamine. The solution was cooled to +10° and 0.238 ml of ethylchlorocarbonate was added. After 15 minutes at this temperature a solution of 187 mg of glycine in 2.5 ml of *N* sodium hydroxide was added at once and the mixture rapidly mixed. After 15 minutes when the gas evolution had subsided and the solution had set to

a gel, enough water was added to give a clear solution which was evaporated *in vacuo* to a syrup; the latter was redissolved in about 20 ml water. Acidification with hydrochloric acid gave a sticky precipitate that was washed by decantation with water until free from HCl. When triturated with a little ethyl acetate it rapidly set to a mass of crystals that were filtered and dried. Yield 880 mg of practically pure glycocholic acid. m.p. 162° *cf.*^{8,13}. For analysis a sample was recrystallized twice from ethanol-ethyl acetate and dried at 110° *in vacuo*. (Found: C 66.9; H 9.4; N 2.9. Calc. for C₂₆H₄₅O₅N: C 67.0; H 9.3; N 3.0.)

Tauroolithocholic acid (Lithocholytaurine): 0.983 g of lithocholic acid and 0.59 ml of tri-*n*-butylamine was dissolved in 5 ml of dioxane, 0.238 ml of ethyl chlorocarbonate was added as described above. After 15 minutes at +10°, 312 mg of taurine in 2.5 ml *N* sodium hydroxide was added. After 1 hour 10 ml of water was added and the solution evaporated to a syrup *in vacuo*. After addition of about 15 ml of water and acidification to pH 1 with hydrochloric acid a precipitate formed that was filtered off. The dried precipitate was dissolved in 10 ml hot ethanol and then diluted with 15 ml ethyl acetate. After one more recrystallization from ethanol-ethyl acetate the m.p. was 212–213° (hot stage, corr.) and remained unchanged on further recrystallizations. Yield 45%. Dried at 110° *in vacuo* for analysis. (Found: C 64.3; H 9.5; N 2.8; S 5.9. Calc. for C₂₆H₄₅NO₅S: C 64.6; H 9.4; N 2.9; S 6.6.)

Microanalyses by K. Pääbo at this institute and B. Rolander at the Medical Nobelinstitute, Stockholm.

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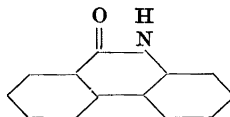
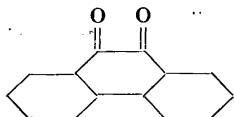
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The Stoichiometry of the Reaction between Hydrazoic Acid and Phenanthrenequinone

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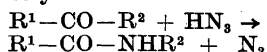
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Caronna¹ states that phenanthrenequinone and retenequinone dissolved in concentrated sulphuric acid reacts with hydrazoic acid to give phenanthridone and 1-methyl-7-*iso*-propyl-phenanthridone respectively.



The initial stage of this reaction is possibly the same as in the Schmidt reaction in which compounds containing a carbonyl group react with hydrazoic acid in the presence of strong Lewis acids to give amides. (For a review of the Schmidt reaction see H. Wolff².) The sequence of reactions following the initial stage of the reaction considered here must, however, be different from that of the Schmidt reaction as the overall reaction in the former case is the substitution of a NH group for a CO group, whereas in the latter an amide is formed by interposing a NH group between the CO group and a group attached to it. Accordingly the stoichiometry of the two reactions appears to be entirely different.

The Schmidt reaction has the following stoichiometry



as side reactions are probably responsible for the small production of ammonia

observed. In the reaction studied by us we observed formation of ammonia to a considerably greater extent, and we interpret this as a property in the stoichiometry.

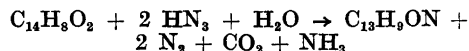
The stoichiometrical proportions were determined in the following way: The reaction was carried out with dilute solutions (0.1 M) of phenanthrenequinone and hydrazoic acid in concentrated sulphuric acid. After some hours the reaction mixture was diluted with water, the residual hydrazoic acid distilled off in a Kjeldahl-apparatus and titrated with 0.1 N ceric sulphate³. The liquid was made alkaline and the ammonia was distilled off and titrated in the usual way.

The molar ratio between converted hydrazoic acid and the ammonia formed is calculated, and, when hydrazoic acid is present in excess, the molar ratio between converted hydrazoic acid and converted phenanthrenequinone can be calculated as well.

We found	Mean value	Standard deviation
Hydrazoic acid : Ammonia	1.95	0.06
Hydrazoic acid : Phenanthrenequinone	2.18	0.17

These ratios are independent of the ratio between the initial concentrations of hydrazoic acid and phenanthrenequinone within the range examined (0.5–6.0 for $\text{HN}_3 : \text{NH}_3$; 2.0–6.0 for $\text{HN}_3 : \text{C}_{14}\text{H}_8\text{O}_2$).

The gas evolved from the sulphuric acid solution was analysed and found to be pure nitrogen. Only after the addition of water an evolution of carbon dioxide took place. This suggests the following stoichiometry for the overall reaction:



When ice is added together with water to dilute the reaction mixture a black tarry intermediate product is often observed, which within a certain time decomposes, forming the white phenanthridone. The same stoichiometry is found when the reaction is carried out with retenequinone instead of phenanthrenequinone. We have prepared 1-methyl-7-*iso*-propylphenanthridone as follows: