A New Non-enzymatic Route to Chenodeoxycholic Acid

Olga Bortolini, Umberto Cova,† Giancarlo Fantin, and Alessandro Medici*
Dipartimento di Chimica, Via Borsari 46, 44100 Ferrara, Italy
†*ICE srl, Via Sicilia 8/10 Reggio Emilia, Italy*

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A novel route for the production of the chenodeoxycholic acid is described, based on the selective non-enzymatic reduction of dehydrocholic acid. The relative reactivity scale was established to be in the order: 12-keto (1), 7-keto (2), 3-keto (17.5).

Chenodeoxycholic acid **3** possess an important therapeutic effect related to the ability to dissolve cholesterol gallstones.[1] This chemotherapeutic property is displayed in greater extent by ursodeoxycholic acid **4**, usually obtained from the naturally occurring chenodeoxycholic acid through selective α-β C-7 OH inversion. The preparation of these steroids, summarized in Scheme 1, has been achieved through different synthetic pathways,[2,3] that utilize the relatively inexpensive cholic acid **1** as starting material. Hofman[4] described a seven-step sequence **I** that represents the pure chemical approach to this problem. More recently, Sawada *et al.*[5] reported the preparation of chenodeoxycholic acid **3** by bioconversion of dehydrocholic acid **5** mediated by bacteria **II**. On the other hand, the enzymatic approach to this problem *via* the reactions described in sequence **III** has been elucidated by Carrea and co-workers.[6] Ursodeoxycholic acid is industrially prepared using the seven-step chemical synthesis, however the microbiologic and enzymatic routes, in particular the latter, possess several attractive potentialities, currently under detailed investigation for a possible future use. Worthy of note in the synthesis **I-III** is the occurrence of the 12-keto steroid as key intermediate, that is obtained through two different approaches: i) the oxidation of cholic acid following selective protection or ii) the selective reduction of carbonyl function following complete oxidation. The selective oxidation of C-12 OH, in the former case, must be preceded by protection of the additional C-3 and C-7 hydroxyl functions, since the oxidation aptitude follow the order C-7>C-12>C-3, whereas the protection attitude decreases in the order C-3>C-7>C-12.[7] In the latter case, the selective reduction of C-3 and C-7 from the dehydrocholic acid is obtained by stereospecific enzymatic bioconversions.[6]

In this communication we describe a different route for the production of the chenodeoxycholic acid based on the selective non-enzymatic reduction of dehydrocholic acid. This simple and effective synthetic procedure exploits the reactivity order toward reduction or hydrogenation that has been reported to be C-3>C-7>C-12.[7] The sequence is described in Scheme 2. The commercially available cholic acid **1** was oxidized to dehydrocholic acid **5** according to a literature procedure.[7,8] Dehydrocholic acid, 3 mmols dissolved in 40 mL of a saturated solution of $NaHCO_3$, has been directly reduced by action of $NaBH_4$ (2x2.7 mmols) at 0 °C in 24 h. The reaction mixture was acidified (HCl 20%) and extracted with ethyl acetate. GC analysis[9] of the crude product showed 70% of 12-ketocholic acid **2**, 8% of 7-ketocholic acid as well as 4% of 3-ketocholic acid. Of interest is the relative reactivity scale with respect to carbonyl reduction that can be deduced from these results, in the order: 12-keto (1), 7-keto (2), 3-keto (17.5). The 12-keto steroid may be separated from the isomeric ketocholic acids by flash column chromatography using a mixture of isoctane:ethyl acetate:acetic acid (50:50:1) as eluent, in 62% yield. Alternatively, the crude product may be converted into the 12-azine **7** by reaction with NH_2NH_2·HCl in refluxing ethanol for 4 h.[11] A white crystalline precipitate is obtained upon partial evaporation (1/3) of the solvent, identified as the azine of 12-ketocholic acid[12] in 65% yield. Of interest is the observation that the 7-keto and 3-keto steroids, unwanted products representing the 12% of the total yield and present in the mother liquor, may be recovered and recycled through oxidation, further $NaBH_4$ reduction and azine formation. The azine is quantitatively transformed into the 12-ketocholic acid by acid hydrolysis using acetone-HCl (5%). Attempts to transform directly the azine into the chenodeoxycholic acid **3**, by means of Wolff-Kishner reaction,[7] were unsuccessful. The 12-ketocholic acid obtained either by column chromatography purification or *via* azine formation can be converted into the chenodeoxycholic acid by means of the Wolff-Kishner reduction.[7] A final overall yield of ca. 54% is obtained based on the initial amount of dehydrocholic acid employed.

The absence of protection steps for the different products, the mild conditions employed and the rather good chenodeoxycholic acid yield are the major advantages of this promising synthetic procedure.

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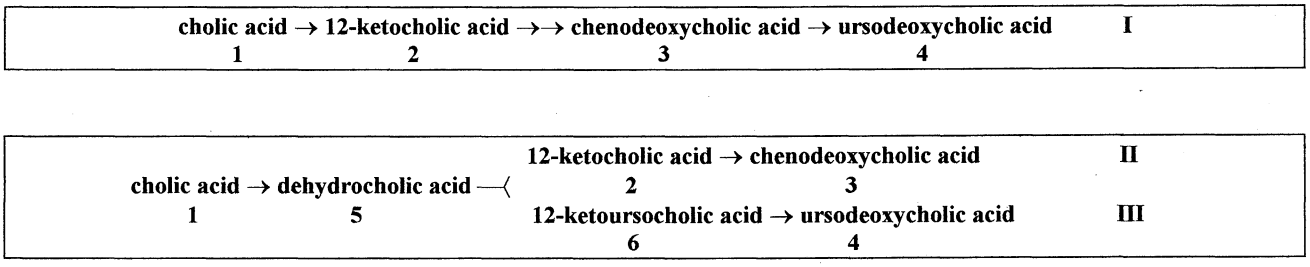
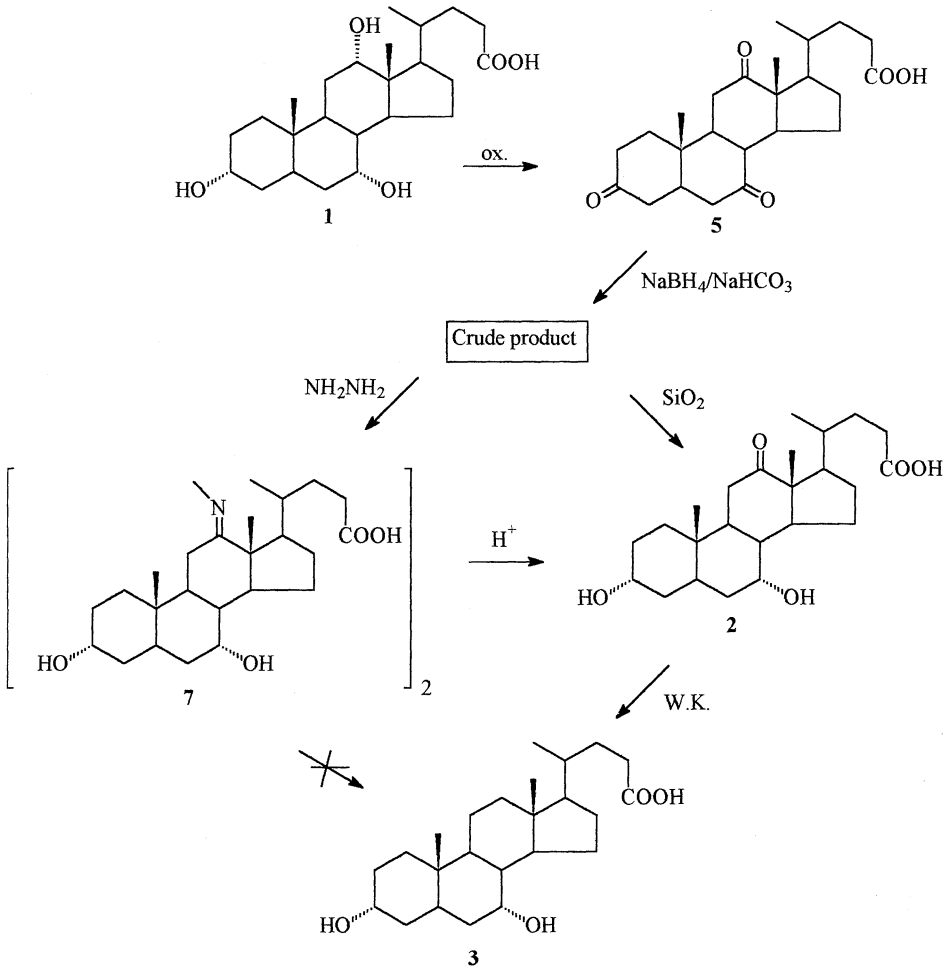


Scheme 1.



Scheme 2.

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10 All compounds gave satisfactory analytical and spectroscopic data, in agreement with literature reports.

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12 Selected ^{1}H NMR (CD_3OD) δ 3.85 (br s 1H), 3.30 (m, 1H), 1.0 (m, 9H). Selected ^{13}C NMR (CD_3OD) δ 178.4 (COOH), 172.7 (C=N-), 71.4 (CHOH), 68.5 (CHOH). Alternative synthetic pathways have been investigated in order to obtain the azine from 12-ketochenodeoxycholic acid and NH_2NH_2·HCl in the presence of $NaHCO_3$ / H_2O, CH_3COONa / CH_3CH_2OH, H_2SO_4 / CH_3CH_2OH / H_2O, respectively. These attempts, however, did not give rise to quantitative reactions.