Antimicrobial activity of basic cholane derivatives. X. Synthesis of 3α- and 3β-amino-5β-cholan-24-oic acids

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A simple and convenient route to 3α- and 3β-amino-5β-cholan-24-oic acids was developed via Leuckart-Wallach amination reduction and subsequent acid hydrolysis. Two epimeric formylamino derivatives were produced (α and β), approximately in a 1 : 1 ratio, as determined by ^{13}C nuclear magnetic resonance spectroscopy. The two isomers were separated by making use of their different solubilities in ethyl ether. The absolute configuration of the two amino acids was assigned by comparison with authentic reference samples. (*Steroids* **56**:395–398, 1991)

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Introduction

Basic groups have been introduced on a bile acid molecule either in the side chain[1–3] and/or in the steroid body.[4,5] This new set of basic cholane derivatives showed interesting and, in some cases, extremely high antimicrobial activity as a function both of the nature of the steroid rings and of N-substituents. In each case considered, the acidic function of the bile acids used as substrates was lost, as were some of their peculiar physiologic properties, e.g., hepatic uptake.[6] With the aim of examining which new properties could be introduced by the concomitant presence of a basic and an acid moiety in these systems, we started a program of preparation of amino bile acids, analogs of the common hydroxy bile acids.

We present the synthesis of 3α- and 3β-amino-5β-cholan-24-oic acid analogs of the 3α-hydroxy-5β-cholan-24-oic acid (lithocholic acid), by means of a Leuckart-Wallach reaction, starting from the 3-oxo-5β-cholan-24-oic acid ethyl ester. This reaction proved particularly suitable because it gave rise to both of the possible epimers at comparable amounts during a single preparation.

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Experimental

Materials

3α-Hydroxy-5β-cholan-24-oic acid (lithocholic acid) was a commercial sample (Fluka, Buchs, Switzerland) and was used as purchased. All other reagents and solvents were of analytic grade.

Infrared (IR) spectra were recorded on a Perkin Elmer 197; ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 200, using tetramethysilane as internal standard; optical rotatory power was measured by a Perkin-Elmer polarimeter at 20 C and λ 589 nm at 1% concentration in 1N methanolic potassium hydroxide.

Synthesis

3-Oxo-5β-cholan-24-oic acid (I). Five grams of lithocholic acid were dissolved in 500 ml pure acetic acid and added dropwise with stirring to 5 g of chromic anhydride in 50 ml glacial acetic acid; the mixture was allowed to react for 3 hours. After the addition of a large volume of water and ice, the precipitate was filtered. The solid was dissolved several times in acetic acid and reprecipitated with water. Yield, 45%; melting point (mp), 116 to 118 C (aqueous acetic acid).

3-Oxo-5β-cholan-24-oic acid ethyl ester (II). Five grams of compound I were dissolved in 100 ml absolute

ethanol saturated with gaseous and dry hydrochloric acid. The solution was refluxed for 4 hours. At the end of this time, a large volume of water was added to precipitate the ester. Yield, 96%; mp, 85 to 88 C.[7]

3-Formylamino-5β-cholan-24-oic acid ethyl ester epimers (III and IV). A total of 0.01 mol of compound **II** and 40 ml formamide were mixed in a three-neck flask. The mixture was heated, with stirring, to an internal temperature of 155 C by means of a silicone oil bath. A 0.1-mol amount of formic acid (4.5 ml) dissolved in 30 ml of formamide was added dropwise over 3 hours. Heating was further continued for 2 hours. The temperature was then allowed to drop; water was added until precipitation was complete, then the solid material was vacuum filtered and purified by column chromatography, using ethyl acetate/methanol (8 : 2 v/v) as eluting mixture. Yield, 80%; mp of the final product, 42 to 45 C. The IR spectrum showed absorption bands at 3,400, 1,740, 1,670, and 1,520 cm^{-1}. ^{13}C NMR showed ($CDCl_3$), among others, two characteristic signals at 161.6 and 164.1 ppm (HCONH, formylamino groups). These signals suggested the presence in the final product of two epimers, further confirmed by the ester signal (174 ppm), not well resolved, but appearing as two signals fused together. The ratio of the two forms, as calculated from the relative intensity of the signals, was 45 : 55. Again, in the ^1H NMR spectrum ($CDCl_3$), two signals at 6.0 and 6.4 ppm suggested the N-H protons of the two epimers: the signal disappeared after the addition of D_2O.

Separation of the epimeric mixture

A total of 0.5 g of the epimeric formylamino derivative mixture was treated with ethyl ether, achieving a partial dissolution and therefore separation, after filtration, into two parts, which proved different when analyzed. The ^{13}C NMR spectra (C_6D_6) of the two fractions showed that the separation was quantitative: the formylamino signals at 159.8 (insoluble part) and 160.8 (soluble part) ppm in the two spectra were clean and precise. The epimer insoluble in ether (0.24 g) had an mp of 192 to 196 C (**III**); the soluble part (0.20 g) had an mp of 38 to 40 C (**IV**).

3α- And 3β-amino-5β-cholan-24-oic acids (V and VI). The two fractions recovered after treatment with ethyl ether were dissolved in 30 ml of aqueous methanol (1 : 1 v/v) mixed with 5 ml of 6N HCl aqueous solution and refluxed for 6 hours. The hydrochlorides of the two aminoacidic epimers were precipitated after the addition of ethyl ether. α Isomer: mp, 265 to 266 C; $[\alpha]_{589} = +31°$. β Isomer: mp, 285 to 287 C; $[\alpha]_{589} = +24°$. The absolute configuration of the two compounds was assigned by comparison with two authentic samples obtained by different synthetic routes (see below).

3α-Amino-5β-cholan-24-oic acid (V). 3-Oxo-5β-cholan-24-oic acid, 0.01 mol, was treated with equimolar amounts of hydroxylamine hydrochloride in ethanol in the presence of sodium acetate. The solution was refluxed for 24 hours. On cooling, the corresponding oxime crystallized pure (mp, 195 to 197 C from ethyl acetate/methanol [9 : 1 v/v]). The 3-oxo-5β-cholan-24-oic acid oxime, 0.01 mol, was dissolved in 150 ml of boiling amyl alcohol in the presence of an excess of metal sodium (6 g). After refluxing for 3 hours, the cooled mixture was diluted with ethyl ether; on washing to neutralize with water, pure 3-α-amino-5β-cholan-24-oic acid precipitated. Found: C, 76.68; H, 11.28; N, 3.72%. $C_{24}H_{41}O_2N$ requires C, 76.75; H, 11.00; N, 3.73%, and has an mp of (hydrochloride) 265 to 267 C.[8]

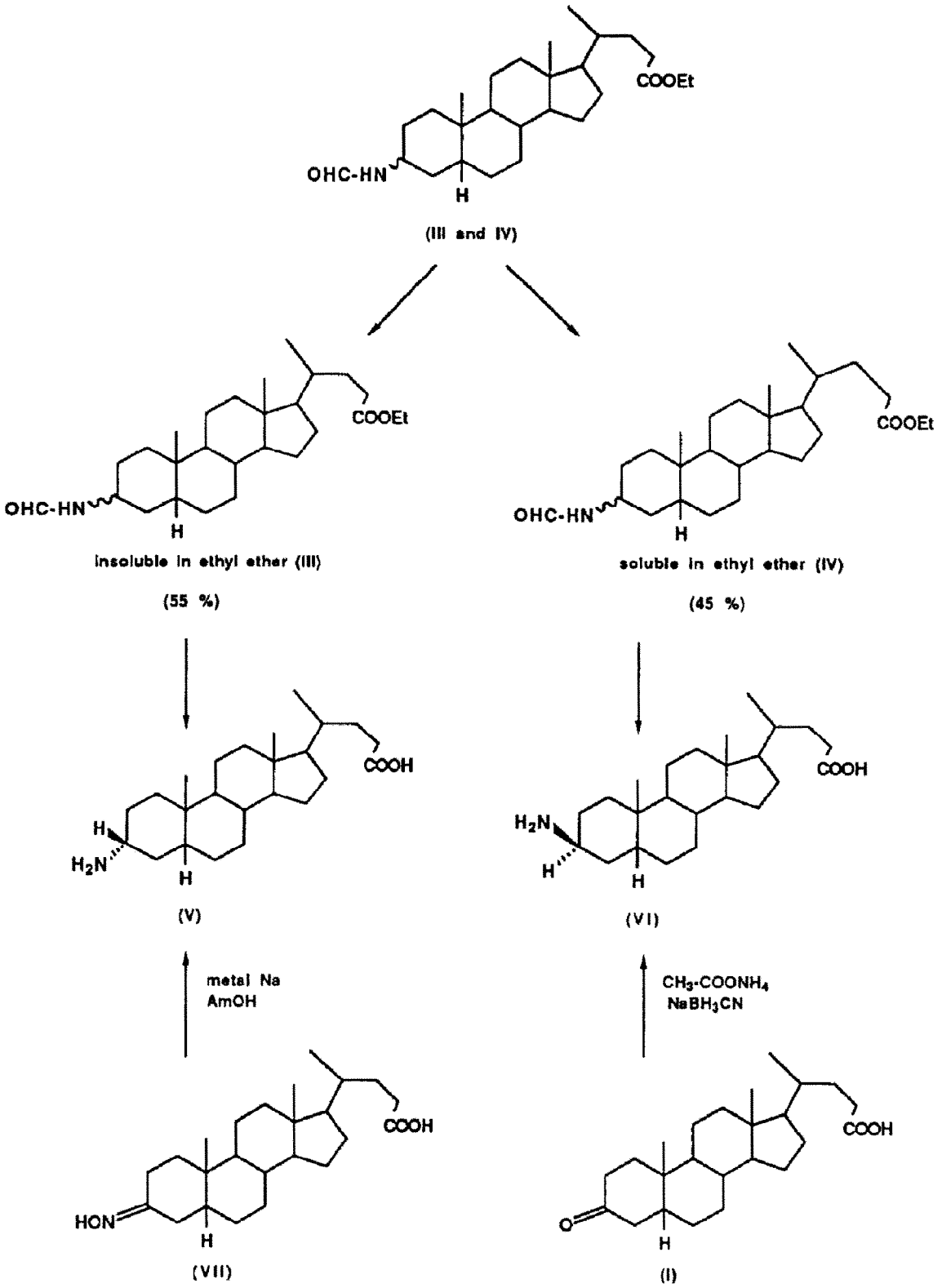
3β-Amino-5β-cholan-24-oic acid (VI). A 0.013-mol amount of sodium acetate and 0.02 mol of sodium cyanoborohydride were added to 0.01 mol of 3-oxo-5β-cholan-24-oic acid (**I**) dissolved in 100 ml of methanol; the mixture was left for 24 hours at room temperature. From the mixture, on dilution with water, 3β-amino-5β-cholan-24-oic acid precipitated together with 2% of the α epimer. Found: C, 76.73; H, 11.22; N, 3.76%. $C_{24}H_{41}O_2N$ requires C, 76.75; H, 11.00; N, 3.73%. The hydrochloride had an mp of 285 to 287 C.[7]

Results and Discussion

In a previous paper,[7] we reported the preparation of 3β-aminocholan-24-oic acid, starting from the 3-oxocholan-24-oic acid by means of an amination reduction reaction, using ammonium acetate and sodium cyanoborohydride in methanol. This reaction proved highly stereospecific (98% of the β epimer) and, when applied to polyoxocholan-24-oic acids, also regioselective, only the 3 position was involved.[5]

To obtain monoamino derivatives of the cholan-24-oic acid in steroid positions other than 3 or polyamino derivatives, we considered using the amination reduction reaction according to Leuckart-Wallach, using formamide and formic acid as reagents: a formylamino derivative is obtained which is hydrolyzed to amino group with strong mineral acids. This reaction has been reported in the literature[9,10] as stereospecific on polyoxo-5β-cholan-24-oic acids, yielding 3α- (equatorial), 7α- (axial), and 12α- (axial) amino derivatives. However, the same reaction carried out on the 3,12-dioxocholan-24-oic acid ethyl ester afforded a mixture of compounds (Bellini AM, unpublished observations).

After column chromatography separation, we obtained two compounds that showed different melting points and thin-layer chromatography rf values, but the same elemental analysis and IR spectra. However, ^{13}C NMR spectra suggested that each compound actually contained two epimers, as demonstrated by four different signals in the 159 to 164 ppm range due to four formylamino groups. The finding of the four possible epimers, in relevant amounts, starting from a dioxo compound indicated that the Leuckart-Wallach reaction is not as stereospecific as reported in the literature.[9,10]


COOEt
OHC-HN
H
(III and IV)
COOEt
OHC-HN
H
insoluble in ethyl ether (III)
(55 %)
COOEt
OHC-HN
H
soluble in ethyl ether (IV)
(45 %)
COOH
H
H2N
H
(V)
COOH
H2N
H
H
(VI)
metal Na
AmOH
CH3-COONH4
NaBH3CN
COOH
HON
H
(VII)
COOH
O
H
(I)

Scheme I

To restrict the formation of α and β formylamino epimers only in the 3 position and with the aim of their separation and the attribution of the absolute configurations, we prepared the 3-oxo-5β-cholan-24-oic acid ethyl ester (**II**) and performed the Leuckart-Wallach reaction on this simpler system. Separation of the two epimers was carried out, taking advantage of the different solubility in ethyl ether of the two 3-formylamino-5β-cholan-24-oic acid ethyl esters. Each pure epimer presented signals of the HCONH at 160.8 and 159.8 ppm in the ^{13}C NMR spectra; these signals were assigned to the epimers indicated as soluble or insoluble in ethyl ether, because it was not possible to attribute the absolute configuration by ^{13}C NMR spectroscopy.

A bidimensional nuclear Overhauser experiment was unsuccessful because the proton signal of each formylamino group is unaffected by other protons in its neighborhood, at least in the experimental conditions selected. Therefore, the absolute configuration was assigned by comparison with pure samples obtained by different synthetic pathways known to yield pure α and β epimers.

Practically pure samples of the 3α-amino-5β-cholan-24-oic acid hydrochloride were obtained by reduction of the 3-oxocholan-24-oic acid oxime with metal sodium in amyl alcohol, while the β epimer was obtained by reductive amination with ammonium acetate and sodium cyanoborohydride in absolute methanol. Melting points, thin-layer chromatography rf values, IR and NMR spectra confirmed the attribution of the configurations. The synthetic pathways are shown in Scheme 1.

Studies are continuing with the aim of preparing either epimeric monoamino cholan-24-oic acids in the 6, 7, and 12 positions or polyamino cholan-24-oic acids derived from common bile acids.

Acknowledgments

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