STEROIDS

PART III. REDUCTION OF OXIMINOCHOLANIC ACIDS[1]

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ABSTRACT

The reduction of 12-oximino-, 3,12-dioximino-, and 7,12-dioximino-cholanic acids using sodium in alcohol resulted in the formation of 12β-amino-, 3α,12β-diamino-, and 7β,12β-diamino-cholanic acids respectively. The reaction of 3,12-dioximinocholanic acid with lithium aluminum hydride provided cholane-3ξ,12ξ,24-triol, while similar treatment of 7,12-dioximinocholanic acid afforded 7ξ-amino-12-oximinocholan-24-ol.

DISCUSSION

Basic derivatives of bile acids and esters, having amino functions located in positions 3 (1, 2), 6 (3), 7 (2, 4, 5), 11 (6), and 12 (2, 5, 7) of the steroidal nucleus, have been described. Their synthesis has been accomplished for the most part by reduction of the corresponding oximes with sodium in alcohol, and has been prompted primarily by the possibility of such compounds being physiologically active.

In the present investigation, a study has been made of the reduction of 12-oximino- (I) (8), 3,12-dioximino- (II) (9), and 7,12-dioximino-cholanic acids (III) (10), using sodium in alcohol and lithium aluminum hydride. In the reductions using sodium, *n*-propyl and *n*-butyl alcohols gave the best yields of purest products. The color of reaction mixtures in isoamyl alcohol was darker, and the crude products contained appreciable amounts of tarry impurities. Since reductions of oximes with alkali metals and proton donors are known to give predominantly the equatorial conformation (11, 12), the conformation of amino groups in the steroidal amino acids synthesized by reductions with sodium in alcohol were assigned on the basis of mode of preparation. Preliminary experiments of oxime reductions using lithium aluminum hydride in tetrahydrofuran showed that two- to five-fold molar excesses of lithium aluminum hydride led to the formation of mixtures of products. When a 10-fold excess of the reagent was used, definite reaction products could be isolated in all cases.

12-Oximinocholanic acid (I) was prepared from 3α,12α-dihydroxycholanic acid (IV) by oxidation of IV to 3,12-dioxocholanic acid (V) (13), Clemmensen reduction of V to 12-oxocholanic acid (VI) (14, 15, 16), and preparation of the oxime (I). The oxidation of the dihydroxy acid (IV) to the dioxo derivative (V) was carried out using the procedure described by Heilbron (17) for the oxidation of acetylenic carbinols. This method gave consistent yields of over 90% of pure product as compared with the 70% yield obtained by Wieland's method (13) of oxidation in acetic acid. As expected, the reduction of the oximino acid (I) with sodium in alcohol provided 12β-aminocholanic acid (VII) in good yield. That VII existed as a dipole ion was confirmed by the infrared spectrum which showed bands characteristic of the carboxylate ion at 1550 cm^{-1} and of the NH_3^+ group at 1625 cm^{-1}. Several treatments of VII with concentrated hydrochloric acid resulted in the formation of the corresponding hydrochloride. The infrared spectrum of the latter showed suppression of the band due to the carboxylate ion and release of the carboxyl

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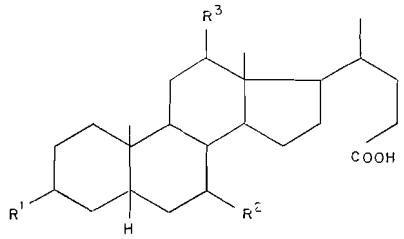


(VII) $R^1 = R^2 = H$; $R^3 = NH_2$

(VIII) $R^2 = H$; $R^1 = R^3 = NH_2$

(XII) $R^1 = H$; $R^2 = R^3 = NH_2$

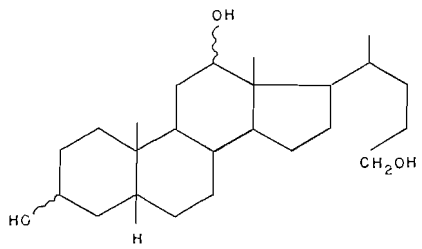
band at 1705 cm^{-1}. Treatment of the oximino acid (I) with 3% sodium amalgam resulted in the transformation of the oxime group to the keto group. The keto acid (VI) was identified by a mixed melting point determination with an authentic sample.

3,12-Dioximinocholanic acid (II) was prepared from 3α,12α-dihydroxycholanic acid (IV) by oxidation of IV to 3,12-dioxocholanic acid (V) and oximation of V to the dioxime (II). The reduction of II with sodium in alcohol resulted in the formation of 3α,12β-diaminocholanic acid (VIII) in good yield. Possessing one carboxyl and two amino groups, this diaminocarboxylic acid behaved as a base and formed a dihydrochloride. The infrared spectrum confirmed the presence of the NH_2 group (1660 cm^{-1}), the NH_3^+ ion (1623 cm^{-1}), and the carboxylate ion (1575 cm^{-1}).

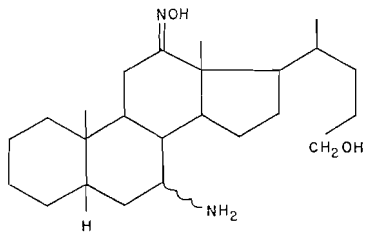
A product containing no nitrogen and insoluble in both acids and bases was formed in the reaction of the oximino acid (II) with lithium aluminum hydride. The infrared spectrum had a very strong band at 3340 cm^{-1}, which was attributed to absorption by associated OH groups, and a series of high intensity bands in the region 1075–1011 cm^{-1}. The latter could be assigned to C—O stretching vibrations in a steroid alcohol. On the basis of the infrared spectrum and the analytical results, the compound was formulated as cholane-3ξ,12ξ,24-triol (IX). The steric course of reductions with lithium aluminum hydride being uncertain, the conformations of the hydroxyl groups at 3- and 12-positions were not assigned.

The formation of IX was quite unexpected, although a somewhat similar result had been obtained by Redel and his co-workers (2) in their attempts to reduce methyl 3α,12α-diformoxy-7-oximinocholanate in ethanolic solution by catalytic hydrogenation under pressure using Raney nickel. Mignonac (18, 19) and Paul (20) also had observed the formation of secondary alcohols on reductions of oximes. It was considered that the reaction of the oximino acid (II) with lithium aluminum hydride proceeded via an unstable imine, which, under hydrolytic conditions, was transformed into the corresponding ketone. The latter was, in turn, reduced to the secondary alcohol (IX) by the lithium aluminum hydride still present in the reaction mixture.

Clemmensen reduction of 3,7,12-trioxocholanic acid (X) to 7,12-dioxocholanic acid (XI) and oximation provided 7,12-dioximinocholanic acid (III). The reduction of III with sodium in *n*-propyl or *n*-butyl alcohol produced 7β,12β-diaminocholanic acid (XII) in high yields, as anticipated. When the reduction of III was carried out under similar conditions to those used for the reduction of the dioximino acid (II) with lithium



(X)



(XIII)

aluminum hydride, a nitrogen-containing product was obtained. In the infrared spectrum there was no carbonyl absorption, but the band at 3300 cm^{-1} indicated the presence of an alcoholic function. The medium intensity band at 1660 cm^{-1} could be due to absorption by an amine, an oxime, or both. Acidic hydrolysis of the reaction product gave a material which contained nitrogen, and in the infrared exhibited a carboxyl band at 1700 cm^{-1}, and bands at 3300 cm^{-1} and 1655 cm^{-1}. The hypothesis of an amino, oximino carbinol fully agreed with the analytical results. Since the 12-position is more sterically hindered than the 7-position, the reaction product was postulated as 7ξ-amino-12-oximinocholan-24-ol (XIII) and its hydrolysis product as 7ξ-amino-24-hydroxycholan-12-one (XIV).

EXPERIMENTAL

The melting points were determined in a Thiele–Dennis melting point tube containing Dow Corning silicone fluid No. D.C. 550, and are uncorrected. The analyses were carried out in the W. Manser laboratory, Zurich, Switzerland. Infrared spectra were determined in potassium bromide on a Perkin–Elmer Model 21 double-beam instrument equipped with a sodium chloride prism.

3,12-Dioxocholanic Acid (V)

To a solution of chromium trioxide (13.35 g) in water (20 ml) concentrated sulphuric acid (11.5 ml) was added dropwise, with stirring so as to avoid the formation of any precipitate. The cold solution was diluted to 50 ml. 3α,12α-Dihydroxycholanic acid (IV) (30 g) was dissolved in acetone (2.2 l.), and the resulting solution was cooled to 20° in an ice-water bath. The oxidizing solution was then added dropwise from a microburette until an orange-brown color persisted (about 45 ml of reagent was required). The solution

was decanted from the inorganic residue, and was diluted with water to a volume of 6 l. The product (23.4 g) precipitated immediately, and was isolated. A second crop (5.35 g) was obtained by diluting the filtrate with another 2 l. of water. The 3,12-dioxocholanic acid (V) melted at 184–185°.

12β-Aminocholanic Acid (VII)

In a nitrogen atmosphere, sodium (2.5 g), in small pieces, was added over a period of 3.5 hours to a refluxing solution of 12-oximinocholanic acid (I) (0.5 g) in dry *n*-propyl alcohol (58 ml) with stirring. Then the reaction mixture was cooled in an ice-water bath, and made slightly acidic with 4% sulphuric acid. The propyl alcohol was distilled off *in vacuo* in a nitrogen atmosphere, and some precipitation occurred. The precipitation was made complete by adjusting the pH to 5.6–5.8 with 1% sulphuric acid. The yellow solid (0.48 g) was isolated. It melted at 122–126° to an opaque viscous liquid which cleared at 138–140°. The product was dissolved in hot acetone (20 ml), and the yellow solution filtered with suction through celite and carbon Nuchar C190-N. The colorless filtrate was concentrated under reduced pressure to a 10-ml volume, and heated to boiling. Hot water was added dropwise until the solution became slightly turbid. Upon cooling to room temperature, needles were deposited from solution. Recrystallization from acetone–water provided 12β-aminocholanic acid (VII) melting at 115–116° to a liquid which became transparent at 120°. ν_{max} 3440 (O—H), 3040 (sh) ($\overset{+}{N}H_3$), 1625 ($\overset{+}{N}H_3$), 1550 cm^{-1} (COO^-). Anal. Calc. for $C_{24}H_{41}O_2N \cdot 3H_2O$: C, 67.13; H, 10.95; N, 3.24. Found: C, 67.20; H, 10.88; N, 3.53%. Hydrochloride: Addition of concentrated hydrochloric acid (0.5 ml) to VII (0.4 g) and evaporation to dryness under reduced pressure was repeated 5 times. The white residue obtained, on recrystallization from methanol–water, gave the hydrochloride as a white powder, m.p. 257–258°. ν_{max} 1705 (C=O), 1610 cm^{-1} ($\overset{+}{N}H_3$).

Attempted Reduction of 12-Oximinocholanic Acid (I) *with Sodium Amalgam in Absolute Ethanol*

Sodium amalgam (3%, 26.6 g) was added in a nitrogen atmosphere to a refluxing solution of I (0.2 g) in absolute ethanol (20 ml), and the mixture was stirred for 6 hours. The major part of the amalgam seemed to have reacted. The supernatant solution was decanted, allowed to cool, diluted with water to 50 ml, and neutralized with 4% sulphuric acid. A white product (0.18 g) which precipitated was isolated. It melted at 178–179° to a milky liquid which cleared completely at 198–201°. Recrystallization from ethanol provided platelets, m.p. 180–182°, which were identified as 12-oxocholanic acid (VI) by a mixed melting point determination (183–184°) with an authentic sample of the keto acid. An oxime derivative of the reaction product was prepared, and a mixed melting point determination with an authentic sample of I showed no depression.

3α,12β-Diaminocholanic Acid (VIII)

To a refluxing solution of 3,12-dioximinocholanic acid (II) (0.4 g) in dry *n*-propyl alcohol (60 ml) were added, with stirring in a nitrogen atmosphere, small pieces of sodium (4.0 g) over a period of 4 hours. The reaction mixture was cooled in an ice-water bath, and treated with 4% sulphuric acid until pH 9 was reached. The propyl alcohol was evaporated under reduced pressure and the pH of the aqueous solution was further adjusted with 1% sulphuric acid to 8.6–8.8. A yellow product (0.25 g) precipitated and was isolated. Recrystallization from methanol–water gave 3α,12β-diaminocholanic acid

(VIII), m.p. 244–245°. ν_{max} 3380 (O—H, NH_2), 1660 (sh) (NH_2), 1623 ($\overset{+}{N}H_3$), 1575 cm^{-1} (COO^-). Anal. Calc. for $C_{24}H_{42}O_2N_2 \cdot H_2O$: C, 70.58; H, 10.78; N, 6.86. Found: C, 70.35; H, 10.63; N, 6.59%.

Reduction of 3,12-Dioximocholanic Acid (II) *with Lithium Aluminum Hydride in Tetrahydrofuran*

To a refluxing suspension of lithium aluminum hydride (0.37 g) in dry tetrahydrofuran (20 ml) in a nitrogen atmosphere was added dropwise, and with stirring, a solution of II (0.3 g) in dry tetrahydrofuran (30 ml). The resulting mixture was stirred and refluxed for a further period of 2 hours, then was cooled to room temperature. A saturated solution (50 ml) of sodium potassium tartrate was added dropwise with stirring. The tetrahydrofuran was removed by evaporation under reduced pressure, and a semisolid material separated in the aqueous phase. It was extracted with three 40-ml portions of *n*-butyl alcohol. The alcoholic extract was washed with 1% sulphuric acid, and with water, then was dried over anhydrous magnesium sulphate. After filtration, the solvent was removed *in vacuo* leaving an amorphous residue (0.23 g), which contained no nitrogen. The product was dissolved in 2 ml of boiling ethanol, and hot water was added dropwise until a slight cloudiness was observed. The solution was seeded with crystals obtained by cooling a few drops of the solution in a dry ice – acetone mixture for 2–3 minutes, and keeping the resulting solid in a refrigerator overnight, and long needles were obtained. Recrystallization from acetone–water yielded cholane-3ξ,12ξ,24-triol (IX), m.p. 166–169°. ν_{max} 3340 cm^{-1} (H—O), 1075, 1055, 1042, 1011 cm^{-1} (C—O). Anal. Calc. for $C_{24}H_{42}O_3$: C, 76.19; H, 11.11. Found: C, 75.86; H, 11.45%.

7β,12β-Diaminocholanic Acid (XII)

In a nitrogen atmosphere, sodium (2.0 g) was added in small pieces, with stirring, to a refluxing solution of 7,12-dioximinocholanic acid (III) (0.2 g) in dry *n*-propyl alcohol (40 ml) over a period of 3 hours. The reaction mixture was cooled in an ice-water bath, and adjusted to pH 9 with 4% then 2% sulphuric acid. The propyl alcohol was removed under reduced pressure, and 1% sulphuric acid was added until precipitation occurred at pH 8.3–8.6. The crude product (0.16 g) was dissolved in 8 ml of ethanol, and the yellow solution was filtered through celite and carbon Nuchar C190-N. The filtrate was concentrated to 4 ml and, when it was left to stand at room temperature, needles were deposited and isolated. Recrystallization from acetone–water afforded 7β,12β-diaminocholanic acid (XII), m.p. 128–130°. ν_{max} 3400 cm^{-1} (O—H), 1635, 1560, 1630 cm^{-1} (NH_2, $\overset{+}{N}H_3$), 1550 cm^{-1} (COO^-). Anal. Calc. for $C_{24}H_{42}N_2O_2 \cdot 1.5H_2O$: C, 69.06; H, 10.31; N, 6.71. Found: C, 68.52; H, 10.32; N, 6.57%.

7ξ-Amino-12-oximinocholan-24-ol (XIII)

A solution of 7,12-dioximinocholanic acid (III) (0.60 g) in dry tetrahydrofuran (80 ml) was added in a nitrogen atmosphere to a refluxing suspension of lithium aluminum hydride (0.73 g) in dry tetrahydrofuran (50 ml), with stirring, over a period of 40 minutes. The reaction mixture was stirred under reflux for a further 2.5 hours. When the suspension had cooled to room temperature, it was hydrolyzed by the dropwise addition of 100 ml of a saturated sodium potassium tartrate (Rochelle salt) solution. The tetrahydrofuran was removed under reduced pressure, and a white precipitate separated from the aqueous solution. The product was extracted with four 50-ml portions of *n*-butanol, and the extract was washed once with 1% sulphuric acid, twice with water, and then was dried over anhydrous magnesium sulphate. The solution was filtered, and the butanol was removed

under reduced pressure leaving a crystalline residue (0.45 g) which melted at 203–209° and became transparent at 215°. Recrystallization from ethanol gave 7ξ-amino-12-oximinocholan-24-ol (XIII) in shiny plates, m.p. 234–236°. ν_{max} 3300 cm^{-1} (O—H), 1660 cm^{-1} (C=N, NH_2). Anal. Calc. for $C_{24}H_{38}O_2N_2$: C, 71.79; H, 9.74; N, 7.17. Found: C, 71.15%; H, 10.17; N, 7.15%.

7ξ-Amino-12-oxocholan-24-ol (XIV)

7ξ-Amino-12-oximinocholan-24-ol (XIII) (15 mg) was dissolved in ethanol (3 ml), and one drop of 4% hydrochloric acid was added to the solution. The reaction mixture was refluxed for 20 minutes, then was concentrated to a volume of 1 ml *in vacuo*, and the needles which separated when the mixture was left to stand at room temperature were isolated; the needles melted at 180–183°. Recrystallization from ethanol–water provided 7ξ-amino-12-oxocholan-24-ol (XIV) in the form of needles, m.p. 183–184°. ν_{max} 3300 cm^{-1} (O—H); 1700 cm^{-1} (C=O); 1655 cm^{-1} (NH_2). Anal. Calc. for $C_{24}H_{37}ON \cdot 1.5H_2O$: C, 71.39; H, 9.95. Found: C, 70.83; H, 9.98%.

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