UDC 547.92/.93

106. Ken'ichi Takeda, Taichiro Komeno, and Jun'ichi Kawanami : Bile Acids and Steroids. XIV.*2 Thiosteroids. (3). Reduction of the 11β,12β-Episulfide.

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In a previous paper,[1] it was reported that methyl 3α-acetoxy-11α,12α-epoxycholanate could be cleaved by thiocyanic acid. The present paper describes the results of further studies on the thiocyanato-hydrin which was obtained by the above-mentioned reaction.

Methyl 3α-acetoxy-11β-thiocyanato-12α-hydroxycholanate (I) could not be halogenated by phosphorus tribromide or phosphoryl chloride, which reaction is known in general to be accompanied by inversion of configuration,[2] but could be chlorinated by thionyl chloride to give a thiocyanato-chloride (IIa), m.p. 146~148°. Since retention of configuration is usual with this reagent,[3] the product (IIa) was assumed to be methyl 3α-acetoxy-11β-thiocyanato-12α-chlorocholanate. Gallagher[4] reported that methyl 3α-(2-carboxyethylcarbonyloxy)-12β-hydroxy-11-oxocholanate was not halogenated by thionyl chloride but was brominated by phosphorus tribromide. These two results are contradictory and show that the approach of halogenation reagents is possible only from the rear side of the steroid molecule due to steric hindrance of angular methyl groups at C-10 and C-13.

When the thiocyanato-hydrin (I) was treated with pyridine and mesyl chloride (methanesulfonyl chloride) an amorphous thiocyanato-mesylate (IIb) was obtained in a quantitative yield. Treatment of the thiocyanato-chloride (IIa) or -mesylate (IIb) with alkali gave the same acid (IIIa), m.p. 213~214°, containing a sulfur atom. This acid was assumed to be 3α-hydroxy-11β,12β-epithiocholanic acid, since it has been reported[5] that treatment of 1,2-halothiocyanates with sodium sulfide gave episulfides in general. Its methyl ester acetate (IIIc), m.p. 150~152°, was reduced with zinc-acetic acid to methyl 3α-acetoxy-11-cholenate (IV), while it was desulfurized by Raney nickel to methyl 3α-acetoxycholanate (V). The methyl ester (IIIb) was oxidized with chromium trioxide-pyridine complex to methyl 3-oxo-11β,12β-epithiocholanate (VI), m.p. 152~154°, without affecting the 11β,12β-episulfide ring.

Subsequently, reduction of the 11β,12β-episulfide was attempted. Reduction of either the 11β,12β-episulfide (IIIa or IIIc) with lithium aluminium hydride or of the 11β-thiocyanato-12α-chloride (IIa) or -12α-mesylate (IIb) with the same reagent gave the identical compound (VIIa), m.p. 163~164°. From the results of the following reaction it was assumed that the compound (VIIa) was not a mercapto derivative but 11β,12β-epithiocholane-3α,24-diol formed only by reduction of the side chain. 11-Lithocholenyl alcohol 3,24-diacetate (VIIIb) was oxidized by perbenzoic acid to 11α,12α-epoxide (IX), m.p. 85~87°, which was converted with thiocyanic acid to an amorphous thiocyanato-hydrin (X). When the compound (X) was mesylated, followed by saponification with alkali, a substance of m.p. 168~170° was obtained (62% overall yield from the 11α,12α-epoxide (IX)). The former substance of m.p. 163~164° and this substance of m.p. 168~170°, prepared

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*2 Part XIII. K. Takeda, T. Kubota, J. Kawanami : This Bulletin, 8, 617(1960).

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by two different methods, were assumed as the dimorphs of 11β,12β-epithiocholane-3α, 24-diol. The reason for this assumption was as follows: (a) The mixed melting point determination of these compounds showed no depression, (b) the difference of their infrared spectra in Nujol disappeared in chloroform solution, and (c) these compounds gave the same diacetate (VIIb), m.p. 108~110°, by acetylation with pyridine-acetic anhydride. From such results it may be deduced that lithium aluminium hydride reacts at the 11β-thiocyanato group at first to give the mercapto-ol derivative and this vicinal *trans*-diaxial mercapto-ol compound forms the episulfide ring, but the episulfide so-formed cannot be further reduced to an 11β-thiol because the position is extremely hindered.*[3]

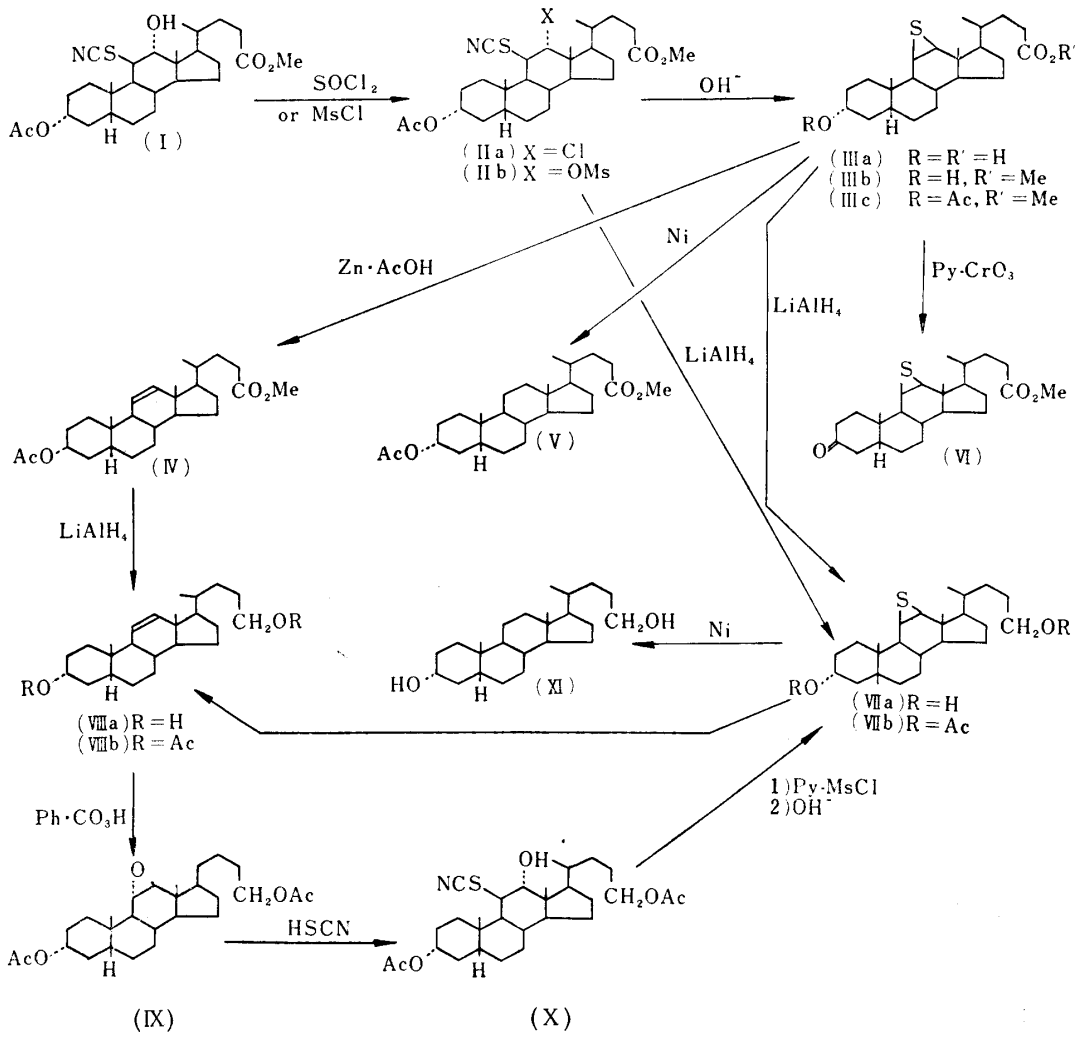

TABLE I. Ring Cleavage Reaction of Methyl 3α-Acetoxy-11β,12β-epithiocholanate (IIIc) by Various Reagents

Reagent	Product
HCl-dioxane, HBr-dioxane, Na-amalgam, Na_2S, $CrCl_2$, H_2/Pt.	Not affected
$LiAlH_4$ in tetrahydrofuran or Bu_2O, $LiAlH_4$-$AlCl_3$	11β,12β-Epithiolithocholanyl alcohol (VIIa)
HBr-AcOH, Clemmensen reduction	Methyl 11-Lithocholenate acetate (IV)
Na-MeOH, Li-$EtNH_2$-liq. NH_3, I_2-EtOH*	11-Lithocholenyl alcohol (VIIIa)

* (VIIa) was used as a starting material instead of (IIIc).

*[3] R. C. Arnold, A. P. Lien, and R. M. Alm (J. Am. Chem. Soc., **72**, 731(1950)) reported that dibutyl disulfide was reduced to butanethiol but di-*tert*-butyl disulfide was not affected by reason of steric hindrance.

11β,12β-Epithiocholane-3α,24-diol (VIIa) was also desulfurized by Raney nickel to lithocholanyl alcohol (XI).

Finally, various reactions to cleave the episulfide ring of (IIIc) were attempted but the expected 11β-mercapto derivative was not obtained. The reagents used and the results are cited in Table I.

Experimental*4

Methyl 3α-Acetoxy-11β-thiocyanato-12α-chlorocholanate (IIa)—A mixture of 700 mg. of the thiocyanato-hydrin (I) in 2 cc. of $SOCl_2$ was allowed to stand for 16 hr. at room temp. The brown reaction mixture was decomposed by adding ice and extracted with Et_2O. The Et_2O solution was washed with Na_2CO_3 solution and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue (800 mg.) was chromatographed over 24 g. of Al_2O_3. The eluate (700 mg.) with petr. ether-benzene (2:1, 1:1) and benzene was crystallized from MeOH to 450 mg. of crystals, m.p. 145~148°, which were further recrystallized with MeOH to long prisms (IIa), m.p. 146~148°. $[\alpha]_D^{31}$ +73.5°±4° (c=0.642, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{42}O_4NClS$: C, 64.16; H, 8.08; Cl, 6.76; S, 6.12. Found: C, 64.43; H, 8.17; Cl, 6.73; S, 5.97. IR ν_{max}^{Nujol} cm^{-1}: 2174 (SCN), 1736, 1259, 1250 (OAc), 720 (C-Cl).

In another run, the first eluate of chromatography gave fine prisms, m.p. 125~126°, which were identified as a dimorph of (IIa). $[\alpha]_D^{21}$ +76°±3° (c=0.756, dioxane). *Anal.* Calcd. for $C_{28}H_{42}O_4NClS$: C, 64.16; H, 8.08. Found: C, 64.55; H, 8.07. IR ν_{max}^{Nujol} cm^{-1}: 2183 (SCN), 1739, 1241 (O-Ac), 738 (C-Cl). The infrared spectra of these compounds were in full agreement in CS_2 solution.

Methyl 3α-Acetoxy-11β-thiocyanato-12α-mesyloxycholanate (IIb)—To a solution of 1.15 g. of (I) in 10 cc. of pyridine, 1.2 cc. of mesyl chloride was added under cooling. The mixture was allowed to stand overnight at room temp., H_2O was added, and extracted with Et_2O. The Et_2O solution was washed to neutrality, dried over Na_2SO_4, and evaporated to dryness. The residue (1.35 g.) was used for the next step.

3α-Hydroxy-11β,12β-epithiocholanic Acid (IIIa)—(a) From (IIa): A solution of 1.1 g. of (IIa) dissolved in 100 cc. of 5% KOH-MeOH, was allowed to stand overnight at room temp., evaporated *in vacuo*, H_2O added, and acidified with dil. HCl. The precipitate was collected by filtration and recrystallized from $CHCl_3$-MeOH to 800 mg. of long needles, m.p. 213~214°. $[\alpha]_D^{30}$ +40.9°±4° (c=0.579, dioxane). *Anal.* Calcd. for $C_{24}H_{38}O_3S$: C, 70.89; H, 9.42; S, 7.89. Found: C, 71.16; H, 9.57; S, 7.99. IR ν_{max}^{Nujol} cm^{-1}: 3311, 2725, 2581 (OH), 1706 (C=O).

(b) From (IIb): A solution of 1.35 g. of the above oily mesylate (IIb) dissolved in 50 cc. of 5% KOH-MeOH was treated as cited above and gave 795 mg. of (IIIa), m.p. 213~214°, which showed no depression on admixture with (IIIa) prepared by method (a).

Methyl 3α-Acetoxy-11β,12β-epithiocholanate (IIIc)—The acid (IIIa) (850 mg.) was esterified with CH_2N_2 and then acetylated with pyridine-Ac_2O. The product was recrystallized from acetone-MeOH to 950 mg. of leaflets (IIIc), m.p. 150~152°. $[\alpha]_D^{30}$ +61.8°±4° (c=0.668, $CHCl_3$). *Anal.* Calcd. for $C_{27}H_{42}O_4S$: C, 70.09; H, 9.15; S, 6.93. Found: C, 70.25; H, 9.26; S, 6.87. UV λ_{max}^{EtOH} ca. 250 mμ (shoulder, ε 58). IR ν_{max}^{Nujol} cm^{-1}: 1736, 1259, 1245 (O-Ac).

Methyl 3α-Acetoxy-11-cholenate (IV) from (IIIc)—To a solution of 300 mg. of (IIIc) in 10 cc. of AcOH, 3 g. of Zn dust was added, the mixture was heated under reflux for 3 hr., H_2O was added, and extracted with Et_2O. The Et_2O solution was washed with Na_2CO_3 solution and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue (290 mg.) was chromatographed over 10 g. of Al_2O_3. The eluate with petr. ether-benzene (49:1~47:3) gave 130 mg. of leaflets (IV), m.p. 118~120°, from MeOH, which was identified by mixed m.p. and the infrared spectrum. From the eluate with petr. ether-benzene (9:1~1:1), 130 mg. of (IIIc), m.p. 145~150°, was recovered.

Methyl 3α-Acetoxycholanate (V) from (IIIc)—To a solution of 200 mg. of (IIIc) in 10 cc. of dioxane, 3 g. of Raney nickel was added, the mixture was heated under reflux for 10 hr., and filtered. The filtrate was added to H_2O and extracted with Et_2O. The Et_2O solution was washed with Na_2CO_3 solution and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from MeOH to 160 mg. of (V), m.p. 133~135°, which was identified by mixed m.p. and the infrared spectrum.

Methyl 3-Oxo-11β,12β-epithiocholanate (VI)—The methyl ester (4.2 g.) prepared from the acid (IIIa) with CH_2N_2 was dissolved in 50 cc. of pyridine and added to a mixture of 50 cc. of pyridine and 5 g. of CrO_3. The mixture was allowed to stand for 18 hr. at room temp., poured into H_2O,

*4 All m.p.s were determined in a capillary tube and are uncorrected. Infrared spectra were measured with a Perkin-Elmer Single-beam Infrared spectrophotometer, Model 12C, and ultraviolet spectra were taken with a Beckman Spectrophotometer, Model DU.

and extracted with Et_2O-benzene (1:1). The organic solution was washed to neutrality, dried over Na_2SO_4, and evaporated to dryness. The residue (4.2 g.) was crystallized from Et_2O to 2.8 g. of crystals, which was further recrystallized from Me_2CO to 2.3 g. of crystals (VI), m.p. 152~154°. $[\alpha]_D^{31}$ +43.6°±4°(c=0.651, $CHCl_3$). *Anal.* Calcd. for $C_{25}H_{38}O_3S$: C, 71.73; H, 9.15; S, 7.66. Found : C, 71.72; H, 9.43; S, 7.65. IR ν_{max}^{Nujol} cm^{-1} : 1737, 1712 (C=O).

11β,12β-Epithiocholane-3α,24-diol (VIIa)—(a) From (IIb) : A solution of 2.48 g. of the oily mesylate (IIb) in 50 cc. of anhyd. Et_2O was added dropwise with stirring into a suspension of 1.2 g. of $LiAlH_4$ in 20 cc. of anhyd. Et_2O over a period of 30 min. After refluxing for 2 hr., the mixture was washed with cold water to destroy excess of reagent and dil. HCl. The Et_2O solution was washed with Na_2CO_3 solution and H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue (1.85 g.) was recrystallized from Me_2CO to 1.32 g. of plates (VIIa), m.p. 163~164°. $[\alpha]_D^{27}$ +43.8°±2°(c=0.930, dioxane). *Anal.* Calcd. for $C_{24}H_{40}O_2S$: C, 73.42; H, 10.27; S, 8.17. Found : C, 73.41; H, 10.32; S, 7.93. UV λ_{max}^{EtOH} 257 mμ (ε 61). IR ν_{max}^{Nujol} 3311 cm^{-1}(OH).

(b) From (IIa) : The chloride (IIa)(110 mg.) was treated with $LiAlH_4$ in ether as above and gave 70 mg. of (VIIa), m.p. 163~164°.

(c) From (VIIIb) : 3α,24-Diacetoxy-11-cholene (VIIIb)(1.80 g.) was oxidized by perbenzoic acid in $CHCl_3$ as usual. The product was recrystallized from hydr. MeOH to 1.50 g. of 3α,24-diacetoxy-11α,12α-epoxycholane (IX), m.p. 85~87°. $[\alpha]_D^{30}$ +43.8°±3°(c=0.672, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{44}O_5$: C, 73.01; H, 9.63. Found : C, 73.35; H, 9.58.

The epoxide (IX)(1.177 g.) was treated with the HSCN-Et_2O solution for 64 hr. as described in the previous paper[1] and gave an oil (X) in a nearly quantitative yield. IR ν_{max}^{Nujol} cm^{-1} : ca. 3420 (broad, OH), 2150 (SCN). A portion of (X) was chromatographed over neutral Al_2O_3 but a crystalline product was not obtained from any fractions.

The crude thiocyanato-hydrin (X)(860 mg.) was mesylated with 9 cc. of pyridine and 0.9 cc. of mesyl chloride to give 950 mg. of an oily mesylate, which was heated under reflux for 1 hr. in 20 cc. of 5% KOH-MeOH and extracted with $CHCl_3$. The extract was crystallized from Et_2O to 400 mg. of crystals and recrystallized from Me_2CO to (VIIa), m.p. 168~170°. *Anal.* Calcd. for $C_{24}H_{40}O_2S$: C, 73.42; H, 10.27; S, 8.17. Found : C, 73.51; H, 10.50; S, 8.06.

This compound was identified to be a dimorph of the compound of m.p. 163~164°, prepared as above, since the mixed m.p. showed no depression and the difference of the spectra in Nujol disappeared in $CHCl_3$ solution.

3α,24-Diacetoxy-11β,12β-epithiocholane (VIIb)—The compound, m.p. 163~164°, was acetylated with pyridine-Ac_2O and gave the diacetate as needles (from MeOH), m.p. 108~110°. $[\alpha]_D^{26}$ +61.0°±2° (c=0.927, dioxane). *Anal.* Calcd. for $C_{28}H_{44}O_4S$: C, 70.55; H, 9.30; S, 6.73. Found : C, 70.30; H, 9.34; S, 6.85. IR ν_{max}^{Nujol} 1740 cm^{-1}(O-Ac).

The compound of m.p. 168~170° was also acetylated with pyridine-Ac_2O similarly and gave the same compound, m.p. 108~110°, from MeOH, which was identified by mixed m.p. and the infrared spectrum.

Lithocholanyl Alcohol (XI) from (VIIa)—To a solution of 200 mg. of (VIIa) in 20 cc. of dioxane, 3 g. of Raney nickel was added. The mixture was heated under reflux for 20 hr. The product was recrystallized twice from Me_2CO to 120 mg. of (XI), m.p. 175~177°, which was identified by mixed m.p. and the infrared spectrum. *Anal.* Calcd. for $C_{24}H_{42}O_2$: C, 79.50; H, 11.68; O, 8.83. Found: C, 79.83; H, 11.58; O, 9.20.

Attempted Ring-opening of (IIIc)—(a) HCl-dioxane : Dry HCl gas was introduced into a solution of 88 mg. of (IIIc) in 3 cc. of dioxane for 1 hr., the mixture was added to Et_2O, and treated as usual. The product was reacetylated with pyridine-Ac_2O and gave (IIIc), m.p. 148~150°, from MeOH.

(b) HBr-dioxane : To a solution of 135 mg. of (IIIc) in 5 cc. of dioxane, 0.05 cc. of 47% HBr-AcOH solution was added. The mixture was stirred for 1 hr. at room temp. and H_2O was added. The precipitate (118 mg., m.p. 134°) was recrystallized from MeOH to (IIIc), m.p. 146~148°.

(c) Na-Hg : To a solution of 43 mg. of (IIIc) in 1 cc. of MeOH and 3 cc. of EtOH, 1.5 g. of 3% Na-Hg and AcOH were added in small quantities under cooling over a period of 45 min. After agitation for 2 hr., dil. HCl was added to the mixture and extracted with Et_2O. The extract was crystallized from MeOH to 35 mg. of (IIIc), m.p. 145~146°.

(d) $CrCl_2$: To a solution of 54 mg. of (IIIc) in 5 cc. of AcOH, 3 cc. of 5% $CrCl_2$ solution was added dropwise in CO_2 atmosphere. The mixture was agitated for 4 hr., allowed to stand overnight at room temp., and extracted with Et_2O. The extract was reacetylated with pyridine-Ac_2O and gave 55 mg. of (IIIc), m.p. 143~146°. This reaction was also carried out in acetone and the results were the same.

(e) H_2-Pt : The episulfide (IIIc)(300 mg.) was hydrogenated over Pt catalyst in 20 cc. of AcOH and absorbed 12 cc. of H_2. The filtrate was evaporated and the residue crystallized from MeOH to 250 mg. of (IIIc), m.p. 145~148°.

(f) Na_2S : A solution of 46 mg. of (IIIc) and 500 mg. of Na_2S in a small amount of H_2O was heated at 140° for 7 hr., acidified with dil. HCl, and extracted with Et_2O. The extract was crystallized

from $CHCl_3$-MeOH to 23 mg. of (IIIa), m.p. 208~210°.
(g) $LiAlH_4$-$AlCl_3$: To a suspension of 50 mg. of $LiAlH_4$ in 3 cc. of anhyd. Et_2O, a solution of 45 mg. of (IIIc) and 150 mg. of $AlCl_3$ in 3 cc. of anhyd. Et_2O was added in small quantities. The mixture was refluxed for 2 hr. and treated as above. The residue was reacetylated and chromatographed over 1 g. of Al_2O_3. The eluate with petr. ether-benzene (9:1~1:1) crystallized from MeOH to 28 mg. of (VIIb), m.p. 104~106°.
(h) $LiAlH_4$-Bu_2O : The episulfide (IIIc) (200 mg.) was reduced with 136 mg. of $LiAlH_4$ in Bu_2O at 80° for 2 hr. The product was crystallized from MeOH to (VIIa), m.p. 156~158°, which was acetylated with pyridine-Ac_2O to the diacetate (VIIb), m.p. 100~105°.
(i) Na-MeOH : To a solution of 100 mg. of (IIIc) in 50 cc. of MeOH, 5 g. of Na was added in small portions over a period of 1.5 hr. The mixture was further heated under reflux for 1.5 hr., H_2O added, and extracted with Et_2O. The Et_2O layer was separated, washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was recrystallized from AcOEt to 10 mg. of 11-lithocholenyl alcohol (VIIIa), m.p. 190~193°. The H_2O layer was acidified with dil. HCl and extracted with Et_2O. The extract was esterified with CH_2N_2, followed by acetylation and gave 50 mg. of crystals, which were separated by chromatography to 10 mg. of (IV), m.p. 110°, and 8 mg. of (IIIc), m.p. 150~153°.
(j) Li-$EtNH_2$-liq. NH_3 : To a solution of 100 mg. of (IIIc) in 20 cc. of $EtNH_2$, 100 mg. of Li was added in small portions. The mixture was allowed to stand for 2 hr. at −20°, but Li did not dissolve completely. Then 10 cc. of liq. NH_3 was added and Li dissolved to give a clear solution. The mixture was allowed to stand for 2 hr. at −40°, 700 mg. of NH_4Cl was added, and NH_3 was allowed to evaporate at room temp. The residue was extracted with Et_2O. The neutral fraction gave 57 mg. of crystals, m.p. 183~187°, which were recrystallized from MeOH to (VIIIa), m.p. 193~194°.
(k) HBr-AcOH : To a solution of 84 mg. of (IIIc) in 4 cc. of AcOH, 0.1 cc. of 30% HBr-AcOH was added. The mixture was allowed to stand overnight at room temp. Treatment as usual gave 60 mg. of an oil, which was isolated by chromatogarphy to 14 mg. of (IV), m.p. 110°.
(l) Clemmensen Reduction : To a solution of 200 mg. of (IIIc) in toluene, Zn-amalgam prepared from 4.3 g. of 5% $HgCl_2$ solution and 1 g. of Zn was added. To the mixture, 6 cc. of HCl was further added and heated under reflux. During refluxing 1 g. of Zn-Hg and HCl were added every 8 hr. After a total refluxing period of 60 hr., the mixture was filtered and evaporated. The residue was dissolved in Et_2O. Et_2O solution was washed to neutrality. Et_2O residue gave 167 mg. of an oil, which was chromatographed over 5 g. of Al_2O_3. From the eluate with petr. ether, 43 mg. of a hydrocarbon, m.p. 50°, was obtained, which was not studied further. The eluate with petr. ether-benzene (9:4~4:1) gave 52 mg. of (IV), m.p. 115~118°, and the eluate with petr. ether-benzene (4:1~1:1) gave 8 mg. of (IIIc), m.p. 150~151°.
(m) I_2-EtOH : To a solution of 500 mg. of (VIIa) in 20 cc. of EtOH, 160 mg. of I_2 was added. The mixture was heated under reflux for 85 hr., evaporated, and extracted with Et_2O. The Et_2O solution was washed with Na_2CO_3 solution and H_2O, dried over Na_2SO_4, and evaporated. The Et_2O residue was crystallized from AcOEt to 300 mg. of (VIIIa), m.p. 190~192°.
The products prepared by various methods described in the sections (a)~(l) were identified respectively by mixed m.p. and comparison of infrared spectra with those of authentic samples.

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Summary

Treatment of methyl 3α-acetoxy-11β-thiocyanato-12α-chloro(or -mesyloxy)cholanate with alkali or lithium aluminium hydride afforded 11β,12β-episulfides, which could not be further reduced to the 11β-thiol by various reagents.

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