UDC 547.92/.93

105. **Ken'ichi Takeda, Tokuo Kubota, and Jun'ichi Kawanami**: Bile Acids and Steroids. XIII. Thiosteroids. (2).[1] Addition of Thiocyanic Acid to Steroidal α,β-Unsaturated Ketones.

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It was reported in the preceding paper[1] that the treatment of steroidal 11α,12α-, 11β,12β-, and 9β,11β-epoxides with thiocyanic acid yielded the corresponding thiocyanatohydrins. In the course of this investigation, when 17α-hydroxy-21-acetoxy-9β,11β-epoxy-4-pregnene-3,20-dione (I) was treated with a chloroform solution of thiocyanic acid prepared by Bück's method,[2] a by-product of m.p. 180~182° (decomp.) was isolated besides the objective thiocyanatohydrin (II), m.p. 154~156° (decomp.). Its infrared spectrum showed no absorption of α,β-unsaturated ketone but a broad, very strong band, compared with thiocyanates, at 2077 cm^{-1} corresponding to isothiocyanates.[3] From this fact the probable structure of the latter compound would be 3-oxo-5ξ-isothiocyanate (III) formed by addition of isothiocyanic acid to 4-en-3-one group. However, it would be difficult to confirm its structure because of the complicity of the functional groups in this compound.

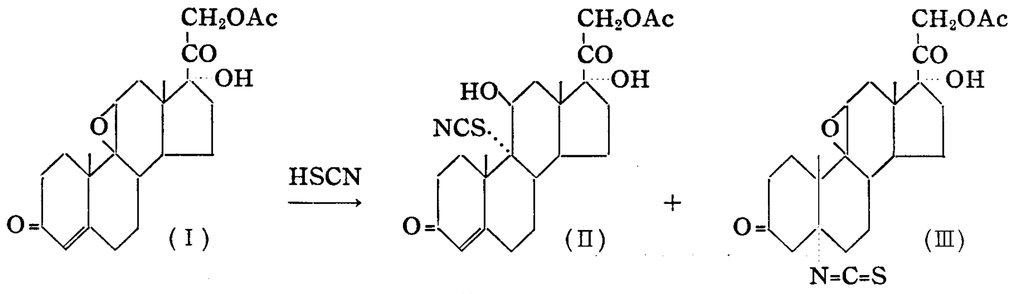

Chart 1.

For the purpose of clarifying the structure of this by-product (III), reaction of 4-cholesten-3-one (IV) with thiocyanic acid was carried out. A product, m.p. 154~155° (decomp.) (V), analogous to the above-mentioned by-product, was obtained. The ultraviolet spectrum of this product shows an absorption maximum at 248 mμ (ε 1,440) due to isothiocyanato group,[4] instead of a strong absorption of α,β-unsaturated ketone group. Also, its infrared spectrum exhibits no absorption of α,β-unsaturated ketone group but a very strong absorption at 2066 cm^{-1} corresponding to the isothiocyanato group and a strong band at 1709 cm^{-1} indicating the presence of an unconjugated ketone. Thus, it is almost certain that a 1,4-addition of thiocyanic acid, in the form of isothiocyanic acid, to the conjugated system of steroidal α,β-unsaturated ketone took place.

It is well known that thiocyanic acid exists in a tautomeric form as $N\equiv C-SH \rightleftarrows HN=C=S$, and addition of thiocyanic acid to a double bond yields thiocyanates or isothiocyanates.[3] However, reaction between thiocyanic acid and α,β-unsaturated ketones has been reported in the field of simple aliphatic compounds such as mesityl oxide and of substituted cyclohexenones. Although their assignment seems to be doubtful, those pro-

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1) Part (1). Ken'ichi Takeda, Taichiro Komeno: This Bulletin, **8**, 468(1960).

2) U. Bück: Z. anorg. Chem., **77**, 51(1912).

3) L.S. Luskin, G.E. Gantert, W.E. Craig: J. Am. Chem. Soc., **78**, 4965(1956).

4) R. Charonnt, R. Bourdon: Bull. soc. chim. France, **1958**, 719; E. Svátek, R. Zahradnik, A. Kjaer: Acta Chem. Scand., **13**, 442(1959).

ducts were described[5] as normal thiocyanato compounds. Also, the fission of steroidal epoxides by thiocyanic acid afforded normal thiocyanato compounds, as described in the preceding paper.[1]

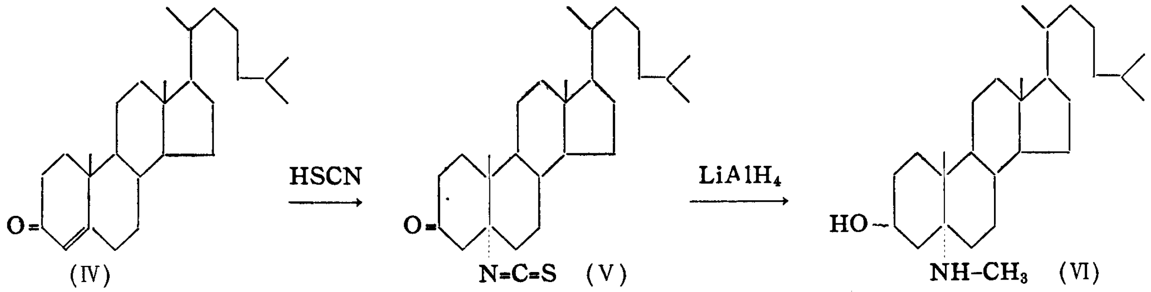


Chart 2.

The reaction product of thiocyanic acid and 4-cholesten-3-one was thus subjected to lithium aluminium hydride reduction in order to confirm the isothiocyanate group. There was a recognized evolution of hydrogen sulfide and a methylamino compound (VI), $C_{28}H_{51}ON$, m.p. 110~111°, was obtained as expected. The group introduced into C-5 therefore was verified to be isothiocyanate and the data in the above-mentioned infrared and ultraviolet spectra are also in agreement.

The isothiocyanato group was assigned an α-configuration by the inference that the addition of an isothiocyanic acid molecule to the double bond should occur more readily from the rear side since β-side would be hindered by the methyl group in C-10. This assignment was further supported by the fact that the rotatory dispersion curve[*2] of isothiocyanatocholestanone (V) shows identical positive Cotton-effect Curve as that of ordinary 3-keto-5α-steroids.[6] (Fig. 1).

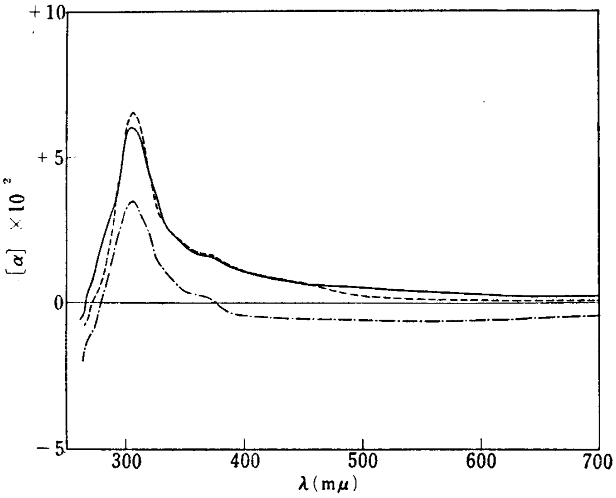


Fig. 1.
Rotatory Dispersion Curves (in MeOH)

——— 5α-Isothiocyanatocholestan-3-one (V)
–·–·–· 5α-Isothiocyanato-17α-methyl-17β-hydroxyandrostan-3-one (XIII)
-------- 5α-Isothiocyanato-17α-ethynyl-17β-hydroxy-19-norandrostan-3-one (XVII)

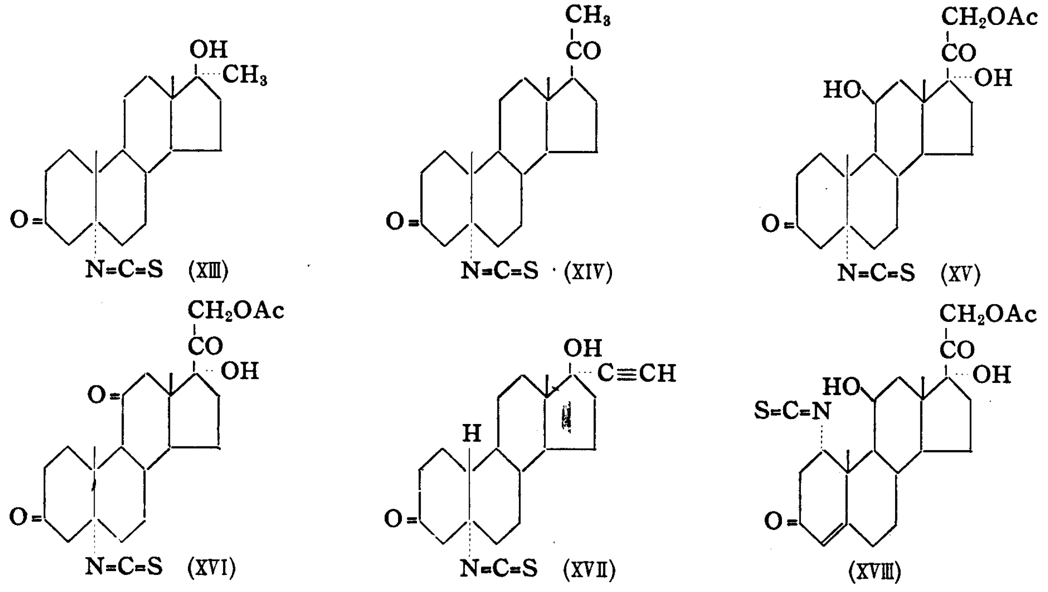
It has now been established that thiocyanic acid reacted with 4-cholesten-3-one (IV) to give 5α-isothiocyanatocholestan-3-one (V). Accordingly, the structure of the by-product in the reaction of 17α-hydroxy-21-acetoxy-9β,11β-epoxy-4-pregnene-3,20-dione (I) with thiocyanic acid is found to be 3-oxo-5-isothiocyanate (III).

[*2] Rotatory dispersion curves were measured at Stanford University through the kind offices of Dr. C. Djerassi.

5) H. A. Bruson: U.S. Pat. 2,395,453 (C. A. **40**, 3467(1946)); R. A. Mathes, F. D. Stewart, F. Swedish, Jr.: J. Am. Chem. Soc., **70**, 1452(1948).

6) C. Djerassi: Bull. soc. chim. France, **1957**, 741.

In the steroid series, addition of thiocyanic acid to α,β-unsaturated ketones, such as that described above, had been unknown up to that time,[*3] and so this addition reaction of thiocyanic acid was carried out on a number of steroidal hormones, i.e. methyltestosterone (VII), progesterone (VIII), hydrocortisone acetate (IX), cortisone acetate (X), 19-norethynyltestosterone (XI), and prednisolone acetate (XII). As expected, the corresponding isothiocyanato derivatives were obtained in all cases.



(XIII) (XIV) (XV) (XVI) (XVII) (XVIII)

Chart 3.

In these products, there is little doubt that the derivatives obtained from (VII), (VIII), (IX), and (X) are the 3-oxo-5α-isothiocyanates (XIII), (XIV), (XV), and (XVI), respectively, as in the case of 4-cholesten-3-one (IV) except from 19-norethynyltestosterone (XI). As it has no methyl group in C-10, the configuration of the isothiocyanato group in C-5 of the reaction product of (XI) was somewhat doubtful. However, it was confirmed that this product (XVII) has the same 5α-configuration as the others, because the rotatory dispersion curve of the product (XVII) closely resembles that of (V) (Fig. 1).

Although prednisolone acetate (XII) possesses 1,4-dien-3-one system, analysis of its product suggested an addition of only one mole of thiocyanic acid and the ultraviolet spectrum showed absorption maximum at 242 mμ (ε 13,000) indicating the retention of a conjugated ketone system. Therefore, the isothiocyanato group has been assigned the 1α-position by analogy with known addition of alkanethiolic acid to 1,4-diene-3-keto-steroids[7)] and the product has the structure of (XVIII).

Further, 5α-isothiocyanatoallopregnane-3,20-dione was submitted to lithium aluminium hydride reduction and the reduction product was examined. The basic fraction of the reaction mixture afforded a small amount of a by-product, $C_{22}H_{37}O_2N$, m.p. 242～244°, in addition to the methylamino compound (XIXa), m.p. 178～180°. The former compound showed absorptions at 1635, 889, and 878 cm^{-1} corresponding to a methyleneimino group in the infrared spectrum and the structure was presumed to be (XXa). However, this compound resisted lithium aluminium hydride reduction or catalytic reduction and also gave no picrate. On the other hand, purification of the neutral fraction yielded a small amount of a compound of m.p. 284～286°. It contains sulfur in the molecule and

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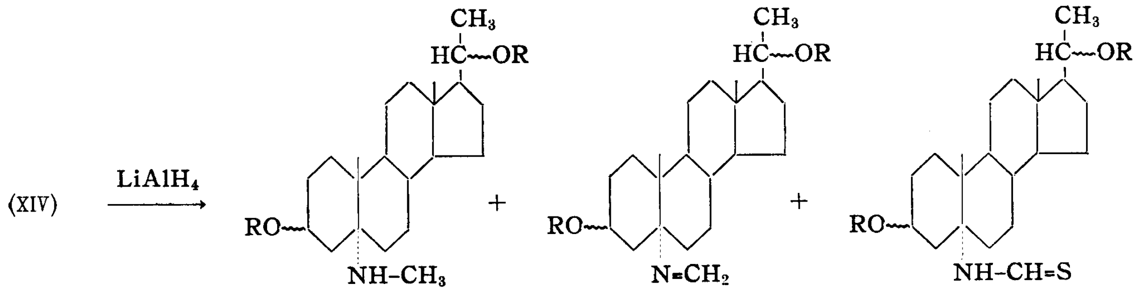
[*3] After the principal part of this investigation was finished (Patent Applied, October, 10, 1958), preparation of such isothiocyanato-steroids was found in U.S. Pat. 2,872,462 (February 3, 1959).

7) R.M. Dodson, R.C. Tweit: J. Am. Chem. Soc., 81, 1224(1959).

the elemental analysis of this product agreed well with $C_{22}H_{37}O_2NS$. The compound has a strong absorption maximum at 251 mμ (ε 18,000) in the ultraviolet spectrum and a band at 1530 cm^{-1} considered to be due to a thioureido group in the infrared spectrum. The structure of the neutral product was presumed to be (XXIa) but further investigation has not been carried out.



(XIXa) R=H (XXa) R=H (XXIa) R=H
(XIXb) R=Ac (XXb) R=Ac (XXIb) R=Ac

Chart 4.

The 5α-isothiocyanato group in these steroids was easily eliminated by the action of alkali or acid and these compounds afforded the parent 4-ene-3-keto-steroids.

In the progestational activity, the compounds (XIV) and (XVII) were approximately equal to the original progesterone (VIII) and 19-norethynyltestosterone, respectively. Also, (XVI) had the same order of glucocorticoid activity as cortisone acetate (X). However, the glucocorticoid activity of (XV) was only about ¼ to ⅒ of the parent hydrocortisone acetate (IX).

It is still doubtful whether the 3-oxo-5α-isothiocyanate hormone analogs themselves are biologically active or it is because of their easy conversion to the original 4-ene-3-ketones in the organism.

Experimental[*4]

Preparation of HSCN-CHCl$_3$ Solution—A slurry of 7.3 g. of powdered KSCN in 30 cc. of CHCl$_3$ was triturated with 11.2 g. of KHSO$_4$ in a mortar for 5 min. After HSCN-CHCl$_3$ solution was decanted, an additional 10 cc. of CHCl$_3$ was added to the solid mixture. The mixture was triturated for an additional 5 min. and then filtered through cotton. The CHCl$_3$ solution was combined to give 33 cc. and 1 cc. of this solution was shown to contain 48.6 mg. of HSCN by titration with 0.1*N* NaOH solution.

5α-Isothiocyanatocholestan-3-one (V)—To 26 cc. (8 moles) of HSCN-CHCl$_3$ (48 mg./1 cc.) solution, 1.00 g. of 4-cholesten-3-one (IV) was added and dissolved. The solution was allowed to stand at room temperature, protected from light, for 48 hr. and filtered. The filtrate was washed with H_2O, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was crystallized from acetone-MeOH to 420 mg. of crystals, m.p. 145~148°(decomp.). Recrystallization from acetone gave pure 5α-isothiocyanatocholestan-3-one (V) as needles, m.p. 154~155°(decomp.), $[\alpha]_D$ +54°(c=0.87, CHCl$_3$). *Anal.* Calcd. for $C_{28}H_{45}ONS$: C, 75.80; H, 10.22; N, 3.16; S, 7.21. Found: C, 75.85; H, 10.59; N, 3.13; S, 6.96. UV λ_{max}^{EtOH}: 248 mμ (ε 1,440). IR ν_{max}^{Nujol} cm^{-1}: 2066 (-N=C=S), 1709 (C=O).

5α-Isothiocyanato-17α-methyl-17β-hydroxyandrostan-3-one (XIII)—Methyltestosterone (VII) (1.00 g.) was dissolved in 33 cc. of HSCN-CHCl$_3$ solution, the solution was stored in a refrigerator for 48 hr., and freed from the precipitated polymerization product by filtration. The filtrate was washed with H_2O, dried, and evaporated to dryness *in vacuo*. The oily residue was crystallized from acetone-hexane to 730 mg. of crystals, m.p. 133~136°(decomp.), which was recrystallized twice from the same solvent to needles, m.p. 143~144°(decomp.). $[\alpha]_D$ +19°(c=0.96, CHCl$_3$). *Anal.* Calcd. for $C_{21}H_{31}O_2NS$: C, 69.77; H, 8.65; N, 3.88; S, 8.85. Found: C, 69.63; H, 8.69; N, 3.55; S, 8.77. UV λ_{max}^{EtOH}: 249 mμ (ε

[*4] All m.p.s 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.

1,470). IR ν_{max}^{Nujol} cm^{-1} : 3510 (OH), 2137～2065 (-N=C=S), 1713 (C=O).

5α-Isothiocyanatoallopregnane-3,20-dione (XIV)—The reaction of 10.5 g. of progesterone (VIII) with HSCN-$CHCl_3$ solution was processed as described above for (V). Trituration of the crude oily product with MeOH afforded 5.0 g. of the crystalline product, m.p. 139～141°(decomp.), which was recrystallized from MeOH and then from AcOEt to yield 4.1 g. of the pure material as prisms, m.p. 175～176° (decomp.). $[\alpha]_D$ +135°(c=1.04, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{31}O_2NS$: C, 70.75; H, 8.37; N, 3.75; S, 8.57. Found : C, 70.51; H, 8.31; N, 3.69; S, 8.33. UV λ_{max}^{EtOH} : 249 mμ (ε 1,350). IR ν_{max}^{Nujol} cm^{-1} : 2127 (-N=C=S), 1715, 1695 (C=O).

5α-Isothiocyanato-11β,17α-dihydroxy-21-acetoxyallopregnane-3,20-dione (XV)—Hydrocortisone acetate (IX) (1.00 g.) was treated with HSCN-$CHCl_3$ solution as described for preparation of (V). After working up as usual, 680 mg. of crude crystals, m.p. 197～208°(decomp.), was obtained from acetone-hexane. The product was dissolved in acetone under warming and crystallized with addition of hexane after removal of the less soluble starting material (IX), m.p. 212～215°. This procedure was repeated twice more to yield 320 mg. of small plates of the pure isothiocyanato derivative (XV), m.p. 182～184°(decomp.). $[\alpha]_D$ +125°(c=0.86, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{33}O_6NS$: C, 62.19; H, 7.18; N, 3.02; S, 6.91. Found : C, 61.73; H, 7.59; N, 2.72; S, 6.64. UV λ_{max}^{EtOH} mμ (ε) : 248 (1,680), 287 (124). IR ν_{max}^{Nujol} cm^{-1} : 3448 (OH), 2132～2092 (-N=C=S), 1715 (C=O), 1264, 1244 (OAc).

5α-Isothiocyanato-17α-hydroxy-21-acetoxyallopregnane-3,11,20-trione (XVI)—Cortisone acetate (X) (1.00 g.) was treated with HSCN-$CHCl_3$ solution by the usual method and the product was purified as described above for (XV) to yield 250 mg. of (XVI) as needles, m.p. 197～199°(decomp.). $[\alpha]_D$ +151° (c=0.96, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{31}O_6NS$: C, 62.45; H, 6.77; N, 3.04; S, 6.94. Found : C, 62.39; H, 6.81; N, 3.47; S, 6.49. UV λ_{max}^{EtOH} mμ (ε) : 248 (1,610), 290 (168). IR ν_{max}^{Nujol} cm^{-1} : 3401 (OH), 2079 (-N=C=S), 1698 (C=O), 1274, 1236 (OAc).

5α-Isothiocyanato-17α-ethynyl-17β-hydroxy-19-norandrostan-3-one (XVII)—19-Norethynyltestosterone (XI) (1.00 g.) was treated with HSCN-$CHCl_3$ solution as described for preparation of (XIII). The product, obtained by the usual manner, was recrystallized three times from acetone-hexane to give (XVII) as prisms, m.p. 158～159°(decomp.). $[\alpha]_D$ +52°(c=1.03, $CHCl_3$). *Anal.* Calcd. for $C_{21}H_{27}O_2NS$: C, 70.56; H, 7.61; N, 3.92; S, 8.96. Found : C, 70.39; H, 8.01; N, 3.74; S, 8.83. UV λ_{max}^{EtOH} : 248 mμ (ε 1,490). IR ν_{max}^{Nujol} cm^{-1} : 3386 (OH), 3300 (-C≡CH), 2096 (-N=C=S), 1708 (C=O).

1α-Isothiocyanato-11β,17α-dihydroxy-21-acetoxy-4-pregnene-3,20-dione (XVIII)—The reaction of 1.00 g. of prednisolone acetate (XII) with HSCN-$CHCl_3$ solution and purification of the product was carried out in the manner described above for preparation of (XV). There was obtained 120 mg. of the isothiocyanato-steroid (XVIII) as prisms, m.p. 225～227°(decomp.). $[\alpha]_D$ +195°(c=0.92, $CHCl_3$). *Anal.* Calcd. for $C_{24}H_{31}O_6NS$: C, 62.45; H, 6.77; N, 3.04; S, 6.94. Found : C, 62.86; H, 7.22; N, 3.23; S, 6.56. UV λ_{max}^{EtOH} : 242 mμ (ε 13,000). IR ν_{max}^{Nujol} cm^{-1} : 3472, 3401 (OH), 2165, 2132 (-N=C=S), 1727, 1742 (C=O), 1618 (⊨), 1263, 1244 (OAc).

Reduction of 5α-Isothiocyanatocholestan-3-one (V) with $LiAlH_4$—A solution of 500 mg. of (V) in 30 cc. of dehyd. Et_2O was added dropwise over a period of 40 min. into a suspension of 500 mg. of $LiAlH_4$ in 10 cc. of refluxing dehyd. Et_2O with stirring. The mixture was refluxed for an additional 3 hr. After cool, a small portion of H_2O was added carefully to decompose the complex and then 5% HCl was added to dissolve an amorphous metal hydroxide, when needles of the hydrochloride of the organic compound precipitated. The crystals were collected by filtration, washed with dilute HCl and Et_2O, and dried. The resulting hydrochloride (480 mg.) was added into NaOH-Et_2O mixture and the mixture was shaken to free the base. The Et_2O solution was washed with H_2O and dried over Na_2SO_4. The solvent was removed to give 425 mg. of a crystalline residue, m.p. 107～108°, which was recrystallized from acetone to 3ξ-hydroxy-5α-methylaminocholestane (VI) as needles, m.p. 110～111°. $[\alpha]_D$ +38°(c=0.95, $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{51}ON$: C, 80.51; H, 12.31; N, 3.35. Found : C, 80.26; H, 12.28; N, 3.23. IR ν_{max}^{Nujol} : 3356 cm^{-1} (OH).

The filtrate after separation of the above-mentioned hydrochloride was combined. The Et_2O layer was separated, washed with $NaHCO_3$ solution and H_2O, dried, and evaporated to yield 56 mg. of vitreous neutral product which was not investigated further.

Reduction of 5α-Isothiocyanatoallopregnane-3,20-dione (XIV) with $LiAlH_4$—To a suspension of 500 mg. of $LiAlH_4$ in 100 cc. of dry Et_2O, a solution of 980 mg. of (XIV) in 100 cc. of dry tetrahydrofuran was added dropwise under stirring. After stirring was continued for 48 hr. at room temperature, a small portion of H_2O was cautiously added to the reaction mixture to decompose the complex. The mixture was then acidified with addition of dil. HCl and shaken with Et_2O. To the aqueous acid layer, solid KOH was added under chilling to free the base and the mixture was extracted with Et_2O. The Et_2O solution was washed with H_2O, dried, and evaporated to dryness. The crystalline residue (786 mg.) was separated into 17 mg. of less-soluble prisms and 328 mg. of more-soluble needles by fractional recrystallization from acetone. The former is the methyleneimino compound (XXa) of m.p. 242～244°, $[\alpha]_D$ −36°(c=1.04, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{37}O_2N$: C, 76.03; H, 10.73; N, 4.03. Found : C, 75.53; H, 10.69; N, 4.11. IR ν_{max}^{Nujol} cm^{-1} : 3484 (OH), 1635, 889, 878 (-N=CH_2).

Diacetate : Acetylation of the above compound with Ac_2O-pyridine and recrystallization of the product from acetone afforded the diacetate (XXb), m.p. 156～158°. $[\alpha]_D$ +20°(c=0.42, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{41}O_4N$: C, 72.35; H, 9.58; N, 3.25. Found : C, 72.28; H, 9.47; N, 3.45. IR ν_{max}^{Nujol} cm^{-1}: 1729, 1255, 1244 (OAc), 1649, 877 (-N=CH_2).

The latter more-soluble compound is the methylamino compound (XIXa) of m.p. 178～180°, $[\alpha]_D$ +29°(c=1.03, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{39}O_2N$: C, 75.59; H, 11.25; N, 4.01. Found : C, 75.58; H, 11.36; N, 4.14. IR ν_{max}^{Nujol} : 3390 cm^{-1} (OH).

Diacetate : Acetylation of this compound by standing with Ac_2O-pyridine at room temperature for 48 hr. and recrystallization of the product from MeOH yielded the diacetate (XIXb) as scales of m.p. 182～184°. $[\alpha]_D$ +8°(c=0.51, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{43}O_4N$: C, 72.01; H, 10.00; N, 3.23. Found : C, 71.86; H, 10.18: N, 3.15.

The Et_2O layer, after separation of the above-mentioned basic fraction, was washed with $NaHCO_3$ solution and H_2O, dried, and evaporated. The crystalline residue (129 mg.) was repeatedly recrystallized from EtOH to 22 mg. of the thioureid (XXIa) as prisms, m.p. 284～286°(decomp.). $[\alpha]_D$ +26°(c=0.85, $CHCl_3$). *Anal.* Calcd. for $C_{22}H_{37}O_2NS$: C, 69.62; H, 9.83; N, 3.69; S, 8.43. Found : C, 69.38; H, 9.46; N, 3.95; S, 8.34. UV λ_{max}^{EtOH} : 251 mμ (ε 18,000). IR ν_{max}^{Nujol} cm^{-1} : 3336 (OH), 1530 (-NH-CH=S).

Diacetate : Acetylation of this compound with Ac_2O-pyridine and recrystallization of the product from $CHCl_3$-MeOH gave the diacetate (XXIb) as needles, m.p. 288～290°(decomp.). $[\alpha]_D$ + 81°(c=0.60, $CHCl_3$). *Anal.* Calcd. for $C_{26}H_{41}O_4NS$: C, 67.36; H, 8.91; N, 3.02; S, 6.90. Found : C, 67.62; H, 8.87; N, 3.12; S, 7.08. UV λ_{max}^{EtOH} : 251 mμ (ε 18,000). IR ν_{max}^{Nujol} cm^{-1} : 3291 (NH), 1731 (AcO), 1503 (-NH-CH=S).

The authors are grateful to Dr. C. Djerassi of Stanford University for determination of the rotatory dispersion curves. Thanks are also due to Messrs. H. Miyazaki and Y. Matsui for ultraviolet and infrared spectral measurements, and to the members of the analysis room of this Laboratory for elemental analyses.

Summary

The reaction between 4-cholesten-3-one (IV) and thiocyanic acid was investigated and it has been confirmed that thiocyanic acid was added to α,β-unsaturated ketone system to yield 5α-isothiocyanatocholestan-3-one. By this addition reaction, a number of isothiocyanato derivatives of steroidal hormones were prepared.

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