Anal. Calcd. for $C_{19}H_{25}BrO_2$: C, 62.46; H, 6.90. Found: C, 62.47; H, 6.94.

The bromohydrin, 0.22 g. in 20 ml. of acetic acid, was refluxed with stirring and treated with three 2-g. portions of zinc dust at 30-min. intervals. The mixture was cooled, filtered, and concentrated; it was extracted with 1:1 ether–petroleum ether, the extracts being washed, neutralized, dried, and evaporated. Chromatography of the residue on 4 g. of silica and elution with 1:1 benzene–petroleum ether gave 130 mg. of semicrystalline material, recrystallized to 80 mg. of X, m.p. 67–69°, identical with earlier preparations.

Finally, treatment of XXI with alcoholic alkali by the procedure described earlier yielded 3-methoxyestra-1,3,5(10)-trien-16-one, XIX, identical with other samples.

SKOKIE, ILL.

[CONTRIBUTION FROM THE RESEARCH LABORATORY, SHIONOGI & CO., LTD.]

Angular-Substituted Polycyclic Compounds. I. Cyanation of Δ^4-Cholesten-3-one

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Optimum conditions for the cyanation of Δ^4-cholesten-3-one were found to be in dimethylformamide in the presence of ammonium chloride. By this method we have been able to synthesize pure samples of 5α- (IV) and 5β-cyanocholestanone (V). The rate of hydrolysis of these compounds has been examined and the respective configurations determined. Some interesting properties of the corresponding acid amides (II and III) of the 5α- and 5β-cyano compounds are discussed.

The introduction of a C-substituent to the angular position of a condensed alicyclic-ring system has been already achieved by many authors.[1] As far as it is known the C_5-substituted cholestane derivatives are Westphalen's diol,[2] Claisen rearrangement products of 3β-vinyloxy-Δ^4-cholestene,[1c] and the product from the fission of 5(6) β-epoxycholestan-3β-ol by a Grignard reagent.[3]

The action of a Grignard reagent on Δ^4-cholesten-3-one[4] or 7-ketocholesterol[5,6] produced only 1,2-addition products and not the anticipated 1,4-addition products. This clearly shows that in the case of a sterically hindered C_5 position the introduction of a bulky substituent, such as the solvated-Grignard reagent, is difficult. Therefore, we attempted the 1,4-addition of the small yet sufficiently nucleophilic CN^- ion on Δ^4-cholesten-3-one. It is well known that the 1,4-addition reaction of potassium cyanide to α,β-unsaturated ketones is a very useful preparative method in organic chemistry.[7]

When Δ^4-cholesten-3-one (I) was treated with potassium cyanide in boiling methanol, four reaction products together with some starting material were obtained and separated by chromatography on alumina. Following the order of the elution, cholestenone, 5α-cyanocholestanone (IV), the dimer (IX), 3α-amino-3β-hydroxy-5α-carboxycholestane lactam (II), and finally 3β-amino-3α-hydroxy-5β-carboxycholestane lactam (III) were obtained in 17.8%, 21.2%, 3.6%, 2.3%, and 26.2% yield, respectively. 5α-Cyanocholestanone (IV) could also be separated by direct crystallization from the reaction mixture before chromatography. It melts at 181–183° and the analytical values are in good agreement with the formula $C_{28}H_{45}ON$. The infrared spectrum in chloroform solution showed absorption bands at 2237 cm.$^{-1}$ (nitrile) and 1723 cm.$^{-1}$ (six-membered ring ketone) but no band corresponding to the α,β-unsaturated ketone. It did not exhibit selective absorption in the ultraviolet spectrum.

The (5 → 3) α-lactam (II) melted at 249–251° and in chloroform solution in the infrared it exhibited bands at 3697 cm.$^{-1}$ (free-OH), 3477 cm.$^{-1}$ (free —NH), 3327 cm.$^{-1}$ (bonded —N—H), 1705 cm.$^{-1}$ (lactam carbonyl), and 1682 cm.$^{-1}$ (associated lactam carbonyl). The (5 → 3)β-lactam (III) (m.p. 200–202°) displayed similar bands in the infrared, *i.e.* 3605 cm.$^{-1}$ (free —OH), 3445 cm.$^{-1}$ (free —N—H), 3300 cm.$^{-1}$ (bonded —NH), 1702 cm.$^{-1}$ (lactam carbonyl), and 1690 cm.$^{-1}$ (associated lactam carbonyl). Neither lactam showed the band of the noncyclic amide in the 1510–1620 cm.$^{-1}$ region.[8] These findings suggest that II and III are C_5 acid amides epimeric at C_5 and furthermore that they exist in the hemiketal

(1) (a) A. J. Birch and R. Robinson, *J. Chem. Soc.*, 501 (1943); (b) R. B. Woodward, *J. Am. Chem. Soc.*, **62**, 1208 (1940); (c) A. W. Burgstahler and J. C. Nordin, *J. Am. Chem. Soc.*, **81**, 3151 (1959); (d) M. S. Ahmad, G. Baddeley, B. G. Heaton, and J. W. Rasburn, *Proc. Chem. Soc.*, 395 (1959).

(2) B. Ellis and V. Petrow, *J. Chem. Soc.*, 2246 (1952).

(3) Y. Urushibara and M. Chuman, *Bull. Chem. Soc.* (Japan), **22**, 69 (1949).

(4) O. C. Musgrave, *J. Chem. Soc.*, 3121 (1951).

(5) S. Weinhouse and M. S. Kharasch, *J. Org. Chem.*, **1**, 490 (1936).

(6) B. Baun, I. M. Heilbron, and F. S. Spring, *J. Chem. Soc.*, 1274 (1936).

(7) (a) H. H. Inhoffen, S. Chütz, P. Rossberg, O. Berges, K. H. Nordsiek, H. Plenio, and E. Höroldt, *Chem. Ber.*, **91**, 2626 (1958) and previous papers; (b) J. Romo, *Tetrahedron*, **3**, 37 (1958); (c) U. R. Ghatak, *Tetrahedron Letters*, **1**, 19 (1959); (d) E. Adlerová, L. Novák, and M. Protiva, *Coll. Czechoslov. Chem. Commun.*, **23**, 681 (1958).

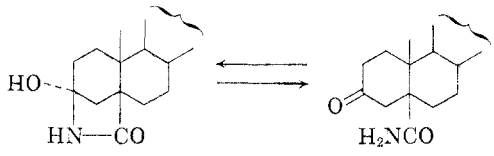

Chart 1

form,[9] even in solution. The optical rotatory dispersion curves of II and III showed only plane curves[10]; this is further support for the assigned structures. On the other hand, while the ultraviolet spectrum of II did not show an absorption band corresponding to the carbonyl group that of III showed very weak absorption at 280 mμ (ε 9.6) in ethanol solution. From these findings, the following equilibrium is thought to occur in alcohol solution in the latter case.

The analytical values of the second eluted compound, m.p. 196–198°, agreed with the empirical formula $C_{56}H_{90}O_3N_2$ (Mol.wt. 839.3) and its infrared spectrum showed absorption bands corresponding to a hydroxyl group (3683 cm.$^{-1}$), imide (3443 cm.$^{-1}$), bonded imine (3240 cm.$^{-1}$), nitrile (2255 cm.$^{-1}$), lactam carbonyl (1705 cm.$^{-1}$) and associated lactam carbonyl group (1685 cm.$^{-1}$). Although the molecular weight determination by *Rast* showed only 623.5, the structure of this compound was assumed to be a dimer having either formula IXa, IXb, or IXc.

In this experiment, 5β-cyanocholestanone was not isolated but (5 → 3)β-lactam (III) was obtained in considerable yield. Since the 5α-cyano group is both axial to ring A and B, while its β-epimer is axial to ring A but equatorial to ring B as shown in the Fig. 1, hydrolysis of the 5β-cyano group may

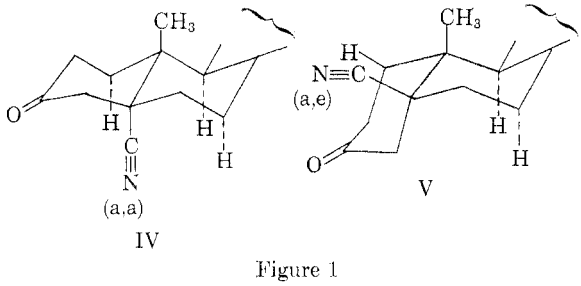

Figure 1

be much more easier than that of the 5α-epimer. For this reason, it is suggested that 5β-cyanocholestanone was more easily hydrolyzed[11] than the 5α-epimer by the action of potassium cyanide as a strong base and yielded (5 → 3)β-lactam (III). In order to avoid this side reaction, cyanation of I was carried out with two mole equivalents of potassium cyanide and 1.5 mole equivalents ammonium chloride in dimethylformamide. As the excess ammonium hydroxide, which was produced by the consumption of cyanide anion, was liberated as ammonia by heating, the reaction mixture was maintained at minimal basicity. Under these conditions, a mixture of 5α- and 5β-cyanocholestanone and a small amount of lactam[12] were obtained in *ca.* 82% (total yield), as expected. Neither starting material nor the dimer (IX) was isolated from the reaction mixture. The separation of each 5α- and 5β-epimer from the reaction mixture by repeated chromatography on alumina and recrystallization was almost always unsatisfactory. The 5β-cyano ketone was eluted together with its 5α-epimer at the same time or after some elution of the 5α-cyano ketone from the alumina column. A similar result was observed in the case of the epimeric pair of (5 → 3)-lactams. Such exceptions have been reported in the literature.[13] However, it was found that the tosyl hydrazones of the 5-cyano epimers were suitable for separation by either chromatography on alumina or by recrystallization. Hydrolysis of each epimer with pyruvic acid then gave the pure 5α- and 5β-cyano compounds. 5β-Cyanocholestanone (V), m.p. 127–128° was thus obtained and showed absorption bands in the infrared in chloroform solution at 2237 cm.$^{-1}$ (CN) and 1723 cm.$^{-1}$ (six-membered ring ketone) and the corresponding monosemicarbazone had m.p. 155–160°. The ratio of the yield of the 5α and 5β derivatives is about 1:1.[14]

From the conformational considerations discussed above, it can be seen that the 5β-cyano compound should be more easily saponified than the 5α-epimer. This was confirmed experimentally by examining the hydrolysis[15,16] rate of both the 5α- and 5β-cyanocholestanone using the absorption band at 2237 cm.$^{-1}$ in the infrared spectrum as a measure of the concentration of the nitrile group. The intensity of the band at 2237 cm.$^{-1}$ of each

(8) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Methuen & Co., Ltd., London, second edition, 1958, p. 203.

(9) It is known that several amides of β-benzoylpropionic acid exist in the hemiketal form in solution. (*cf.* N. H. Cromwell and K. E. Cook, *J. Am. Chem. Soc.*, **80**, 4573 (1958) and other references cited there).

(10) We are most grateful to Dr. C. Djerassi for measuring the optical rotatory dispersions.

(11) Ref. D. H. R. Barton and R. C. Cookson, *Quart. Rev.*, **10**, 60 (1956).

(12) It was observed that the mixture of 5α- and 5β-cyano ketone gave (5 → 3)β-lactam in *ca.* 25% yield after chromatography on basic alumina. This observation suggested also the sensitivity of the 5β-cyano ketone (V) towards alkali indicating that the actual yield of the formed III was much lower than that actually isolated.

(13) For example, J. A. Cella, E. A. Brown, and R. R. Burtner, *J. Org. Chem.*, **24**, 743 (1959).

(14) See Experimental.

(15) The use of the word "saponification or hydrolysis" may not be adequate here. Hence, in this case these words should be interpreted as the decomposition rate of the cyano group, especially in the case of 5α-cyano ketone.[14]

(16) For the measurement of the optical densities, a "Koken DS 301" (double beam) was used. All measurements of infrared spectra were performed by Mr. Y. Matsui in this laboratory, to whom we wish to express our profound thanks.

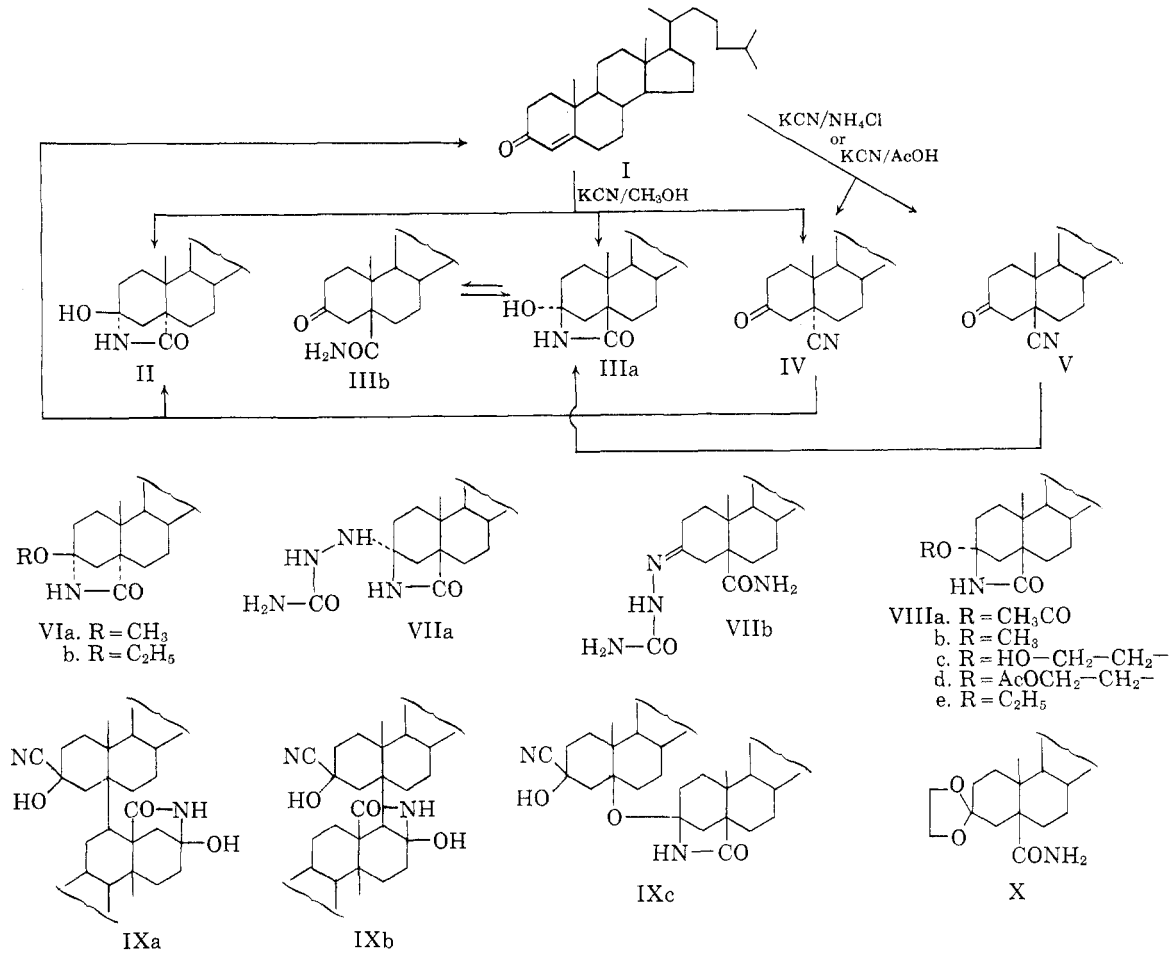

Chart 2

5-cyano derivative obeyed Beer's law below an optical density of 0.2 (Fig. 5). Saponification[15,16] of each 5-cyanocholestanone was carried out with 0.08*N* sodium hydroxide solution in 95% ethanol solution at 55°. There is a marked difference in the saponification rate between the 5α- and 5β-cyano derivatives and 90% of the former was saponified in six and a half hours while the same per cent of the latter saponified in only two hours as shown in Fig. 2. The plotted values of log Co/C against time almost formed a straight line (Fig. 3) so this is applicable to the equation—$1/t$ log Co/C=0.4343 K—when the hydroxide concentration is constant. These results indicate that this reaction proceeds by a base catalyzed pseudo first order reaction. Also the values 0.00609 min.$^{-1}$ and 0.0203 min.$^{-1}$ were given as saponification rate constants $K^{55°}$ of the 5α- and 5β-cyano ketones, respectively. The velocity ratio of the 5α to the 5β compound is 1:3.5. The assignment of configuration to the C_5 epimers from these results is in good agreement with the results of the rotatory dispersion curves[10] (Fig. 4).

In order to confirm the configurations of the two epimeric lactams, 5β-cyanocholestanone was saponified by treatment with sodium hydroxide in boiling methanol for one hour and afforded the (5 → 3)β-lactam in 70% yield, identical with the above-mentioned lactam (III), m.p. 202°. 5α-Cyanocholestanone, on the other hand, gave 50% of the anticipated (5 → 3)α-lactam, identical with the lactam (II), m.p. 251°, and 25% of Δ^4-cholesten-3-one by the action of sodium hydroxide in refluxing methanol for twenty hours. It seems reasonable that cholestenone was obtained by the β-elimination of 5α-cyano ketone and that there exists an equilibrium between the 5α-cyano derivative and cholestenone (Chart 3). These facts were also supported by the

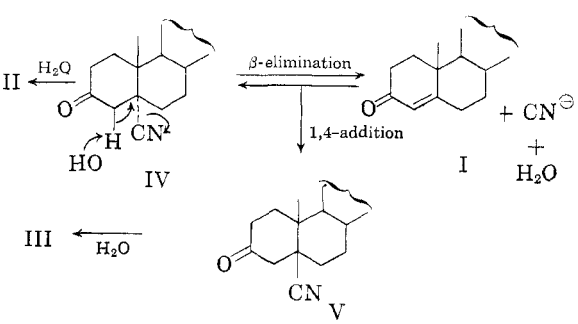

Chart 3

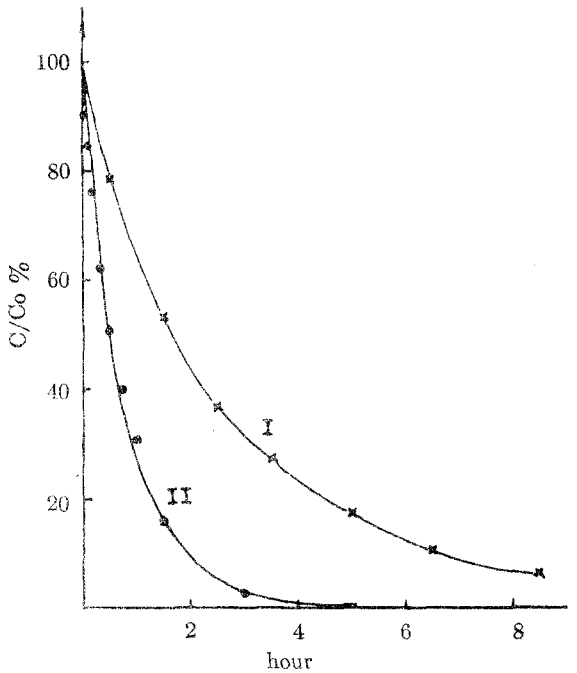

Fig. 2. C/Co values against time in the hydrolysis of 5α- (I) and 5β-cyanocholestan-3-one (II) with 0.08*N*-sodium hydroxide at 55° ± 1°
Co = initial concn. of cyano ketone in weight %
C = concn. of cyano ketone in weight %

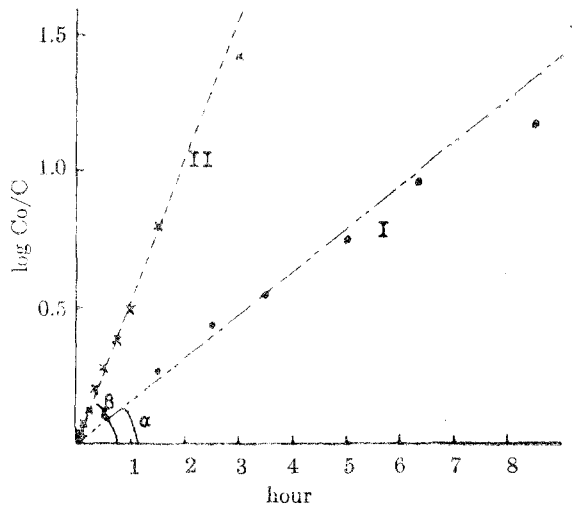

Fig. 3. Pseudo-first-order rate plots for the disappearance of the CN group of each 5-cyanocholestanone
I 5α-cyanocholestan-3-one
II 5β-cyanocholestan-3-one

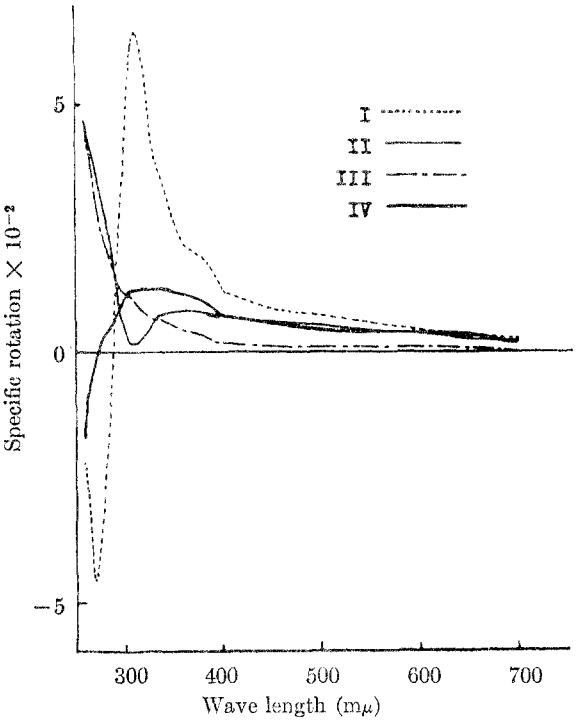

Fig. 4. Optical rotatory dispersion curves of
I 5α-cyanocholestan-3-one (C=0.090, 700–267.5 mμ)
C=0.018, 265–260 mμ)
II 5β-cyanocholestan-3-one (C=0.100, 700–260 mμ)
III 5α-amidocholestan-3-one (C=0.080, 700–262.5 mμ)
IV 5β-amidocholestan-3-one (C=0.085, 700–260 mμ)
in CH_3OH

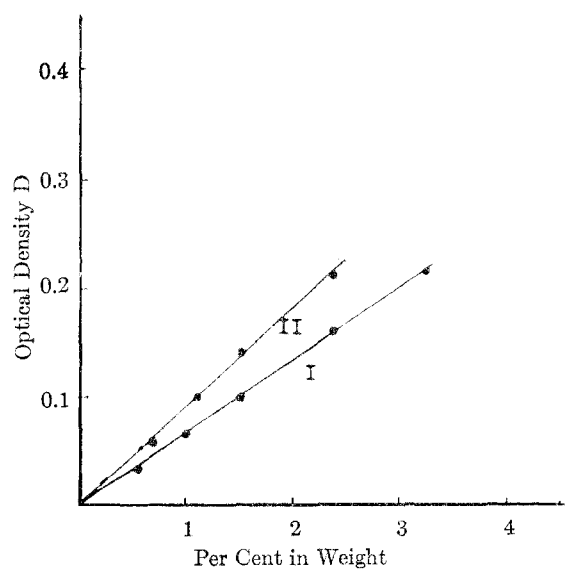

Fig. 5. Calibration curves for determining the concentration of 5α- (I) and 5β-cyanocholestan-3-one (II). The linear relation of the optical density of CN-Absorption at 2237 cm.$^{-1}$ in chloroform to percent in weight

following experiments. When 5α-cyanocholestanone was treated with 10% sodium hydroxide in dimethylformamide under vigorous conditions it gave cholestenone in 14.7% yield, the epimeric (5 → 3)β-lactam (III) in 10% yield and the starting material in 17.9% yield but no (5 → 3)α-lactam (II) was isolated. If the 5α-cyanide were treated with potassium hydroxide in ethanol in the presence of potassium cyanide and heated under reflux for twelve hours, there were obtained 40% of II and 15% of III but no cholestenone. The most probable explanation of the formation of the epimeric (5 → 3)β-lactam (III) from the 5α-cyanide is as follows: Cholestenone and CN^- ion, both liberated from 5α-cyanocholestanone by the β-elimination in the presence of alkali, reacted with each other and afforded the 5β-cyano derivative which then

TABLE I

INFRARED SPECTRA IN CARBON TETRACHLORIDE, CM.$^{-1}$

	Free OH	Free NH	Bonded NH	Acetyl	Lactam CO	Bonded Lactam CO
III	3600	3490	3350, 3130	—	1726	1676
VIIIa	—	3495	—, —	1741	1727	—
VIIIb	—	3490	3230, 3084	—	1722	1702
VIIIc	3610	3482	3250, 3090	—	1722	1695
VIIId	—	3487	3214, 3089	1746	1728	1702

TABLE II

MOLECULAR ROTATION OF 5*X*-CHOLESTANONE AND 5*X*-COPROSTANONE

	X	$[\alpha]_D$ Chf	$[M]_D$ Chf	$\Delta(M)_D^{(CN-H)}$	$\Delta[M]_D^{(5\alpha-5\beta)}$
Cholestanone	H	+41°	+158.5°		>+19.3°
Coprostanone	H	+36°	+139.2°		
5α-Cyanocholestanone	CN	+47.0°	+193.5°	+35.0°	>+80.7°
5β-Cyanocholestanone	CN	+27.4°	+112.8°	−26.4°	

underwent saponification to yield the (5 → 3)β-lactam.

These differences between the epimeric 5-cyano compounds with alkali were also due to the configuration of the 5α- and 5β-cyano groups and in the case of the more hindered 5α-cyano group, the reagents attacked not only the 5α-cyano radical but also the neighboring activated 4β-hydrogen atom.

The (5 → 3)α-lactam afforded its methyl and ethyl ether (VIa and b) when treated with methanol and hydrogen chloride or with ethanol-benzene in the presence of *p*-toluenesulfonic acid, respectively. Similarly, the (5 → 3)β-epimer gave the methyl and ethyl ether (VIIIb and VIIIe) in the same way. It formed an acetate (VIIIa) with acetic anhydride and pyridine or better with acetic anhydride and *p*-toluenesulfonic acid. The (5→3)β-lactam afforded a compound (VIIIc), m.p. 185–187°, when treated with ethylene glycol and a catalytic amount of *p*-toluenesulfonic acid in benzene. The infrared spectrum of this compound shows an absorption band at 3600 cm.$^{-1}$ in chloroform solution corresponding to the hydroxyl group and this was confirmed by the formation of its acetate (VIIId) with acetic anhydride-pyridine at room temperature. From these facts, a reasonable structure of the above-mentioned compound is represented by the formula VIIIc rather than the normal ethylene ketal structure X.

The infrared spectra of these lactam derivatives, VIa, VIb, VIIIa, VIIIb, VIIIc, VIIId, and VIIIe were all lacking the absorption band corresponding to the 2nd band of the non-cyclic amide.[8] All these findings support the hemiketal structures.

The infrared absorption bands due to the intermolecular association were identified from other bands by measurement in carbon-tetrachloride solution and by dilution[17] as in Table I. Thus the assigned absorption band corresponding to the lactam carbonyl group existed between 1722 cm.$^{-1}$ and 1728 cm.$^{-1}$, and this region is located at the shorter wave-length region than that of the primary amide. This gave further support to the assumed hemiketal structure mentioned above.

The contributions of the C_5 substituted cyano group to the molecular rotatory power were summarized in Table II. As shown in the table the effect of the cyano group is opposite to that of the hydroxyl group.[18] As mentioned above the 3-keto group in the 5-acid amide derivatives has a hemiketal type structure, the rotatory powers of these derivatives can not be compared.

EXPERIMENTAL

Melting points were measured on a Kofler-block "Monoscope" (Hans Bock Co., Frankfurt am Main, Germany) and are corrected. Unless otherwise stated, specific rotations were measured in chloroform solution and ultraviolet spectra in 95% ethanol. For rotation and elemental analysis, the samples having the melting points up to 120°, 180°, and over 180° were dried for 3 hr. over phosphorus pentoxide *in vacuo* (1–2 mm.) at room temperature to 60°, 70–90° and 100–120° respectively. Chromatography was usually performed according to the method described by Reichstein and Shoppee.[19]

Reaction of Δ^4-cholesten-3-one with potassium cyanide. A. *With potassium cyanide in methanol.* A solution of potassium cyanide (0.65 g., 0.01 mole) in water (2 ml.) was added to a hot solution of Δ^4-cholesten-3-one (I) (1.93 g., 0.005 mole) in methanol (50 ml.) and refluxed for 6 hr. After cooling, the crystals were separated, washed with ether, and recrystallized from ethanol, to afford 5α-cyanocholestan-3-one (IV), m.p. 181–184°, as fine prisms. The filtrates were combined and evaporated *in vacuo*, water was added, and the product

(17) Infrared spectra were measured by use of a 3-mm. cell with the range of the concentration between 20–95% transmission of carbonyl, which was controlled by dilution.

(18) (a) L. F. Fieser and M. Fieser, *Steroids*, Reinhold, New York, 1959, p. 179; (b) D. H. R. Barton and W. Klyne, *Chem. & Ind.*, 27, 755 (1948).

(19) T. Reichstein and C. W. Shoppee, *Disc. Trans. Farad. Soc.*, No. 7, 305 (1949).

was extracted with chloroform. The chloroform extracts were washed with water, dried, and then evaporated *in vacuo*. The residue (2.08 g.) was chromatographed on alumina (60 g., Brockmann, II). Elution with petroleum ether (b.p. 40–60°)–benzene (4:1) afforded recovered Δ^4-cholesten-3-one (I) (343.2 mg., 17.8%), m.p. 80–82°, undepressed on admixture with an authentic sample. Further elution with petroleum ether–benzene (2:1 and 1:1) afforded IV (223.8 mg.), m.p. 176.5–181°, raised by several crystallizations from ethanol to 182–184° [no depression on admixture with the first separated crystals (IV); the combined yield was 431.7 mg. (21.2%)]. $[\alpha]_D^{27}$ +47.0° (*c*, 0.888), $\lambda_{max}^{C_2H_5OH}$ 288 mμ (ε 40), $\nu_{max}^{CHCl_3}$ 2237 and 1723 cm.$^{-1}$

Anal. Calcd. for $C_{28}H_{45}ON$: C, 81.69; H, 11.02; N, 3.40. Found: C, 81.33; H, 11.07; N, 3.49.

5α-Cyanocholestan-3-one semicarbazone, m.p. 250–253° dec., $[\alpha]_D^{18}$ +72.6° (*c* 1.018 in chloroform–glacial acetic acid 1:1 v/v), ν_{max}^{Nujol} 3515, 3225, 2232, 1688, 1661, and 1572 cm.$^{-1}$

Anal. Calcd. for $C_{29}H_{48}ON_4$: C, 74.31; H, 10.32; N, 11.95. Found: C, 74.04; H, 10.17; N, 11.97.

Further elution with benzene-chloroform (2:1) gave dimer (IX) (73.2 mg., 3.6%), m.p. 186–192°, raised by crystallization from ethanol to 196–198°, $[\alpha]_D^{20}$ +25.2° (*c*, 0.957), $\nu_{max}^{CHCl_3}$ 3683, 3443, 3240, 2255, 1705, and 1685 cm.$^{-1}$

Anal. Calcd. for $C_{56}H_{90}O_3N_2$: C, 80.00; H, 10.73; N, 3.35. Found: C, 80.19; H, 10.70; N, 3.97.

Molecular weight measurement (*Rast*) gave 623.5.

Further elution with chloroform and chloroform-methanol (199:1) afforded crystals (49.2 mg., 2.3%), m.p. 238–245°. Several crystallizations from ethanol gave 3α-amino-3β-hydroxy-5α-carboxycholestane lactam (II), m.p. 249.5–252°, $[\alpha]_D^{26}$ +10.4° (*c*, 1.022), $\nu_{max}^{CHCl_3}$ 3697, 3477, 3327, 1705, and 1682 cm.$^{-1}$

Anal. Calcd. for $C_{28}H_{47}O_2N \cdot H_2O$: C, 75.12; H, 11.03; N, 3.13. Found: C, 74.96; H, 11.07; N, 2.98.

Further elution with chloroform-methanol (99.5:0.5, 99:1, and 49:1) afforded 3β-amino-3α-hydroxy-5β-carboxycholestane lactam (III) (562.2 mg., 26.2%) as fine prisms from ethanol, m.p. 200–205°, $[\alpha]_D^{26}$ +33.6° (*c*, 1.088), $\lambda_{max}^{C_2H_5OH}$ 280, mμ (ε 9.6), $\nu_{max}^{CHCl_3}$ 3605, 3445, 3300, 1702, and 1690 cm.$^{-1}$

Anal. Calcd. for $C_{28}H_{47}O_2N$: C, 78.27; H, 11.03; N, 3.26. Found: C, 77.34; H, 10.96; N, 3.16.

Tosylhydrazone of IV. 5α-Cyano ketone (50 mg.) and tosylhydrazine[20] (27.3 mg.) was dissolved in ether (4 ml.) and allowed to stand at room temperature overnight. The crystals were filtered and recrystallized from ethanol, 43.5 mg. of rods, m.p. 198–203°. From the filtrate, further 5.6 mg. of crystals were obtained. $[\alpha]_D^{19}$ +70.5° (*c*, 1.519), ν_{max}^{Nujol} 3210, 2205, 1638, 1597, 1490, 1347, 1166, and 809 cm.$^{-1}$.

Anal. Calcd. for $C_{35}H_{53}O_2N_3S$: C, 72.50; H, 9.21; N, 7.25; S, 5.52. Found: C, 72.15; H, 9.14; N, 7.06; S, 5.36.

Tosylhydrazone of V. 5β-Cyanocholestan-3-one [see below] (50 mg.) and tosylhydrazine (27.3 mg.) were dissolved in ether (4 ml.) and allowed to stand at room temperature overnight. The solution was washed with 2*N* hydrochloric acid and water, dried and then evaporated *in vacuo*. The residue (84.5 mg.) was crystallized from ethanol, needles (31 mg.), m.p. 117–123°. From mother liquor, further 19.4 mg. of the hydrazone, m.p. 115–119° was obtained. $[\alpha]_D^{13}$ +13.0° (*c*, 1.483), ν_{max}^{Nujol} 3576, 3243, 3094, 2232, 1630, 1602, 1495, 1322, 1164 and 813 cm.$^{-1}$

Anal. Calcd. for $C_{35}H_{53}O_2N_3S \cdot {}^1/_2H_2O$: C, 71.41; H, 9.24; N, 7.14; S, 5.44 or for $C_{35}H_{53}O_2N_3S$: C, 72.50; H, 9.21; N, 7.25; S, 5.52. Found: C, 71.64; H, 9.23; N, 6.96; S, 5.36.

Regeneration of IV *from its tosylhydrazone.* 5α-Cyanocholestan-3-one tosylhydrazone (50 mg.) was dissolved in chloroform (2 ml.) and ethanol (1 ml.), containing pyruvic acid (0.1 ml.) and allowed to stand overnight. The solution was poured onto ice-water and extracted with ether. The ether extract was washed with 2*N* sodium carbonate and water, dried over anhydrous sodium sulfate and then evaporated *in vacuo*. The crude product (43.5 mg.) was crystallized from ethanol, giving IV (10.8 mg.), fine prisms, m.p. 182–184°. The melting point was undepressed upon admixture with an authentic sample. The second crop (18 mg.) melted at 164–174°.

Regeneration of V *from its tosylhydrazone.* 5β-Cyanocholestan-3-one tosylhydrazone (100 mg.) was treated in the same way as described above. After working up as usual, there was obtained 47.7 mg. of V, needles, m.p. 125–126°, undepressed upon admixture with an authentic sample (see below B).

B. *With potassium cyanide and ammonium chloride in dimethylformamide.* Δ^4-Cholesten-3-one (1.93 g., 0.005 mole), potassium cyanide (0.65 g., 0.01 mole) and ammonium chloride (0.393 g., 0.0075 mole) in dimethylformamide (40 ml.) and water (5 ml.) were heated with occasional shaking for 8 hr. at 100°, during which time ammonia gas was evolved. After cooling the solvent was distilled off *in vacuo*. Water was then added and the product was extracted with chloroform. All extracts were washed with water and dried. Evaporation *in vacuo* afforded 2.18 g. of crude product. 2.15 g. of this product was dissolved in ether (100 ml.), to which tosylhydrazine (1.153 g.) was added and the clear solution was allowed to stand overnight and then further refluxed for 1 hr. The ether was evaporated *in vacuo* and the crude oil (3.38 g.) was crystallized from ethanol. The first crop (264 mg. of rods, m.p. 198–202°) and the second (287.5 mg. of rods, m.p. 196–201°) were mainly 5α-cyanocholestan-3-one tosylhydrazone, the third and the fourth crop (514 mg. of needles, m.p. 108–125° and 380.0 mg. of needles, m.p. 100–125°) were apparently a mixture of both 5α and 5β compounds, but with 5β-compound predominating. The rest (oil) was roughly chromatographed on alumina (15 g., Woelm III, neutral), from which there was obtained 31 mg. of rods, m.p. 196–201° (5α-compounds) and 865.6 mg. of crude crystals, m.p. 100–135°. The total yield of crude crystals was 2.34 g. (82%).

The third, the fourth, and the last crops were combined (1.75 g.) and then chromatographed on alumina. This resulted in the isolation of 1.14 g. of needles, m.p. 115–130° (mixed m.p. with a pure sample of 5β-compound was 112–120°) and further 160 mg. of needles, m.p. 100–135°. As a result, 582.5 mg. of 5α-cyanocholestan-3-one tosylhydrazone (A) and 1.30 g. of a mixture (B) of 5β- and 5α-cyanocholestan 3-one tosylhydrazone were obtained. The latter (B) was proved to be an approximately 4:1 mixture of 5β- and 5α-compounds after hydrolytic cleavage with pyruvic acid (see below).

Hydrolytic cleavage of (A). 5α-Cyanocholestan-3-one tosylhydrazone (582 mg.) was dissolved in chloroform (24 ml.) and ethanol (12 ml.), to which pyruvic acid (1.2 ml.) was added and the solution was refluxed for 1 hr. After working up as above i, there were obtained 435 mg. of residue, which gave after one crystallization from ethanol 313.7 mg. of IV, fine prisms, m.p. 182.5–184°, undepressed on admixture with an authentic sample. Further 28.4 mg. of IV, m.p. 168–173° was obtained from the mother liquor. Total crude yield was 81.5%.

Hydrolytic cleavage of (B). A solution of (B) (1.3 g.), and pyruvic acid (2.6 ml.) in chloroform (52 ml.) and ethanol (26 ml.) was allowed to stand at room temperature overnight. After working up as above i, there was obtained 913 mg. of crude product, which upon direct crystallization from ethanol gave 195 mg. of V, needles, m.p. 117–119°, raised by several crystallizations from ethanol to 127–128°. $[\alpha]_D^{29}$ +27.4° (*c*, 0.660), $\lambda_{max}^{C_2H_5OH}$ 288 mμ (ε 19), $\nu_{max}^{CHCl_3}$ 2223 and 1723 cm.$^{-1}$

Anal. Calcd. for $C_{28}H_{45}ON$: C, 81.69; H, 11.02; N, 3.40. Found: C, 81.63; H, 11.02; N, 3.60.

Semicarbazone. Fine prisms, m.p. 155–160°, $[\alpha]_D^{19}$ −6.5° (*c*, 1.013), ν_{max}^{Nujol} 3536, 3222, 2216, 1693, 1670, and 1573 cm.$^{-1}$

(20) K. Freudenberg and F. Blümmeli, *Ann.*, **440**, 51 (1944).

Anal. Calcd. for $C_{29}H_{48}ON_4$: C, 74.31; H, 10.32; N, 11.95. Found: C, 74.08; H, 10.35; N, 11.74.

The rest (672 mg.) was chromatographed on alumina (15 g., Woelm, neutral II), giving 180 mg. of practically pure V, m.p. 119–120° by elution with petroleum ether–benzene (4:1 and 3:1), 140.6 mg. of the mixture of IV and V, enriched with the latter, m.p. 103–120°, 19.3 mg. of the another mixture, m.p. 107–170° and 37.3 mg. of IV, m.p. 179–183° by elution with petroleum ether–benzene (3:1, 2:1, and 1:1), and 26 mg. of 5α-cyanocholestan-3-one tosylhydrazone, m.p. 197–200° by elution with chloroform-methanol (99:1 and 98:2). These results showed that the original mixture (B) was crude 5β-cyanocholestan-3-one tosylhydrazone containing ca. 20% 5α-isomer.

Saponification of 5α-cyanocholestan-3-one (IV) *with base.*[21] (1) 5α-Cyano ketone (IV) (200 mg.) and sodium hydroxide (300 mg.) were dissolved in methanol (98 ml.) and water (2 ml.), which was refluxed for 20 hr., where the disappearance of cyanide group in IV was checked by infrared spectrum. Methanol was distilled *in vacuo*, water added, and extracted with chloroform. The extract was washed with 2*N* hydrochloric acid and with water, dried over anhydrous sodium sulfate and then evaporated *in vacuo*. The crude product (157 mg.: the yield was lowered because of sampling for infrared measurement) gave II (40.4 mg.) as needles from ethanol, m.p. 247–251°. The rest was chromatographed on alumina (Brockmann) and gave further 35 mg. of cholestenone by elution with petroleum ether-benzene (1:1) and benzene, and further 40.0 mg. of II, m.p. 245–251° by elution with chloroform and chloroform-methanol (99:1).

(2) A solution of the 5α-cyano compound IV (500 mg.), sodium hydroxide (1.5 g.) in dimethylformamide (10 ml.) and water (5 ml.) was heated for 7 hr., after which the reaction mixture was neutralized with acetic acid (2.2 g.) and the solvent was evaporated *in vacuo*. The residue was treated in the usual manner. Chromatography of the chloroform extract (oil 512.7 mg.) on alumina (15 g., Brockmann II) gave 66.3 mg. of Δ^4-cholesten-3-one by elution with petroleum ether–benzene (4:1), 84.4 mg. of starting material (IV) by elution with petroleum ether-benzene (2:1) and benzene, and 54.2 mg. of III, m.p. 195–198°, by elution with chloroform-methanol (99.5:0.5).

(3) A solution of IV (280 mg.), potassium cyanide (280 mg.) and potassium hydroxide (280 mg.) in ethanol (20 ml.) and water (2 ml.) was refluxed for 12 hr. After working up as in (2) gave 308 mg. of crude products, which were chromatographed on alumina (8 g., Brockmann II) and the following products were obtained in the same way as (2); 118.3 mg. of II, m.p. 245–250° and 43.2 mg. of III, m.p. 192–198°.

Hydrolysis of 5β-cyanocholestan-3-one V with base.[21] A solution of the 5β-cyano compound (V) (200 mg.), sodium hydroxide (300 mg.) in methanol (98 ml.) and water (2 ml.) was refluxed for 1 hr., where the disappearance of cyanide group was checked by infrared spectrum. Working up in the same way as above furnished 200 mg. of crude product, which was purified by alumina chromatography (6 g., Brockmann II), giving 138.7 mg. of III, m.p. 186–200°, raised by several crystallizations from ethanol to 200–202° [from the fractions eluted with chloroform and chloroform-methanol (98:2)].

3β-Methoxy-3α-amino-5α-carboxycholestane lactam (VIa). (1) (5 → 3) α-Lactam II (395 mg.) was dissolved in 40% (w/w) hydrochloric acid–methanol (64 g.) and allowed to stand for 1.5 days. The methanol was evaporated *in vacuo* and worked up as usual. Chromatography of the residue (405 mg.) on alumina (15 g., Brockmann II) gave 302.6 mg. (74.3%) of VIa by elution with benzene-chloroform (9:1, 4:1, 2:1 and 1:1), needles from ether, m.p. 188.5–190°. $[\alpha]_D^{29}$ +25.8° (*c*, 0.887), ν_{max}^{Nujol} 3320, 3140, and 1700 cm.$^{-1}$

(21) In this experiment each substance was identified with the corresponding authentic sample by mixed melting point determination.

Anal. Calcd. for $C_{29}H_{49}O_2N$: C, 78.50; H, 11.13; N, 3.16. Found: C, 78.67; H, 11.05; N, 3.06.

(2) A solution of II (266 mg.) in 5% (w/w) hydrochloric acid–methanol (30 ml.) was heated for 12 hr. under reflux and worked up as above (1). There were obtained in the same way 195 mg. (56%) of VIa, m.p. 186–187°.

3β-Ethoxy-3α-amino-5α-carboxycholestane lactam (VIb). A solution of II (320 mg.) and *p*-toluenesulfonic acid monohydrate (64 mg.) in absolute ethanol (30 ml.) and absolute benzene (50 ml.) was distilled off slowly within 20 hr. through a fractionating column. A total of 45 ml. of absolute ethanol and 75 ml. of absolute benzene were added dropwise during the distillation to keep the initial volume constant. Sodium acetate (anhydrous, 30 mg.) was then added, followed by evaporation *in vacuo*. The product was extracted with chloroform, washed with water, dried over anhydrous sodium sulfate, and then evaporated *in vacuo*. The residue (349 mg.) was chromatographed on alumina (8 g., Brockmann II), to afford 262.4 mg. (77%) of VIb by elution with petroleum ether–benzene (1:1), benzene and benzene-chloroform (9:1 and 4:1) needles from ether, m.p. 159–160°, raised by two crystallizations to 161–162°. $[\alpha]_D^{29}$ +22.2° (*c*, 0.968), ν_{max}^{Nujol} 3245, 3105, and 1704 cm.$^{-1}$

Anal. Calcd. for $C_{30}H_{51}O_2N$: C, 78.72; H, 11.23; N, 3.06. Found: C, 79.12; H, 11.22; N, 2.90.

Further elution with chloroform-methanol (99:1) gave 10.4 mg. of starting material.

3α-Methoxy-3β-amino-5β-carboxycholestane lactam (VIIIb). (5 → 3) β-Lactam III (2 g.) was dissolved in 30% (w/w) hydrochloric acid–methanol (100 g.) and allowed to stand at room temperature overnight. Working up in the same way as above gave 2.332 g. of crude products, which were purified by chromatography on Florisil (60 g.). Elution with benzene-chloroform (2:1 and 1:1) and chloroform gave 1.675 g. (82.1%) of practically pure VIIIb, silky needles from ether-pentane, m.p. 167–168°, raised by one crystallization to 170–171°. $[\alpha]_D^{23.5}$ +22° (*c*, 1.08), infrared: Table I.

Anal. Calcd. for $C_{29}H_{49}O_2N$: C, 78.50; H, 11.13; N, 3.16. Found: C, 78.06; H, 11.05; N, 3.04.

Further elution with chloroform-methanol (98:2) gave 221 mg. of starting material, m.p. 190–192°.

3α-Acetoxy-3β-amino-5β carboxycholestane lactam (VIIIa). (1) (5 → 3) β-Lactam III (1 g.) was dissolved in acetic anhydride (10 ml.), to which *p*-toluenesulfonic acid monohydrate (0.3 g.) was added, and allowed to stand at room temperature for 48 hr. The crystals (VIIIa) were filtered and washed with a little acetic anhydride. The filtrate was evaporated *in vacuo* after addition of excess anhydrous sodium acetate. The residue was extracted with ether and the ether extracts were washed with 2*N* sodium carbonate and with water, dried over anhydrous sodium sulfate, and then evaporated *in vacuo*. The residue gave upon crystallization from acetic anhydride further crude VIIIa, m.p. 89–95°. The combined crude crystals were recrystallized from acetic anhydride, giving 602.7 mg. (63.2%) of VIIIa, m.p. 115–118°, which upon further recrystallization from acetic anhydride gave pure VIIIa (needles) melting at 116–118°. $[\alpha]_D^{23.5}$ +39° (*c*, 0.992), infrared: Table I.

Anal. Calcd. for $C_{30}H_{49}O_3N$: C, 76.38; H, 10.47; N, 2.97. Found: C, 76.44; H, 10.45; N, 2.93.

(2) III (0.1 g.) was dissolved in pyridine (2.5 ml.) and acetic anhydride (1.5 ml.) and allowed to stand overnight at room temperature. Working up as usual gave 121.4 mg. of crude product, which by chromatography on alumina (4 g., Brockmann II) gave 31.4 mg. (34.5%) of VIIIa, m.p. 115–117°, and 33.8 mg. of starting material, m.p. 187–191°.

3α-(β-Hydroxy)ethoxy-3β-amino-5β-carboxycholestane lactam (VIIIc) (5 → 3β)-Lactam III (600 mg.) was dissolved in absolute benzene (100 ml.), to which ethylene glycol (1 ml.) and *p*-toluenesulfonic acid (50 mg.) were added and the whole was refluxed slowly during 9.5 hr., using a Dean-Stark apparatus to separate the water formed. 2*N* sodium carbonate was added and shaken thoroughly. The water

TABLE III

No.	t, Min.	Residue, Mg.	Chloroform, Mg.	Co, in Wt. %	D,	C, in Wt. %	C/Co, × 100	log Co/C
			5α-Cyanocholestan-3-one					
1	30	50.1	2094	2.34	0.125	1.83	78.2	0.106
2	90	51.9	1782	2.83	0.103	1.51	53.4	0.272
3	150	51.2	1486	3.33	0.084	1.23	36.9	0.434
4	210	50.7	1212	4.01	0.076	1.11	27.7	0.558
5	300	52.1	1145	4.35	0.052	0.76	17.5	0.758
6	390	50.7	1106	4.39	0.032	0.47	10.7	0.972
7	510	52.0	949	4.95	0.021	0.30	6.6	1.182
			5β-Cyanocholestan-3-one					
1	2	51.8	2447	2.07	0.174	1.88	91.0	0.042
2	5	52.3	2254	2.27	0.177	1.92	84.6	0.0719
3	10	53.3	1511	3.41	0.235	2.60	76.3	0.114
4	20	52.1	1219	4.09	0.230	2.53	61.9	0.207
5	30	53.4	1353	3.66	0.175	1.90	52.0	0.288
6	45	52.8	1269	3.84	0.143	1.54	40.1	0.398
7	60	50.7	1320	3.70	0.109	1.18	31.9	0.496
8	90	49.5	1219	3.91	0.058	0.62	15.85	0.795
9	180	49.8	1102	4.32	0.012	0.13	3.01	1.522

layer was extracted further with benzene. The extracts were washed with water, dried over anhydrous sodium sulfate and then evaporated *in vacuo* to afford 694 mg. of crude products (from acetone-ether-pentane, m.p. 177–180°). Chromatography over Florisil (20 g.) gave 512.3 mg. of pure VIIIc, recrystallized from benzene-pentane, m.p. 185–187° and an additional 52.3 mg. from the mother liquor, 179–182°, by elution with chloroform-methanol (99.5:0.5, 99:1 and 99:5). Total yield; 565.6 mg. (85.5%). $[\alpha]_D^{20}$ + 26.0° (*c*, 1.037), infrared: Table I.

Anal. Calcd. for $C_{30}H_{51}O_3N$: C, 76.06; H, 10.85; N, 2.96. Found: C, 75.69; H, 10.84; N, 2.84.

3α-(β-Acetoxy)ethoxy-3β-amino-5β-carboxycholestane lactam (VIIId). VIIIc (230 mg.) were acetylated with acetic anhydride (2 ml.) and pyridine (3 ml.) at room temperature overnight. After working up in the usual way, 281.4 mg. of crude acetate was obtained, which was then chromatographed on alumina (8 g., Brockmann II). The fractions eluted with benzene and benzene-chloroform (9:1, 4:1 and 1:1) gave 162 mg. of VIIId, needles from ether and pentane, m.p. 60–63°/84°, raised by two crystallizations from the same solvent to 64–65°/86°. $[\alpha]_D^{18}$ + 22.9° (*c*, 1.151), infrared: Table I.

Anal. Calcd. for $C_{32}H_{53}O_4N$: C, 74.52; H, 10.36; N, 2.72. Found: C, 74.14; H, 10.51; N, 2.59.

3α-Ethoxy-3β-amino-5β-carboxycholestane lactam (VIIIe). A solution of (5→3)β-lactam III (2.49 g.) and *p*-toluenesulfonic acid monohydrate (560 mg.) in absolute ethanol (100 ml.) and absolute benzene (150 ml.) was slowly distilled within 14 hr. through a fractionating column. A total of 50 ml. of absolute ethanol and 80 ml. of absolute benzene were added dropwise in order to maintain the original volume of the reaction mixture. Working up as in the case of VIb (see above) gave 2.6 g. of crude product, which was purified by filtration through an alumina column (60 g. Woelm II). Elution with petroleum ether–benzene (4:1, 2:1 and 1:1) gave a total of 2.28 g. of crude VIIIe (m.p. 149–151°), which upon further crystallization from ethanol gave pure VIIIe as colorless needles m.p. 153–154°. $[\alpha]_D^{20}$ + 24.7° (*c*, 1.156), ν_{max}^{Nujol} 3476 and 1697 cm.$^{-1}$

Anal. Calcd. for $C_{30}H_{51}O_2N$: C, 78.72; H, 11.23; N, 3.06. Found: C, 78.40; H, 11.22; N, 3.02.

Further elution with a chloroform-methanol (95:5 and 90:10) gave 50 mg. (2%) of starting material, m.p. 195–200°.

Determination of the saponification rate[15] *of 5α- and 5β-cyanocholestan-3-one.* (*1*) *The method for determination.* The determination of rates was carried out by measuring the decrease of optical densities with time of the CN-stretching absorption band at 2237 cm.$^{-1}$ in the infrared spectra.

(*2*) *Preparation of calibration curve.* The calibration curve was obtained by plotting the relationship between the optical density (D) of the C≡N band and concentration. As seen in Figure 5, the plots formed straight lines at optical densities below *ca.* 0.2 showing that Beer's relationship was valid.

(*3*) *Procedure.* A mixture of 2*N* sodium hydroxide (4 ml.) and 95% ethanol (96 ml.) in a 200 ml. reaction flask fitted with a reflux condenser was prewarmed at 55° (±1°) (inner temp.). The 5-cyano compound (500 mg.) was then added. Each aliquot (10 ml.) of the reaction mixture was taken out at various times and was carefully neutralized with 0.4 ml. of 2*N* hydrochloric acid, evaporated *in vacuo*, and extracted three times with chloroform. The chloroform layers were worked up as above. The residue (50–53 mg.) was dissolved in sufficient chloroform to give a solution having an optical density less than 0.2. For the optical densities listed in Table III, a mean value from five measurements was obtained. The concentration C (w/w) of the remaining 5-cyano compounds was obtained from the calibration curve prepared above. These values are illustrated in Fig. 2 and summarized in Table III.

(4) *Results.* As shown in Fig. 3, the curve obtained by plotting the relationship between log Co/C and time for both 5α- and 5β-cyanocholestan-3-one formed straight lines, indicating that the rate of the base catalyzed hydrolysis followed the first order kinetic law and the values of the rate constant K were shown by the equation $1/t \log \mathrm{Co/C} = 0.4343\ K$, followed by $\tan\alpha = 0.4343\ K_{5\alpha}^{55°}$, $K_{5\alpha}$ = 0.00609 min.$^{-1}$ for 5α-cyano ketone and $\tan\beta = 0.4343\ K_{5\beta}^{55°}$, $K_{5\beta}$ = 0.0203 min.$^{-1}$ for 5β-cyano ketone. It was found that the ratio of their rate constants at 55° was 3.5:1.

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