Chem. Pharm. Bull. 20(7)1467—1472(1972)

UDC 547.92.04 : 546.173—35.04

Formation of 6-Acetoximino-3β-acetoxycholestan-5α-ol Nitrite and Its Reduction over Adams' Platinum[1]

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(Received December 22, 1971)

One step formation of 6-acetoximino-3β-acetoxycholestan-5α-ol nitrite (I) from 3β-acetoxycholest-4-ene has been carried out in a fairly good yield. Catalytic reduction of I affords the 6-keto compounds with 5α-OAc in dioxane and the 6β-amino compounds with 5α-OAc in acetic acid, respectively.

It is generally known that a double bond reacts with nitrous acid to form an addition product but this reaction has not been used to date for steroids. During our studies on steroids, we found that the double bond of a steroid reacted with nitrous acid to give an interesting product which underwent a series of interesting reaction. We now wish to report, in full, the reaction described in our earlier communication,[1] together with the result of catalytic reduction of the product.

3β-Acetoxycholest-4-ene reacted with sodium nitrite in acetic acid solution, in the presence of conc. sulfuric acid, and gave several products. Fractional crystallization of the products afforded compound (I), $C_{31}H_{50}O_6N_2$, as the main product and a small amount of compound (II), $C_{29}H_{48}O_4$, whose infrared (IR) spectrum (KBr) showed absorptions at 3410 (OH), 1735 (OAc), and 1710 cm^{-1} (CO), and its nuclear magnetic resonance (NMR) spectrum (100 MHz) had absorptions at δ 5.02 m (W_H = 24 Hz, 3α-H), 1.98 s (3β-OAc), and 0.93 s (10β-Me). From these evidence, II was identified as 3β-acetoxy-5α-hydroxycholestan-6-one.[3]

Treatment of I with methanolic potassium hydroxide solution resulted in its conversion to a hydroxy-ketone (IV), $C_{27}H_{46}O_3$, *via* an oxime (III), $C_{27}H_{46}O_4N_2$. IR spectrum ($CHCl_3$) of IV showed absorptions at 3360 (OH) and 1705 cm^{-1} (CO), and acetylation of IV with acetic anhydride converted it to II. From these evidences, IV was identified as 3β,5α-dihydroxycholestan-6-one.[3] These facts suggest the presence of 3β-OAc, 5α-ONO, and 6=NOAc in I

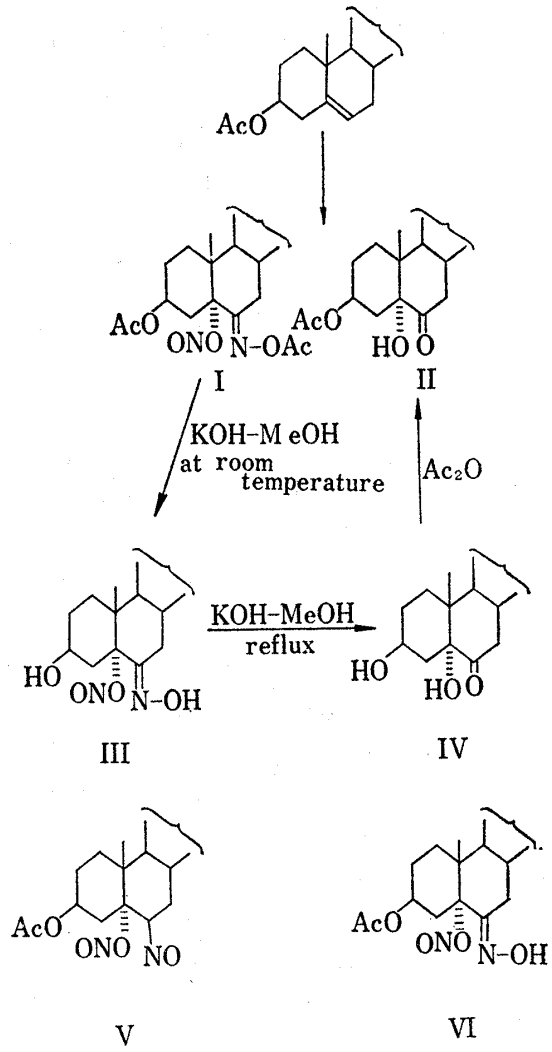


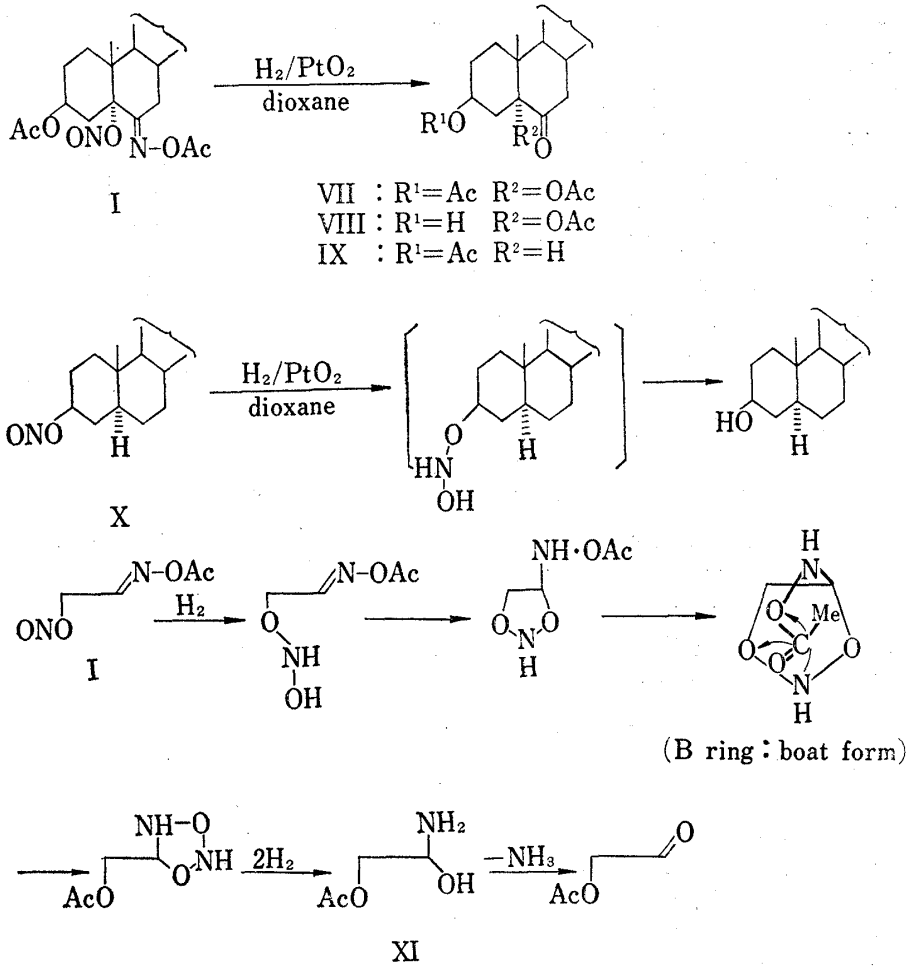
Chart 1

1) Steroids. II. Part I: M. Onda and A. Azuma, *Chem. Pharm. Bull.* (Tokyo), **17**, 859 (1971).
2) Location: *Minato-ku, Tokyo.*
3) R.G. Schultz, *J. Org. Chem.*, **24**, 1955 (1959).

and it is assumed to be 6-acetoximino-3β-acetoxycholestan-5α-ol nitrite. This structure for I is supported by the presence of absorptions at 1734 (OAc), 1633 and 1569 cm^{-1} (ONO) in its IR spectrum ($CHCl_3$) and at δ 4.84 m (W_H=24 Hz, 3α-H), 2.16 s (NOAc), 2.01 s (3β-OAc), and 1.06 s (10β-Me) in its NMR spectrum (100 MHz).

Formation route of I may be considered as follows. 3β-Acetoxy-6β-nitrosocholestan-5α-ol nitrite (V) would be formed by the *trans*(diaxial)-addition of dinitrogen trioxide to the double bond in 3β-acetoxycholest-4-ene, and V would be isomerized to a stable oxime (VI) whose acetylation would give I. Formation of II is assumed to be the result of hydrolysis of I or VI, or by the Claisen degradation of the oxime group in VI by nitrous acid, followed by hydrolysis of the nitrite group.

Catalytic reduction of I over Adams' platinum, carried out as one of the means for its structural elucidation, gave a very interesting result. Reduction of I in dioxane gave a product which exhibited three spots on thin-layer chromatogram. Further chromatography over alumina gave compound (VII), $C_{31}H_{50}O_5$, in 54% yield and compound (VIII), $C_{29}H_{48}O_4$, in 36% yield as the main products, and a small amount of compound (IX), $C_{29}H_{48}O_3$ (5%). VII and IX were identified as 3β,5α-diacetoxycholestan-6-one[4] and 3β-acetoxycholestan-6-one,[5] respectively, by mixed melting point, and IR and NMR spectral comparison. From the IR and NMR spectral data and the fact that acetylation of VIII with acetic anhydride gave VII, VIII was established as 5α-acetoxy-3β-hydroxycholestan-6-one.



XI

Chart 2

4) L.F. Fieser and S. Rajagopalan, *J. Am. Chem. Soc.*, **71**, 3938 (1949).
5) R.M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948).

The acetyl group in 5α-OAc in the main products (VII and VIII) must have formed by rearrangement of the acetyl group in 6=NOAc. No rearrangement occurred when I was stirred with Adams' platinum in the absence of hydrogen in dioxane. 6-Acetoximino-3β-acetoxycholestan-5α-ol (XIX, R=Ac) was not hydrogenated over Adams' platinum in dioxane and cholestanol nitrite absorbed approximately three moles of hydrogen to give cholestanol. From these facts, the first step in the reduction of I would be the reduction of the nitrite group to afford the hydroxylaminoxy group which would add to the double bond of the acetoximino group and an aminohydrin (XI) would be formed after several steps. Liberation of ammonia from XI will give the ketones with 5α-OAc. The fact that this last step did not take place during after-treatment of the reaction mixture is borne by the detection of ammonia[6] at the end of the reduction and failure of VII to undergo reduction over Adams' platinum in dioxane.

Next, reduction of I in acetic acid gave a more complex product. Chromatographic purification of the product gave compound (XII), $C_{29}H_{50}O_2$, in 4% yield, compound (XIII), $C_{31}H_{52}O_4$, in 26% yield, compound (XIV), $C_{31}H_{53}O_4N$, in 50% yield, compound (XV), $C_{29}H_{51}O_2N$, in *ca.* 7% yield, and compound (XVII), $C_{29}H_{51}O_3N$, in 3% yield. XII was identified as 3β-acetoxycholestane by mixed melting point, IR and NMR spectral comparison. From the IR (CCl_4) absorptions at 1750 and 1735 cm^{-1} (OAc), and NMR (60 MHz) absorptions at δ 4.55 m (W_H=24 Hz, 3α-H), 2.13 s (5α-OAc), 2.03 s (3β-OAc), and 1.08 s (10β-Me), XIII was assumed to be 3β,5α-diacetoxycholestane. IR spectrum (CCl_4) of XIV had absorptions at 3450 (NH_2) and 1732 cm^{-1} (OAc) and its NMR spectrum (100 MHz) showed absorptions at δ 4.73 m (W_H=24 Hz, 3α-H), 3.98 m (W_H=12 Hz, 6α-H), 2.06 s (5α-OAc), 2.01 s (3β-OAc), and 1.27 s (10β-Me). Acetylation of XIV gave a triacetate (XVIII), which was proved to be 6β-acetamido-3β,5α-diacetoxycholestane[7] obtained by acetylation of the amino-alcohol (XX) (*vide infra*) with acetic anhydride in the presence of boron trifluoride. Therefore, XIV would be 6β-amino-3β,5α-diacetoxycholestane.[7] XV was a slightly crude compound, whose NMR

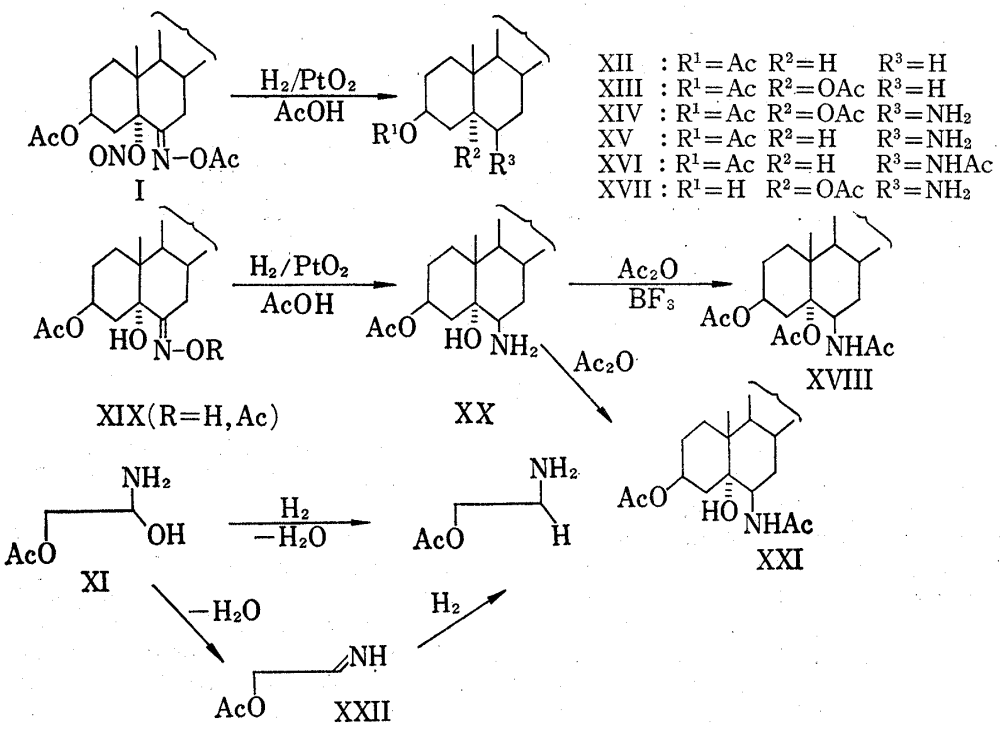
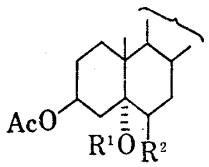


Chart 3

6) Ammonia was trapped as picrate.
7) This configuration was incorrectly reported in our preliminary communication.
8) C.W. Shoppee, D.E. Evans and G.H.R. Summers, *J. Chem. Soc.*, **1957**, 97.

spectrum showed the presence of 3β-OAc and 6β-NH_2. Since its acetate (XVI) was identified with an authentic sample of 6β-acetamido-3β-acetoxycholestane,[8] XV was determined to be 3β-acetoxy-6β-aminocholestane. XVII had absorptions at 3450 (OH and NH_2) and 1720 cm^{-1} (OAc) in its IR spectrum ($CHCl_3$) and at δ 3.75 m (W_H=24Hz, 3α-H), 3.60 m (W_H=10 Hz, 6α-H), 2.01 s (5α-OAc), and 1.21 s (10β-Me) in its NMR spectrum (60 MHz). Since the acetylation of XVII with acetic anhydride gave the triacetate (XVIII), XVII was proved to be 5α-acetoxy-6β-aminocholestan-3β-ol.

Table I. Chemical shifts of 10β-Me in XX and related Compounds



	R^1	R^2	10 β-Me	R^1	R^2	10 β-Me
XX	H	NH_2	1.22	H	OH	1.23
XXI	H	NHAc	1.14	H	OAc	1.19
XIV	Ac	NH_2	1.27	H	α-OAc	1.05
XVIII	Ac	NHAc	1.18			

Reduction of XIX in acetic acid gave 3β-acetoxy-6β-aminocholestan-5α-ol[7] and no compound with 5α-OAc was obtained. The configuration of the amino group at C-6 in XX was determined from the half-height width (10 Hz) of the proton at C-6. Derivation of 5α-OH to 5α-OAc in steroidal compound results in the shift of 6α-H to a lower field by *ca.* 1.15 ppm.[9] The difference in the chemical shift of 6α-H between XX and XIV is −1.21 ppm. Furthermore, the down-field shift (δ 1.22) of 10β-Me due to the interaction with the amino group at C-6 supports the above structure for XX (Table I).

The facts that stirring of I with Adams' platinum in acetic acid gave no product and XX was obtained by reduction of XIX in acetic acid indicate that the acetoxyl group at C-5 in XIV and XVII (XIII) did not originate in the solvent but was formed by intramolecular migration and that it is not possible to consider XIX as an intermediate in the formation of these compounds. It is presumed that the aminohydrin (XI), which was obtained by the same steps as in the case of reduction in dioxane, would directly hydrogenolyze to give the amino compounds with 5α-OAc or would be dehydrated to yield the imino compound (XXII) which is hydrogenated to afford the amino compounds with 5α-OAc.

Experimental

Melting points were determined on a micro hot-stage and were uncorrected. IR spectra were measured with a JASCO Model IR-G. NMR spectra were measured with a Varian HA-100 and T-60 in $CDCl_3$. Mass spectra were taken on a JEOL's JMS-OlS.

Reaction of 3β-Acetoxycholest-4-ene with Nitrous Acid——To a solution of 3β-acetoxycholest-4-ene (27 g) and conc. H_2SO_4 (13.5 ml) in AcOH (810 ml), which was kept at 70°, was added $NaNO_2$ (44 g) over 1 hr. The stirring was continued for 30 min and the reaction mixture was filtered to remove Na_2SO_4. The filtrate was poured into ice–water (5 liter). The precipitate was collected, washed with H_2O and dried *in vacuo* to give yellow solid (34.9 g). Recrystallization from MeOH gave 6-acetoximino-3β-acetoxycholestan-5α-ol nitrite (I) (12.3 g) as colorless needles of mp 168.5—170.5°. *Anal.* Calcd. for $C_{31}H_{50}O_6N_2$: C, 68.10; H, 9.22; N, 5.12. Found: C, 68.05; H, 9.31; N, 5.05. Recrystallization of the residue, which was obtained from the above MeOH-filtrate, from ether gave 3β-acetoxy-5α-hydroxycholestan-6-one (740 mg) (II) as colorless needles of mp 235—238°. The above ether-filtrate gave a syrup (20 g). The chromatography of this syrup (500 mg) over silica gel (10 g) using benzene as eluent gave II (41 mg), mp 235—238°.

9) J.M. Coxon, M.P. Hartshorn and G.A. Lane, *Tetrahedron*, **26**, 841 (1970).

Hydrolysis of I——(a) To a solution of I (500 mg) in MeOH (100 ml) was added a solution of KOH (500 mg) in H_2O (5 ml). The mixture was refluxed for 15 hr. After evaporation, the precipitate was collected and treated with $CHCl_3$. The $CHCl_3$-soluble fraction was recrystallized from MeOH to give 3β, 5α-dihydroxycholestan-6-one (IV) (218 mg) as colorless needles of mp 232—235°. *Anal.* Calcd. for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07. Found: C, 77.76; H, 11.05. Acetylation of IV (244 mg) with Ac_2O (1 ml) gave an acetate (215 mg) as colorless needles of mp 235—236°, which was identified to be II by mixed melting point, IR and NMR spectral comparison. The $CHCl_3$-insoluble fraction gave K-salt of 6-hydroximinocholestane-3β, 5α-diol 5-nitrite (III) (44 mg) as colorless needles of mp 238—241°. The K-salt was acidified with 10% AcOH, filtered and washed with H_2O to give III which was recrystallized from MeOH to afford colorless needles (30 mg) of mp 174—175°. *Anal.* Calcd. for $C_{27}H_{46}O_4N_2$: C, 70.09; H, 10.02; N, 6.05. Found: C, 70.24; H, 10.08; N, 6.14.

(b) To a solution of I (1.0 g) in MeOH (250 ml) was added a solution of KOH (1.0 g) in MeOH (5 ml) and the mixture was allowed to stand at room temperature for 30 min. The reaction mixture was concentrated *in vacuo* to yield K-salt of III (854 mg) of mp 238—241°.

Hydrolysis of III——To a solution K-salt of III (101 mg) in MeOH (16 ml) was added Claisen alkaline (0.3 ml) and refluxing was continued for 9 hr. After concentration, there was obtained colorless needles (65 mg) of mp 232—235° which was identified to be IV by mixed melting point, and IR and NMR spectral comparison.

Reduction of I in Dioxane——A solution of I (1.0 g) in dry dioxane (15 ml) was hydrogenated over platinum black obtained from PtO_2 (500 mg) at room temperature and H_2 (178 ml) was absorbed during 2 hr. After filtration, the filtrate was evaporated *in vacuo*. The residue (986 mg) was chromatographed over Al_2O_3 (neutral, grade III) (50 g). The first fraction, which was eluted with *n*-hexane–benzene (1:1), gave 3β-acetoxycholestan-6-one (IX) (39 mg, 5%) as colorless needles of mp 129—130° from MeOH. IR (CCl_4): 1735 (OAc), 1715 cm^{-1} (CO). NMR (60 MHz): δ 4.67 m (W_H=24 Hz, 3α-H), 2.02 s (3β-OAc), 0.91 s (10β-Me). *Anal.* Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.62; H, 10.99. The second fraction, which was eluted with benzene, gave 3β, 5α-diacetoxycholestan-6-one (VII) (500 mg, 54%) as colorless needles of mp 175—178° from MeOH. IR (CCl_4): 1748, 1739 (OAc). 1729 cm^{-1} (CO). NMR (60 MHz): δ: 4.80 m (W_H=24 Hz, 3α-H), 2.15 s (5α-OAc), 2.00 s (3β-OAc), 0.90 s (10β-Me). *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.02. Found: C, 73.91; H, 10.20. The third fraction, which was eluted with AcOEt–benzene (1:1), gave 5α-acetoxy-3β-hydroxycholestan-6-one (VIII) (305 mg, 36%) as colorless needles of mp 181—183° from MeOH. IR (CCl_4): 3450 (OH), 1750 (OAc), 1725 cm^{-1} (CO). NMR (60 MHz): δ 3.70 m (W_H=24 Hz, 3α-H), 2.12 s (5α-OAc), 0.90 s (10β-Me). *Anal.* Calcd. for $C_{29}H_{48}O_4$: C, 75.61; H, 10.50. Found: C, 75.61; H, 10.54. Acetylation of VIII (57 mg) with Ac_2O (0.3 ml) gave an acetate (57 mg) as colorless needles of mp 175—177°, which was identified to be VII by mixed melting point, and IR and NMR spectral comparison.

Reduction of I in Acetic Acid——A solution of I (1.0 g) in AcOH (14 ml) was hydrogenated over platinum black obtained from PtO_2 (516 mg) at room temperature and H_2 (170 ml) was absorbed during 9 hr. After filtration, the filtrate was evaporated *in vacuo*. The residue was treated with aqueous Na_2CO_3 and extracted with benzene. The benzene residue (935 mg) was chromatographed over silica gel (43 g). The first fraction, which was eluted with benzene, gave 3β-acetoxycholestane (XII) (30 mg, 4%) as colorless plates of mp 108—110° from MeOH. Mass spectrum. Calcd. for $C_{29}H_{50}O_2$: mol. wt., 430.381. Found: M^+, 430.380. The second fraction, which was eluted with benzene–$CHCl_3$ (9:1), gave 3β, 5α-diacetoxycholestane (XIII) (218 mg, 26%) as colorless needles of mp 90—92°, from MeOH. *Anal.* Calcd. for $C_{31}H_{52}O_4$: C, 76.18; H, 10.72. Found: C, 76.62; H, 10.51. The third fraction, which was eluted with benzene–$CHCl_3$ (70:25), gave 6β-amino-3β, 5α-diacetoxycholestane (XIV) (427 mg, 50%) as a syrup. Mass spectrum Calcd. for $C_{29}H_{49}O_2N$: mol. wt., 443.736. Found: M^+ -$C_2H_4O_2$, 443.739. Picrate: mp 218°. *Anal.* Calcd. for $C_{37}H_{56}O_{11}N_4$: C, 60.66; H, 7.65; N, 7.65. Found: C, 60.98; H, 8.06; N, 8.03. The acetylation of XIV (30 mg) with Ac_2O (0.2 ml) gave the triacetate (XVIII) (33 mg) as colorless needles of mp 254—255° from MeOH. *Anal.* Calcd. for $C_{35}H_{59}O_7N$: C, 72.62; H, 10.16; N, 2.56. Found: C, 72.96; H, 10.54; N, 2.33. The fourth fraction, which was eluted with $CHCl_3$–MeOH (9:1), gave 3β-acetoxy-6β-aminocholestane (XV) (126 mg). Acetylation of XV (126 mg) with Ac_2O (1 ml) in the presence of pyridine (1 ml) gave the acetate (XVI) (65 mg) as colorless needles of mp 175—176° from *n*-hexane. IR (CCl_4): 3470 (NH), 1732 (OAc), 1685 cm^{-1} (NAc). NMR (60 MHz): δ 5.27 d (J=10 Hz, NH), 4.68 m (W_H=24 Hz, 3α-H), 4.09 bd (J=10 Hz, 6α-H), 2.01 s (3β-OAc), 1.99 s (6β-NAc), 1.00 s (10β-Me). *Anal.* Calcd. for $C_{31}H_{53}O_3N$: C, 76.38; H, 10.63; N, 2.64. Found: C, 76.39; H, 10.47; N, 2.87. The yield of XV was estimated to be *ca.* 7% from that of XVI. The fifth fraction gave 5α-acetoxy-6β-aminocholestan-3β-ol (XVII) (24 mg, 3%) as a syrup. Mass spectrum Calcd. for $C_{27}H_{47}ON$: mol. wt., 401.366. Found: M^+ -$C_2H_4O_2$, 401.364. The acetylation of XVII with Ac_2O gave a triacetate which was identified to be XVIII by mixed melting point, IR and NMR spectral comparison.

3β-Acetoxy-6β-aminocholestan-5α-ol (XX)——(a) A solution of XIX (R=H) (823 mg) in AcOH (70 ml) was hydrogenated over platinum black obtained from PtO_2 (800 mg) at room temperature and H_2 (95 ml) was absorbed during 2 hr. After work-up, there was obtained XX (657 mg) as colorless needles of mp 194—197° from petr. ether–ether. IR (CCl_4): 3430 (OH, NH_2), 1725 cm^{-1} (OAc). NMR (100 MHz): δ 5.16 m

(W_H=24 Hz, 3α-H), 2.77 m (W_H=10 Hz, 6α-H), 2.02 s (3β-OAc), 1.22 s (10β-Me). *Anal.* Calcd. for $C_{29}H_{51}O_3N$: C, 75.44; H, 11.13; N, 3.03. Found: C, 75.63; H, 11.34; N, 2.92.

(b) The oxime-acetate (XIX, R=Ac) (243 mg) gave XX (208 mg) by the same procedure.

Acetylation of XX——(a) 6β-Acetamido-3β, 5α-Diacetoxycholestane (XVIII): To a suspension of XX (84 mg) in Ac_2O (0.4 ml) was added $BF_3(OEt_2)$ (3 drops) and the mixture was heated on a water bath for 5 min. After cooling, the reaction mixture was poured into ice–water and solid (81 mg) was collected. The chromatography of this solid over Al_2O_3 (neutral, grade III) (4 g) using *n*-hexane–benzene (5:95) as eluent gave XVIII (59 mg) as colorless needles of mp 256—257° from MeOH. IR ($CHCl_3$): 3460 (NH), 1725 (OAc), 1675 cm^{-1} (NAc). NMR (100 MHz): δ 5.53 d (J=10 Hz, NH), 5.25 bd (J=10 Hz, 6α-H), 1.99 s (3β-OAc, 6β-NAc), 1.18 s (10β-Me). Mass spectrum Calcd. for $C_{33}H_{55}O_5N$: mol. wt., 485.387. Found: M^+ -$C_2H_4O_2$, 485.388.

(b) 6β-Acetamido-3β-acetoxycholestan-5α-ol (XXI): A solution of XX (324 mg) and Ac_2O (4 ml) in dry ether (10 ml) was stirred at room temperature for 4 hr. After work-up, there was obtained XXI (300 mg) as colorless needles of mp 196—197° from H_2O–MeOH. IR ($CHCl_3$): 3470 (OH, NH), 1730 (OAc), 1670 cm^{-1} (NAc). NMR (100 MHz): δ 5.62 (J=10 Hz, NH), 5.16 m (W_H=24 Hz, 3α-H), 4.10 bd (J=10 Hz, 6α-H), 2.00 s (3β-OAc, 6β-NAc), 1.14 s (10β-Me). Mass spectrum Calcd. for $C_{31}H_{53}O_4N$: 503.397. Found: M^+, 503.401.