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# HETEROCYCLE STEROIDS

## SYNTHESIS OF A NEW STEROID ALKALOID: 27 NOR-23,26-IMINO-5α-CHOLESTEN-23(N)-3β-OL

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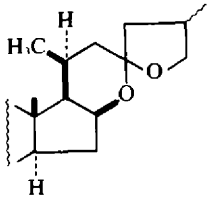
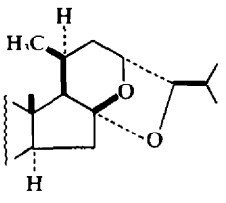
**Abstract**—An original synthetic method, which leads to 27 nor-steroid alkaloid **13** is described. The physical and the spectroscopic properties of this product are examined.

In this paper we describe an original synthetic method which leads easily to a new steroid alkaloid, 27 nor-23,26-imino-5α-cholesten-23 (N)-3β-ol **13**. This problem was included in the work we have been carrying out on the synthesis of new products of potential chemical and biological interest; to this end we have already prepared some new steroids **1**, **2** and **3**, which are at present undergoing pharmacological tests.[1]



**1**



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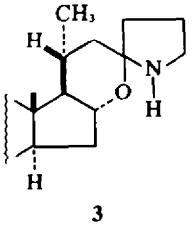


**3**

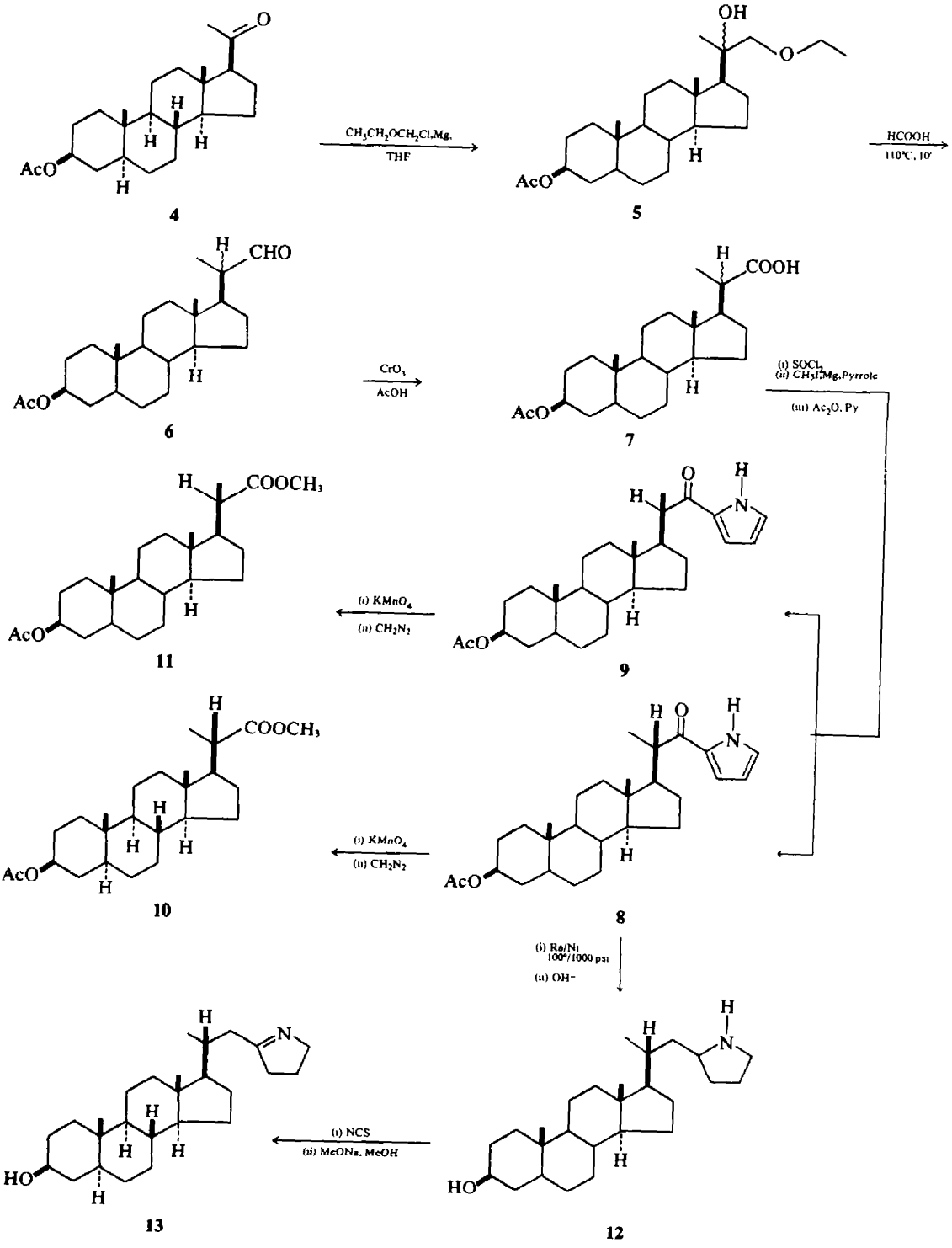
The starting material was 3β - acetoxy - 5α - pregnan - 20 - one **4**, commercially available. The aldehyde **6** (Scheme 1) was the key intermediate in our synthesis, its preparation requires a reaction novel in steroid chemistry, which introduced an aldehyde group into the 20-position of **4**, in almost quantitative yield. In effect, **4** was submitted to a Grignard reaction with ethylchloromethyl ether at −5 to −10°C, yielding quantitatively the glycol monoether **5**, as a mixture of C(20) epimers, as indicated by the large melting point range and by the PMR spectrum (2 quartets centred at 3.53 and 3.55 δ, due to $-O-CH_2-CH_3$ groups of the 2 epimers). The components of this mixture were not separated. By dealkylation of **5** with HCOOH at 110°C for 10 min,† we obtained in almost quantitatively yield the aldehyde **6**. On the basis of the analytical and spectroscopic data the aldehyde **6** appears to be a mixture of C(20) epimers, which might be expected since the acid-catalyzed dealkylation of **5** proceeds via the enol intermediate **5b**, that tautomerizes to the keto form, which is more stable , as a mixture of C(20) epimers (Scheme 2).[2] The 2 epimers were not separated, since they had almost the same $R_f$ values.

The cautious oxidation of **6** with $CrO_3$ in $CH_3COOH$ gave quantitatively the acid **7**, again as a mixture of C(20) epimers with nearly identical $R_f$ values and therefore not separable. Treatment of **7** with $SOCl_2$ gave the corresponding acyl chloride, which was reacted with pyrrolmagnesium iodide at −10°C and reacetylated yielding 2 products **8** and **9**, separated by chromatography. Compound **8**, with the higher $R_f$ value, showed analytical and spectroscopic data in agreement with the 3β-acetoxypyrrole structure.‡ The IR spectrum showed 2 bands at 3440 $cm^{-1}$ (-NH stretching) and 1640 $cm^{-1}$ (ketone C=O), typical of 2-acylpyrroles;[3] the PMR spectrum showed a set of signals, due to the C(24), C(25) and C(26) protons of the pyrrole ring (respectively 7.04 δ, 6.29 δ and 6.95 δ), characteristic of pyrroles with a carbonyl function in the 2-position[4] (Fig. 1).

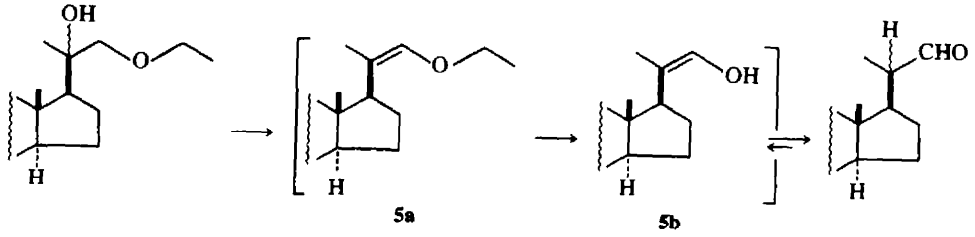
Compound **9**, with the lower $R_f$, was the minor product and the analytical and the spectroscopic data were in agreement with the proposed structure. The IR spectrum showed the same bands as **8** at 3450 and 1643 $cm^{-1}$, and the PMR spectrum showed the same set of signals due to the C(24), C(25) and C(26) protons of pyrrole ring. Oxidation of **8** with $KMnO_4$ in neutral medium and subsequent esterification with $CH_2N_2$, gave the methyl ester **10**, that possessed the same physical properties as the methyl ester of 3β-acethoxy-5α-bisnorcholanic acid.[5] Such correlation allowed the stereochemistry of the various asymmetry centres (3, 5, 8, 9, 10, 13, 14 and 17) to be defined; furthermore it showed that **8** had to possess the natural biogenetic α configuration in the C(20) position, from which it can be seen that no inversion occurred during the reaction. From **9**, by the same

†The monoethers of 1,2-glycols, heated in an acid medium, yield the corresponding aldehydes; see M. de Bottom, *Compt. Rendu* **260**, 478 (1968).

‡The alkylation of pyrrolmagnesium iodide with alkyl halides results in the formation of complex mixtures formed by N-, 2- and 3- alkylpyrroles and polyalkylpyrroles. On the contrary, only 2-acylpyrroles result from the acylation of pyrroles with acyl halides; see L. A. Paquette, *Principles of Modern Heterocycles Chemistry*, p. 318. Benjamin, New York (1963).




Scheme 1.



Scheme 2.

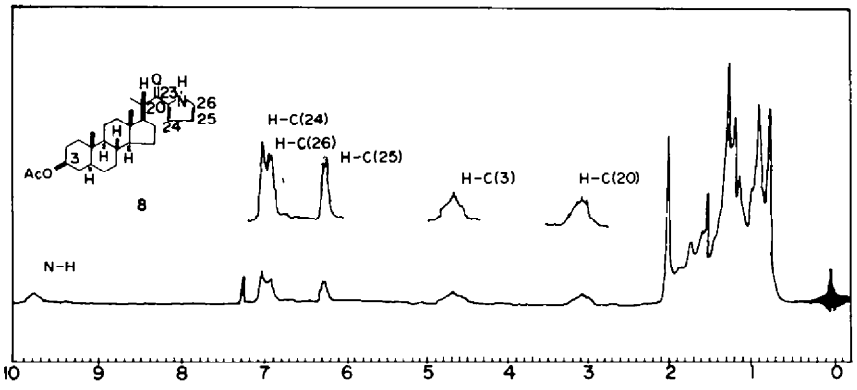
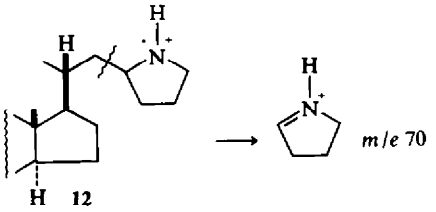


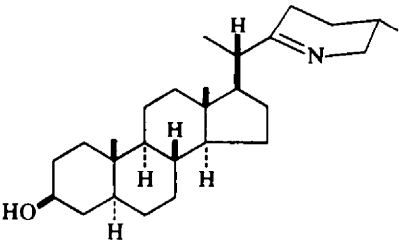

Fig. 1.

procedure, a methyl ester was obtained, probably the methyl ester of 3β - acetoxy - 5α - 20 isobisnorcholanic acid **11**.†

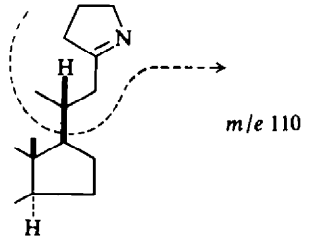
Compound **8**, submitted to catalytic reduction with Raney nickel, and subsequent hydrolysis of the acetate group, undergoes reduction of the pyrrole ring and hydrogenation of the C(22) = 0 group to a methylene group,[6] giving 27 nor - 23,26 - imino - 5α - cholestan - 3β - ol **12**. In the IR spectrum the typical bands of 2-carbonylpyrroles (3440 and 1640 $cm^{-1}$) were replaced by bands at 3580 $cm^{-1}$ (free -OH) and 3450 and 3300 $cm^{-1}$ (broad, -OH and pyrrolidine -NH). The PMR spectrum showed a signal at 4.3 δ (1H, s, -NH). The mass spectrum clearly showed 2 peaks at *m/e* 387 ($M^+$) and *m/e* 386 ($M^+ - 1$) and a base peak at *m/e* 70, due to the $C_4H_8N^+$ ion, the presence of which was in agreement with the proposed structure. Since **12** is prepared from **8** it is probably a mixture of C(23) epimers.[7]



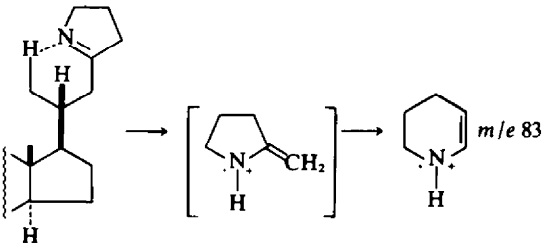

Compound **12** reacted with N-chlorosuccinimide to give the corresponding N-chloramine, from which, in basic medium, the isomerically pure enamine **13** was obtained. This was another new alkaloid and probably corresponds to dihydroverazine **14**, a natural alkaloid.[8]



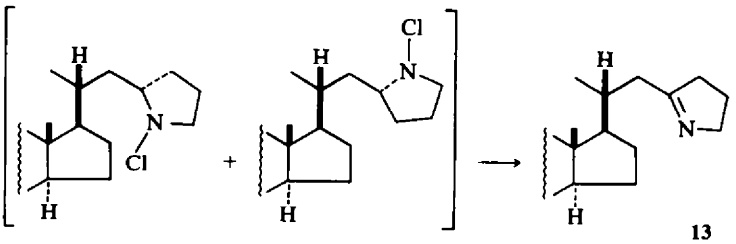

The structure of **13** was confirmed by analytical and spectroscopic data. The mass spectrum of **13** did not give further data for the determination of structure; however, besides the molecular ion at *m/e* 385, it showed 2 intense peaks at *m/e* 110 and 83 (base peak), whose presence could be due to the fragmentation process




and the McLafferty rearrangement.‡




At last, **13** appeared to be sterically pure; this was confirmed by the narrow melting point range and the very clear signals from the C(18) and C(19) methyl groups in its




†Oxidation of **8** with $KMnO_4$ in neutral medium does not cause any inversion of asymmetric centres; see H. O. House, *Modern Synthetic Reactions*, p. 276. Benjamin, New York (1972).

‡This kind of fragmentation often occurrs and is important in the field of sapogenins and steroid alkaloids; see H. Budzikiewicz, C. Djerassi, D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectroscopy*, Vol. 2. p. 114. Holden Day, San Francisco (1964).

PMR spectrum; furthermore, as we could easily observe by Dreiding models, the 2 epimer N-chloramines (not separated) yielded the same enamine **13** by dehydrohalogenation.

## EXPERIMENTAL

M.ps were determined on a Kofler block and are uncorrected. Rotatory powers were determined in $CHCl_3$ solution at room temperature. PMR spectra were taken with a Perkin–Elmer R 32, using $CDCl_3$ soln with TMS as internal standard. IR spectra were taken with a Perkin-Elmer 257 Infracord spectrometer. Commercial Merck silica gel (140–230 mesh) and Woelm alumina were used for column chromatography. Merck precoated silica gel plates were used in TLC. The chromatograms were detected by spraying with 5N $H_2SO_4$ and heating at 110°C for 10 min. Mass spectra were obtained with an AEI MS-12 spectrometer at 70 eV, using direct insertion at source temperature of 150°C.

*Glycol monoether* **5**

$HgCl_2$ (70 mg) was added to Mg (1.6 g), under anhyd THF, and stirred for 30 min under $N_2$. Then, $CH_3CH_2OCH_2Cl$ (4.7 ml), diluted with 4 ml of anhyd THF, and 1 g of **4**, dissolved in the minimum amount of anhyd THF, were added during 75 min at −5 to −10°C. The mixture was stirred overnight, then sat aq $NH_4Cl$ was added and, after 2 hr, the organic layer was separated and the aqueous phase extracted with AcOEt. The neutral organic extracts were dried ($Na_2SO_4$) and, after the removal of the solvent, an oily product (1.5 g) was obtained, this was treated with 20 ml of $Ac_2O$ and 20 ml of pyridine at room temperature overnight. The usual isolation procedure gave 1.2 g of crude product, that was chromatographed on $Al_2O_3$ B III. Benzene/*n*-hexane 9/1 eluted 1.050 g of pure **5**. Recrystallisation from $CH_2Cl_2$/*n*-hexane gave **5** as plates, m.p. 186–90°C. (Found: C, 74.32; H, 10.49. Calc. for $C_{26}H_{44}O_4$: C, 74.24; H, 10.54%). IR, $\nu_{max}$: 3560 (–OH) and 1728 $cm^{-1}$ (acetate C=O). PMR, $\delta$: 4.7 (1H, broad m, C(3)–H), 3.55 and 3.53 (2H, 2 q, $-O-CH_2-CH_3$), 3.2 (2H, s, C(20)–$CH_2$–O–), 2.0 (3H, s, $-OCOCH_3$), 0.84 (6H, s, C(18) $H_3$ and C(19) $H_3$). MS, *m/e*: $M^+$ = 420 in agreement with the proposed formula; other intense peaks at 360 (base peak), 342, 301, 300.

*3β-Acetoxy-5α-bisnorcholan-22-al* **6**

**5** (1.050 g), dissolved in 11 ml of 99% HCOOH, was kept at 110°C for 10 min. Then the cold mixture was diluted with AcOEt and washed with sat aq $NaHCO_3$, $H_2O$ and dried ($Na_2SO_4$). After the removal of the solvent 990 mg of crude product was obtained, this was chromatographed on $Al_2O_3$ B III. Benzene/*n*-hexane 4/1 eluted 920 mg of pure **6**. Recrystallisation from $CH_2Cl_2$/*n*-hexane gave **6** as plates; m.p. 159–63°C. (Found: C, 77.06; H, 10.31. Calc. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23%). IR, $\nu_{max}$: 2720 (aldehydic C-H), 1730 $cm^{-1}$ (aldehydic and acetate C=O). PMR, $\delta$: 9.57 and 9.61 (1H, 2 d, aldehydic C–H), 4.67 (1H, broad m, C(3)–H), 2.0 (3H, s, $-OCOCH_3$). MS, *m/e*: $M^+$ = 374, in agreement with the proposed formula; other intense peaks at 373, 314, 313, 258, 80 (base peak).

*3β-Acetoxy-5α-bisnorcholanic acid* **7**

A soln of 470 mg of $CrO_3$ in 7 ml of AcOH was added in 30 min to 920 mg of **6**, dissolved in 13 ml of AcOH. The mixture was stirred for 15 min at room temperature and then, after the addition of 1 ml of $CH_3OH$ to destroy the excess oxidant, it was diluted with AcOEt, washed with sat aq NaCl and dried ($Na_2SO_4$). The solvent was removed *in vacuo* and 930 mg of crude product was obtained, this was chromatographed on $SiO_2$, washed with dil aq HCl. Benzene/ether 9/1 eluted 900 mg of **7**. Recrystallisation from $CH_2Cl_2$/*n*-hexane gave **7** as plates, m.p. 142–46°C. (Found: C, 73.69; H, 9.81: Calc. for $C_{24}H_{38}O_4$: C, 73.81; H, 9.81%). IR, $\nu_{max}$: 3500–2700 $cm^{-1}$ (broad, acid –OH), 1730–1710 $cm^{-1}$ (acid and acetate C=O). PMR, $\delta$: 9.6 (1H, broad signal, –COOH), 4.64 (1H, broad m, C(3)-H), 1.97 (3H, s, $-OCOCH_3$). MS, *m/e*: $M^+$ = 390, in agreement with the proposed formula; other intense peaks at 330, 329, 315, 57 (base peak).

*Acylpyrroles* **8** *and* **9**

(a) Preparation of methylmagnesium iodide: 2.7 ml of $CH_3I$, diluted with 7.5 ml of anhyd $Et_2O$, were added during 30 min to 1 g of Mg, covered with anhyd $Et_2O$. The mixture was stirred for 2 hr under $N_2$ at room temperature.

(2) Preparation of pyrrolmagnesium iodide: 2.8 ml of pyrrole, freshly distilled from KOH pellets, diluted with 5 ml of anhyd $Et_2O$, were added to the soln of $CH_3MgI$. The mixture was then refluxed for 2 hr.

(c) Preparation of acyl chloride: 900 mg of **7**, dissolved in 15 ml of anhyd benzene, was treated with 15 ml of $SOCl_2$ and refluxed for 2 hr. Then the solvent and the excess reactants were removed under reduced pressure.

(d) The acylation reaction: All the acyl chloride, dissolved in the least amount of anhyd $Et_2O$, was added, at −10°C, to the prepared soln of pyrrolmagnesium iodide and the mixture stirred for 150 min under $N_2$. Then sat aq $NH_4Cl$ was added and the resulting mixture stirred overnight. The organic phase was separated and the aqueous one extracted with AcOEt. The neutral organic extracts were dried ($Na_2SO_4$). After removal of the solvent *in vacuo*, 1.5 g of black crude product was obtained, this was treated with 30 ml of $Ac_2O$ and 30 ml of pyridine at room temperature for 40 hr. The usual isolation procedure yielded 1.6 g of black crude product which was chromatographed on $Al_2O_3$ B III. Benzene/$Et_2O$ 9/1 eluted 610 mg of pure **8**. Benzene/$Et_2O$ 4/1 eluted 240 mg of pure **9**. Recrystallisation of **8** from $CH_2Cl_2$/*n*-hexane gave prisms, m.p. 179–81°C; $[\alpha]_D$ = +27° (c = 1.0) (Found: C, 76.57; H, 9.38; N, 3.27. Calc. for $C_{28}H_{41}NO_3$: C, 76.50; H, 9.40; N, 3.19%). For IR and PMR data see the initial section. MS, *m/e*: $M^+$ = 439, in agreement with the proposed formula; other intense peaks at 123 and 94 (base peak). Recrystallisation of **9** from $CH_2Cl_2$/*n*-hexane gave prisms, m.p. 135–37°C, $[\alpha]_D$ = +12° (c = 1.0) (Found: C, 76.61; H, 9.32; N, 3.11. Calc for $C_{28}H_{41}NO_3$: C, 76.50; H, 9.40; N, 3.19%). IR, $\nu_{max}$: 3450 (pyrrole –NH), 1720 (acetate C=O), 1643 $cm^{-1}$ (ketone C=O). PRM, $\delta$: 9.56 (1H, broad s, –NH; it disappeared after shaking with $D_2O$), 7.31 (1H, s, C(24)-H), 6.74 (1H, s, C(26)–H) 6.65 (1H, s, C(25)–H), 4.65 (1H, broad m, C(3)–H), 3.02 (1H, broad m, C(20)–H), 1.98 (3H, s, $-OCOCH_3$). MS, *m/e*: $M^+$ = 439, in agreement with the proposed formula; other intense peaks at 123 and 94 (base peak).

*3β-Acetoxy-5α-bisnorcholanic acid, methyl ester* **10**

85 mg of powdered $KMnO_4$ were added during 30 min, at room temperature, to a soln of 48 mg of **8** in 6 ml of $CH_3COCH_3$. The mixture was stirred for 7 hr; then it was acidified to Congo red with 2N $H_2SO_4$ and a dil soln of $Na_2S_2O_5$ was added. The mixture was poured into $H_2O$ and the usual isolation procedure gave 56 mg of crude product that was treated with 5 ml of an ether soln of $CH_2N_2$ for 1 hr at 0°C. The solvent was cautiously distilled and 56 mg of crude product was obtained, this was chromatographed on $SiO_2$. Benzene/$Et_2O$ 95/5 gave 17 mg of pure **10**. Recrystallisation from MeOH/$H_2O$ gave **10** as plates, m.p. 130–31°C; $[\alpha]_D$ = −6.2° (c = 0.81). (Lit.[5] m.p. 129–30°C; $[\alpha]_D$ = −7° (c = 1.022)). (Found: C, 74.08; H, 10.05. Calc. for $C_{25}H_{40}O_4$: C, 74.22; H, 9.97%).

*3β-Acetoxy-5α-20-isobisnorcholanic acid, methyl ester* **11**

100 mg of **9** were submitted to the same reactions as its isomer; chromatographic separation gave 25 mg of pure **11**, eluted by benzene/$Et_2O$ 95/5. Recrystallisation from MeOH/$H_2O$, gave **11** as plates m.p. 110–12°C; $[\alpha]_D$ = +4° (c = 0.72). (Found: C, 74.16; H, 9.82. Calc for $C_{25}H_{40}O_4$: C, 74.22; H, 9.97%).

*27 Nor-23,26-imino-5α-cholestan-3β-ol* **12**

300 mg of **8** were dissolved in the least amount of abs EtOH, then Raney nickel was added and the mixture kept under $H_2$ at 100°C and 1000 psi for 24 h. The catalyst was separated and washed with warm EtOH. After the removal of the solvent, 290 mg of crude product was obtained, this, dissolved in 100 ml of 5% KOH methanolic soln, was refluxed overnight. The usual isolation procedure yielded 270 mg of crude product which was chromatographed on $SiO_2$. The azeotropic mixture $CHCl_3$/MeOH/$H_2O$ 81/15/4 eluted 244 mg of **12**. Recrystallisation from $CHCl_3$/MeOH gave **12** as plates, m.p. 191–95°C (dec.). (Found: C, 80.48; H, 11.62; N, 3.66. Calc. for $C_{26}H_{45}NO$: C, 80.56; H, 11.70; N, 3.61%). IR, $\nu_{max}$: 3580 (free –OH), 3450–3300 (broad,

–OH and pyrrolidine –NH). PMR, δ: 4.3 (1H, broad s, –NH; it disappeared after shaking with $D_2O$), about 3.4 (a partially visible signal† due to the protons α to –NH group) 1.4 (4H, s, protons β to –NH group). FOR MS, see the initial section.

27 *Nor-23,26-imino-5α-cholesten-23 (N)-3β-ol* **13**

142 mg of **12** were dissolved in 30 ml of $CH_2Cl_2$. A soln of 45 mg of NCS in 6 ml of $CH_2Cl_2$ was added at −5 to −10°C during 30 min. The mixture was stirred for 2 h. Then the organic phase was washed ($H_2O$) and dried ($Na_2SO_4$). The solvent was removed *in vacuo* at 20°C. The product obtained was dissolved in the least amount of abs MeOH and added to a soln of 300 mg of Na in 16 ml of abs MeOH. The mixture was stirred for 2 h at room temperature. Then the usual isolation procedure yielded 120 mg of crude product, which was chromatographed on $SiO_2$. $Et_2O$ eluted 76 mg of pure **13**. Recrystallisation from $CHCl_3$/*n*-hexane, gave **13** as prisms m.p. 171–72°C; $[\alpha]_D = +25°$ (c = 1.0). (Found: C, 81.04; H, 11.30; N, 3.71. Calc. for $C_{26}H_{43}NO$: C, 80.98; H, 11.24; N, 3.63%). IR, $\nu_{max}$: bands at 3580 (–OH) and 1640 $cm^{-1}$ (–C=N–). PMR, δ: 3.75 (2H, m, =N–$CH_2$–), 3.5 (1H, broad m, C(3)–H), 2.3 (2H, m, C(22)$H_2$), 1.9 (2H, m, C(25)$H_2$), 0.80 and 0.70 (6H, 2s, C(18)$H_3$ and C(19)$H_3$ or viceversa).

†**12** was insoluble in the solvents generally used in PMR spectroscopy. It was dissolved only by a mixture of $CDCl_3/CD_3OD$ 1/1. Therefore this signal was obscurred by the $CHD_2OD$ present in $CD_3OD$.

*Acknowledgements*—We are grateful to Mr. M. Caroni and Mr. A. Santi, who have recorded PMR and mass spectra respectively.

REFERENCES

[1a] G. Piancatelli and A. Scettri, *Gazz. Chim. It.* [illegible] (1974); [b] G. Piancatelli and A. Scettri, *Ibid.* **105**, [illegible] G. Piancatelli and A. Scettri, *Ibid.* **106**, 167 (1976).
[2] R. Breslow, *Organic Reaction Mechanism*, p. 160. Benjamin, New York (1965).
[3] C. N. R. Rao, *Application of IR Spectroscopy*, p. 318. Academic Press, New York (1963).
[4] N. S. Bhacca, L. F. Johnson and J. N. Shoolery, *NMR spectra catalog*, Varian Ass., Palo Alto, spectrum No. 98 (1968).
[5] K. Miescher and J. Schmidlin, *Helv. Chim. Acta* **30**, 1405 (1947).
[6] R. L. Augustine, *Catalytic hydrogenation*, p. 82. Dekker, New York (1965).
[7] R. H. F. Manske, *Ibid.* p. 94.
[8] G. Adam, K. Schreiber, J. Tomko and A. Vassova, *Tetrahedron* **23**, 167 (1967).