Hypocholesterolemic Agents. 8.[1] Synthesis of 25-Azadihydrolanosterol and Derivatives[2]

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Continued interest in azasteroids as inhibitors of cholesterogenesis prompted the synthesis of 25-azadihydrolanosterol (**3d**) and derivatives. This was achieved by ozonolysis of lanosterol acetate which afforded the trisnoracid **2a** which upon treatment with $SOCl_2$ followed by $(CH_3)_2NH$ and subsequent reduction of the amide **2d** gave **3d** in good yield. Compound **3d** could also be obtained directly by the action of DMF and formic acid on the trisnoraldehyde **5**. Compounds **3a** and **3c** were also prepared for biological testing. Compound **3d** showed a marked inhibitory effect on the growth and development of tobacco hornworms.

Cholesterol synthesis in the liver of men and mammals is known to be inhibited by exogenous cholesterol in a feedback mechanism. Previous papers in this series describe a variety of synthetic aza- and diazacholesterol analogs[3] which were prepared in an effort to simulate cholesterol in this feedback mechanism. In addition to the desired inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase[4] with concomitant decrease in plasma total sterol levels, these azasteroids also inhibited desmosterol reductase.[5] Since lanosterol is the initial steroid product resulting from the cyclization of squalene, it was of interest to examine aza analogs of lanosterol not only as possible feedback inhibitors, but also as inhibitors of squalene oxidocyclase. This paper represents a continuation in part of our structure–activity relationship studies and describes the synthesis and biological activities of a number of modified azalanosterol analogs.

The most direct approach to 25-azadihydrolanosterol (**3d**) appeared to be ozonolysis of lanosterol acetate (**1**) with formation of a trisnoracid (**2a**) which is suitably functionalized for further transformations. The ozonolysis of lanosterol acetate was first reported by Ruzicka.[6] The isolation of **2a**, however, was quite tedious since the product could not be isolated directly from the reaction mixture. Consequently, these workers resorted to chromatography of methyl ester **2b** and subsequent hydrolysis to the free alcohol of **2a**. In our hands, however, **2a** was obtained directly in 85% yield by performing the ozonolysis of lanosterol acetate in CH_2Cl_2 and pyridine at $-70°$ and subsequently decomposing the ozonide at 0° with Jones reagent. Treatment of **2a** with $SOCl_2$ followed by Me_2NH gave amide **2d** in good yield. Reduction of **2d** with LAH afforded the desired 25-azadihydrolanosterol (**3d**).

25-Azadihydrolanosterol 3-acetate obtained by acetylation of **3d** could also be prepared by the Leuckart reductive amination of the trisnoraldehyde **5**.[7] Due to the presence of dihydrolanosterol,[8] which was present up to 60% in commercially available lanosterol, it was necessary to develop a new procedure for the synthesis of **5**. Addition of HOBr to **1** in aq dioxane gave a mixture of bromohydrin **4a** and dihydrolanosterol acetate. Chromatography on alumina (activity II) gave dihydrolanosterol acetate and epoxide **4b**. Epoxide **4b** was then cleaved with dil $HClO_4$ in dioxane to the corresponding 24,25-diol **4c** in 80% yield. Treatment of diol **4c** with $Pb(OAc)_4$ in THF solution gave trisnoraldehyde **5** in 85% yield. Leuckart reductive amination of **5** with formic acid and DMF gave 25-azadihydrolanosterol 3-acetate.

For biological reasons, nor derivatives **3a** and **3c** were also prepared from trisnoracid **2a**. Treatment of acid chloride **2c** with NH_3 gave the corresponding amide **2e** which was reduced with LAH to the corresponding amine **3a**. 25-Aza-26-nordihydrolanosterol (**3c**) was obtained by treatment of **3a** with ethyl chloroformate followed by reduction of the corresponding ethyl carbamate with LAH.

Preliminary biological studies have shown that **3d** is superior to 20,25-diazacholesterol as an inhibitor of tobacco hornworm growth.[9] Moreover, **3d** was found to be at least as active as 20,25-diazacholesterol as a hypocholesterolemic agent in rats.[10] Further studies with this compound are in progress and will be reported elsewhere.

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(7) First reported by Akhtar, *et al.*, and prepared directly from lanosterol acetate by selective epoxidation. The resulting 24,25-epoxylanost-8-en-3β-ol acetate was cleaved to the corresponding 24-ol-25-methoxy derivative and photolyzed to give the desired aldehyde **5**. [See M. Akhtar, P. F. Hunt, and M. A. Parvez, *Biochem. J.*, **103**, 616 (1967).] In our hands, however, we could not obtain selectivity in monoepoxide formation.

(8) The usual way of separating dihydrolanosterol from lanosterol consists of bromination of the mixture and subsequent isolation of the less sol 24,25-lanosterol dibromide which is then acetylated and dehydrobrominated with Zn to give **1**. In our present work, however, we found that this procedure was only partially successful in separating lanosterol from dihydrolanosterol since the latter cocrystallizes with lanosterol dibromide and is carried through as an impurity (up to 30%) in all subsequent reactions.

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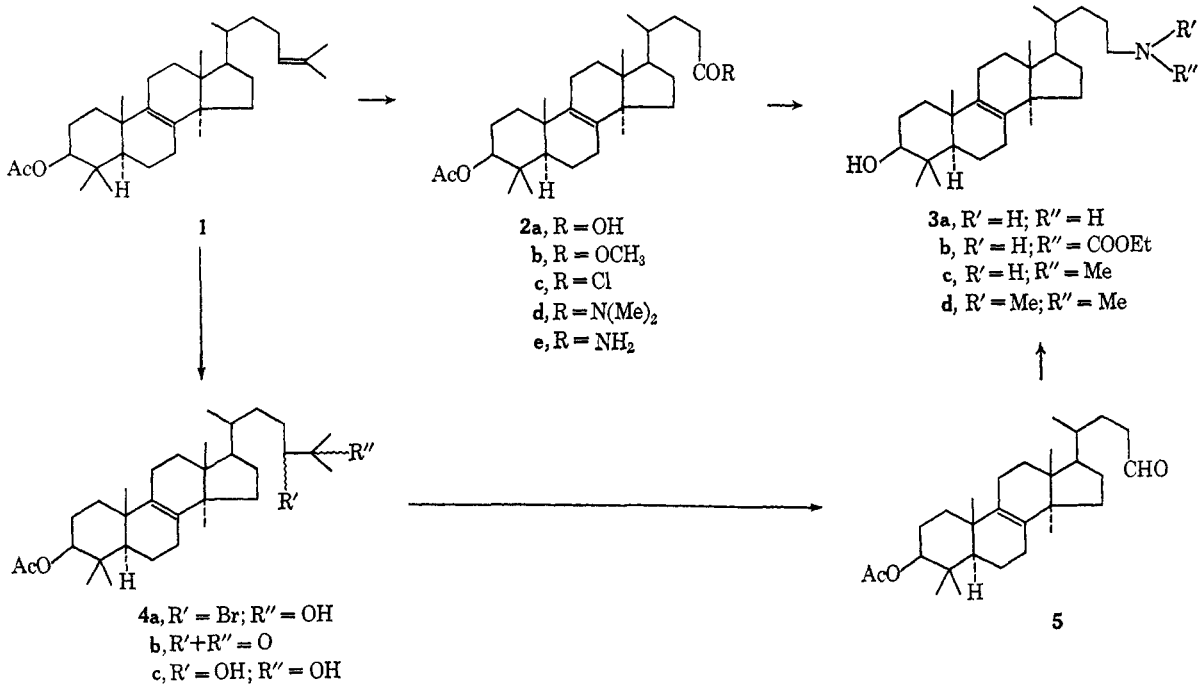

Experimental Section[11]

3β-Acetoxy-25,26,27-trisnorlanost-8-en-24-oic Acid (2a).—Lanosterol acetate[12] (10.0 g, 21.3 mmoles) in CH_2Cl_2 (300 ml) and dry C_5H_5N (3 ml) was ozonized at Dry Ice–Me_2CO temp in the usual manner.[13] The solvent was removed *in vacuo* under N_2 to give the crude ozonide, which was taken up in Me_2CO (300 ml). This was cooled to −10° and Jones reagent added dropwise with cooling and stirring until a brown color persisted (15 ml). The reaction mixture was further stirred for 30 min at 0° and for 10 min at room temp. The entire mixture was then poured into ice–H_2O (100 ml) and the product taken up in Et_2O (5 × 400 ml). The combined Et_2O exts were separated into acidic and neutral parts by treatment with NaOH (5%). The product isolated from the acidic fraction (4.0 g, 82.5%,[14] mp 196–200°) was recrystd from $CHCl_3$–MeOH to give analytically pure **2a**: mp 201.5°–202.5°; $[\alpha]D$ + 54.8°; nmr as expected. *Anal.* ($C_{29}H_{46}O_4$) C, H.

A soln of **2a** (200 mg, 0.43 mmole) in Et_2O was treated with CH_2N_2–Et_2O to yield the corresponding Me ester. Recrystn from $CHCl_3$–MeOH gave pure Me ester **2b** (158.7 mg, 77%), mp 168°–169.5° (lit.[6] mp 168°–170°).

Methanolic KOH (50%) hydrolysis of **2a** (250 mg, 0.54 mmole) yielded, after recrystn ($CHCl_3$–MeOH), pure **3β-hydroxytrisnorlanost-8-en-24-oic acid** (160.1 mg, 70.5%), mp 255–256° (lit.[6] mp 257.5°–259.5°).

3β-Acetoxy-25,26,27-trisnorlanost-8-en-24-oic Acid *N,N*-Dimethylamide (2d).—$SOCl_2$ (6 ml) and C_5H_5N (0.3 ml) were added to a soln of acid **2a** (3.0 g, 6.5 mmoles) in dry C_6H_6 (50 ml). The mixt was stirred at room temp for 3 hr, moisture being excluded. The soln was evapd below 30° under reduced pressure. More C_6H_6 was again added and removed similarly to remove unreacted $SOCl_2$. Crude acid chloride **2c** was then dissolved in 10% Me_2NH–C_6H_6 (100 ml), and the mixture was kept overnight in a pressure bottle at room temp. The mixture was then diluted with CH_2Cl_2 (200 ml) and washed successively with H_2O (3 × 100 ml), 10% HCl (100 ml), 5% $NaHCO_3$ (2 × 100 ml), and with H_2O (2 × 100 ml). The CH_2Cl_2–C_6H_6 soln was dried (Na_2SO_4), filtered, and evapd to yield crude amide **2d** (2.4 g, 77.4%). Recrystn from hexane afforded pure anal. sample: mp 137–139°; $[\alpha]D$ +51.1°; nmr δ 2.96 and 3.04 [6 H, $CON(CH_3)_2$] *Anal.* ($C_{31}H_{51}NO_3$) C, H.

25-Azadihydrolanosterol (3d).—A soln of crude **2d** (2.4 g, 5 mmoles) in purified dioxane (20 ml) was added with stirring to a refluxing slurry of LAH (1.90 g) in purified dioxane (60 ml). The mixture was refluxed for 20 hr and the excess reagent decompd by successive dropwise addition of 80% aq dioxane (10 ml), 20% NaOH soln (2 ml), and H_2O (10 ml). The insol salts were removed by filtration of the hot reaction mixture. The salts were washed with *i*-PrOH, and the filtrate was concd to dryness under reduced pressure. The solid residue was recrystd from dioxane–Me_2CO affording pure **3d** (1.95 g, 90.6%): mp 162–164°; $[\alpha]D$ +60.4°; nmr δ 2.32 [s, 6 H, $N(CH_3)_2$]. *Anal.* ($C_{29}H_{51}NO$) C, H.

Acetylation of **3d** with C_5H_5N and Ac_2O gave the corresponding 3β-acetoxy derivative in quantitative yield: mp 120–121° (hexane); $[\alpha]D$ +52.0°. *Anal.* ($C_{31}H_{53}NO_2$) C, H.

3β-Acetoxy-25,26,27-trisnorlanost-8-en-24-oic Acid Amide (2e).—A soln of acid **2a** (2.0 g, 4.4 mmoles) in dry C_6H_6 (20 ml) was treated with $SOCl_2$ (4.0 ml) and pyridine (0.2 ml) in the usual manner. The crude acid chloride obtained was taken up in dry C_6H_6 and treated with gaseous NH_3 for 2 hr at 0°, and then for 20 hr at room temp. The mixture was diluted with CH_2Cl_2 (200 ml) and washed successively with H_2O (3 × 100 ml), 10% HCl (100 ml), 5% $NaHCO_3$ soln (2 × 100 ml), and with H_2O (2 × 100 ml). The CH_2Cl_2–C_6H_6 soln was dried (Na_2SO_4), filtered, and evapd under reduced pressure to give crude amide **2e** (1.60 g, 80%). Recrystn from $CHCl_3$–hexane gave **2e** as needles, mp 230–232°; $[\alpha]D$ +56.0°; nmr δ 6.37 (broad, 2 H, NH_2). *Anal.* ($C_{29}H_{47}NO_3$) C, H.

25-Aza-26,27-bisnorlanost-8-en-3β-ol (3a).—LAH reduction of amide **2e** in dioxane in the usual manner gave 82.0% yield of pure **3a**: mp 178–180° (aq Me_2CO); $[\alpha]D$ +57.6°; nmr as expected; *m/e* 401. *Anal.* ($C_{27}H_{47}NO \cdot 0.5H_2O$) C, H.

Methylation of 3a.—A soln of **3a** (218 mg, 0.54 mmole), HCO_2H (0.22 ml), and formalin (0.2 ml) was heated on a steam bath for 20 hr. The resulting semisolid was dissolved in MeOH, and the soln was made basic with 25% NaOH soln (2 ml). The mixture was refluxed for 5 min and poured into ice–H_2O (15 ml). The mixture was extd with $CHCl_3$, and the ext was washed with H_2O. The $CHCl_3$ soln was dried (Na_2SO_4), filtered, and evapd under reduced pressure. The solid residue was recrystd from CH_3OH–Me_2CO to give pure **3d** (140 mg, 60%) identical in all respects with that obtained by reduction of **2d**.

25-Aza-26-norlanost-8-en-3β-ol (3c).—A soln of **3a** (204 mg, 0.51 mmole) in dry xylene (20 ml), ethyl chloroformate (0.4 ml), and Et_3N (0.1 ml) was refluxed for 4 days. The mixture was then diluted with $CHCl_3$ (50 ml) and washed with H_2O (2 × 50

(11) Mass spectra were carried out by Dr. R. L. Foltz at the High Resolution Mass Spectrometry Center of the Battelle Memorial Institute, Columbus, Ohio. The nmr spectra were obtained with a Varian A-60A spectrometer in $CDCl_3$ using TMS as an internal standard. The optical rotations were obtained in $CHCl_3$. The melting points were obtained on a Fisher-Johns apparatus and are corrected. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ± 0.4% of the theoretical values.

(12) Lanosterol acetate was prepared from lanosterol in the usual manner. The lanosterol was purchased from Aldrich Chemicals Co., Milwaukee, Wis., which contains up to 60% dihydrolanosterol as an impurity.

(13) The ozonization was carried out in a Welsbach Ozonator at a rate of 3.4 g of ozone per hour.

(14) The yield was calcd based on the 50% purity of the starting lanosterol.

ml). The $CHCl_3$ layer was dried (Na_2SO_4), filtered, and evapd under reduced pressure to give crude carbamate (253.1 mg, 91.7%); the nmr showed peaks at δ 1.24 and 4.15 for the Et group of the ethyl carbamate, ν_{max}^{KBr} (cm^{-1}) 3500 (OH,NH) and 1700 (C=O). This product was used for the subsequent reduction without further purification.

LAH reduction of crude **3b** in dioxane gave 92.0% yield of **25-aza-26-norlanost-8-en-3β-ol (3c)**: mp 164–166° (Me_2CO); $[\alpha]_D$ +54.7°; ν_{max}^{KBr} (cm^{-1}) 3615 (OH) and 3345 (NH); nmr δ 1.67 (**OH,NH**) and 2.49 (s, 3 H, **NCH**$_3$). *Anal.* ($C_{28}H_{49}NO$) C, H.

24ξ-Bromolanost-8-ene-3β,25-diol 3-Acetate (4a).—A soln of NBS (3 g) in H_2O and $HClO_4$ acid (70%, 3 ml) was added to a soln of lanosterol acetate[12] (7 g) in dioxane (300 ml), and the mixture was stirred for 1 hr, poured into H_2O, and filtered. The product was extd into $CHCl_3$, and the organic layer was washed successively with $Na_2S_2O_3$ soln, H_2O, dried, and evapd. The residue was composed of isomeric bromohydrins and dihydrolanosterol acetate. A portion of this mixture (2 g) was chromatographed on silica gel. Elution with hexane–$CHCl_3$ (8:2) gave dihydrolanosterol acetate (0.8 g). Further elution with $CHCl_3$ afforded bromohydrin **4a** (0.8 g): mp 168–169°; nmr δ 1.33 (s, 6 H, C-26, and C-27 Me protons). *Anal.* ($C_{32}H_{53}BrO_3$) C, H.

24,25-Epoxylanost-8-en-3β-ol 3-Acetate (4b).—A portion of the mixture of bromohydrins and dihydrolanosterol acetate (2 g) obtained above was chromatographed on Woelm neutral alumina (Activity II). Elution with hexane–$CHCl_3$ (9:1) gave dihydrolanosterol acetate (0.8 g). Further elution with hexane–$CHCl_3$ (8:2) gave the desired monoepoxide **4b** (0.6 g); mp 188–189°; $[\alpha]_D$ +53° (lit.[7] mp 181–182°; $[\alpha]_D$ +55°); nmr δ 1.25 and 1.30 (C-26 and C-27 Me protons).

Lanost-8-ene-3β,24ξ,25-triol 3-Acetate (4c).—$HClO_4$ (0.28 *N*, 10 ml) was added to a soln of the monoepoxide **4b** (1 g) in dioxane (100 ml), and the mixture was stirred at room temp for 1 hr prior to pouring into H_2O. The ppt was filtered, dried, and recrystd from hexane–CH_2Cl_2 to give diol **4c** (800 mg): mp 187–188°; $[\alpha]_D$ +45°; δ 1.15 and 1.20 (C-26 and C-27 Me protons). *Anal.* ($C_{32}H_{54}O_4$) C, H.

3β-Acetoxy-25,26,27-trisnorlanost-8-en-24-al (5).—$Pb(OAc)_4$ (800 mg) was added to a soln of diol **4c** (1 g) in THF (25 ml), and the mixture was stirred at room temp for 1 hr, filtered, and evapd. The residue was then extd into $CHCl_3$. The org layer was washed with $Na_2S_2O_3$ soln and H_2O, dried, and evapd. The residue was chromatographed on Woelm neutral Al_2O_3 (Activity II). Elution with hexane–Et_2O (1:1) gave the desired aldehyde **5** (730 mg) which was crystd from hexane: mp 144–145°; $[\alpha]_D$ +53° (lit.[7] mp 144–146°; $[\alpha]_D$ +58°); δ 9.72 (t, 1 H, J = 2 cps, CHO).

Leuckart Reductive Amination of 5.—A soln of aldehyde **5** (200 mg) in DMF (1 ml) and HCO_2H (90%, 1 ml) was heated at 140° for 2 hr. The mixture was then cooled, poured into H_2O, and extd into $CHCl_3$. The org layer was then washed with $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evapd. The residue was chromatographed on Al_2O_3, and elution with hexane–Et_2O (7:3) gave the desired azalanosterol derivative (120 mg). Recrystn from hexane gave a product identical in all respects with that obtained by acetylation of **3d**.

Centrally Acting Cyclic Urea, Thiourea, and Their *N,N'*-Dialkyl Derivatives. Structure–Activity Correlations[1a]

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Sixteen cyclic urea and thiourea derivatives were synthesized, 12 of which were new compounds and had not been reported previously. Six of the compounds showed potent convulsant activity, 3 showed potent CNS depressant activity, and 7 of them were found to possess potent respiratory stimulation activity. For these compounds the respiratory stimulation effect was tested in pentobarbital-depressed mice. The pharmacological activities, LD_{50}, HD_{50}, and CD_{50}, were correlated with the partition coefficient (log P) and the dipole moment (μ) by multiple regression analysis using an IBM 360/65 computer. The correlations obtained indicate that there is a parabolic relationship between the pharmacological activity and the partition coefficient of the congeneric cyclic ureas and thioureas. Inclusion of dipole moment further improves the correlations at the 99 percentile level.

Many of the CNS-acting drugs have amide or thioamide linkage as the common molecular structural units.[2,3] Cyclic urea and thiourea derivatives, which also have the same structural features, may be expected to possess CNS activities, although urea and thiourea molecules themselves do not have significant pharmacological activity.

It appears that the pharmacological inertness of urea and thiourea can be attributed to their very high solubility in water and poor solubility in lipid. If appropriate molecular modifications are made on the parent urea and thiourea molecules to raise their lipophilic character, pharmacologically active compounds may result. Relationship between biological activity of different classes of compounds and their lipid solubility has been demonstrated in many cases.[4–6] Meyer and Overton's classical work has been extended by Hansch and his coworkers.[7–10] They have shown that in general a parabolic relationship exists between the biological activity and partition coefficient of a wide variety of compounds. Linear relationship has been considered only as a special case.

Lien and Kumler[11] have reported the CNS activities of 5-, 6-, and 7-membered cyclic ureas, thioureas, and their *N,N'*-dimethyl derivatives. The methylated compounds were reported to be significantly more potent than the unmethylated ones. Also, they showed a

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