6-Amino Derivatives of Stigmastanol and Cholestanol

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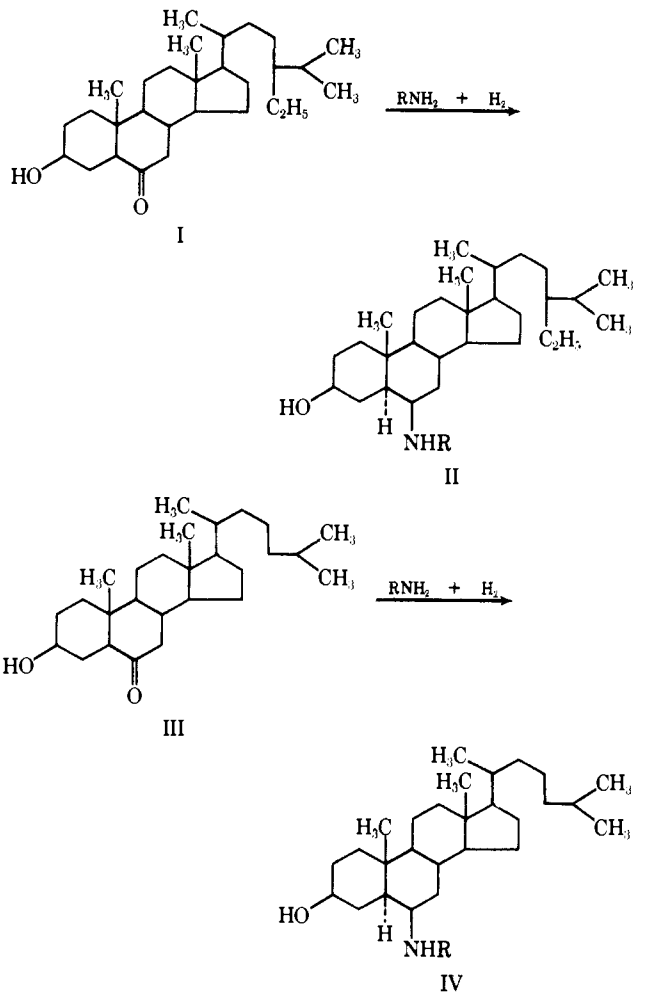
A number of new 6-β-amino and 6-β-(*N*,*N*-dialkylaminoalkyl)amino derivatives of stigmastanol and cholestanol have been prepared by reductive amination of the corresponding 6-keto sterols or reduction of Schiff bases from the same ketones. Several of these amines produce a decrease in the blood cholesterol level in normal rats.

β-Sitosterol (stigmast-5-en-3β-ol) is known to possess some degree of hypocholesterolemia-promoting activity on oral administration and this effect has been ascribed to a partial inhibition of intestinal resorption of dietary cholesterol.[1] Recently, other structural analogs of cholesterol such as trihydroxy derivatives of both stigmastane[2] and cholestane[3] have been reported to show the same type of pharmacologic activity. But with all these compounds very high dose levels are required; and 17-amino derivatives of androstane, some of which are very active,[4] show side effects due to their estrogenicity. It was therefore of interest to investigate other types of amino steroids, and for this purpose we synthesized a number of 6-amino derivatives of stigmastane and cholestane (II and IV), which are readily accessible from β-sitosterol and from cholesterol *via* 3β-hydroxy-6-oxo-5α-stigmastane (I) and 3β-hydroxy-6-oxo-5α-cholestane (III). These two last ketones were either converted with primary amines into Schiff bases which were then catalytically hydrogenated, or submitted directly to catalytic reductive amination.[5] The primary amines used were either monoamines (*n*-$PrNH_2$, phenethylamine, and glycine) or diamines (β-dimethylaminoethylamine, β-diethylaminoethylamine, and γ-dimethylaminopropylamine). Ketone III was obtained by treatment of 3β-acetoxy-6-nitro-Δ^5-cholestene (prepared by Dodson and Riegel's method[6]) with Zn powder in AcOH;[7] a similar reaction sequence was used for the preparation of ketone I from 3β-acetoxy-6-nitro-Δ^5-stigmastene.

The axial configuration of the amino group assumed for the various amines thus prepared is derived from an extension, to reductive amination of ketones I and III, of the rule of "rear attack" as applied to the catalytic hydrogenation of sterically hindered ketones.[8] This assumption was confirmed by the synthesis of 3β-hydroxy-6β-(2-diethylaminoethyl)amino-5α-cholestane (IV, R = $CH_2CH_2NEt_2$) and its 6α epimer, by condensing β-diethylaminochloroethane with, resp, 3β-hydroxy-6β-amino-5α-cholestane (IV, R = H) and its 6α epimer, both of which are known.[9] A similar structure determination was made for 3β-hydroxy-6β-(2-diethylaminoethyl)amino-5α-stigmastane (II, R = $(CH_2)_2NEt_2$), which proved different from its 6β epimer.



I II III IV

The chemical data concerning the monoamines (free bases and monohydrochlorides) and diamines (isolated as dihydrochlorides) obtained are given in Table I. Pharmacological screening for hypocholesterolemia-inducing activity consisted of an evaluation of the decrease in serum cholesterol content of rats treated orally

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(2) H. Pinhas, French Patent 7466 M (June 28, 1968).

(3) Y. Aramiki, T. Kobayashi, Y. Imai, S. Kikuchi, T. Maksukana, and K. Kamazawa, *J. Atheroscler. Res.*, **7**, 653 (1967).

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(5) G. Maume and C. Baron, *Bull. Soc. Chim. Fr.*, 4508 (1967).

(6) R. M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948).

(7) J. Mauthner and W. Suida, *Monatsh. Chem.*, **24**, 648 (1903).

(8) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed, Reinhold, 1949; L. F. Fieser, *Experientia*, **6**, 312 (1950); T. F. Gallagher and T. H. Kritchersky, *J. Amer. Chem. Soc.*, **72**, 882 (1950).

(9) C. W. Shoppee, D. E. Evans, and G. H. R. Summers, *J. Chem. Soc.*, 690 (1955); B. G. Ketcheson and A. Taurins, *Can. J. Chem.*, **38**, 981 (1960).

TABLE I

NEW AMINES AND DIAMINES II AND IV

Compd	R	Mp, °C[a,b]	Formula[c,d]
	6β-Stigmastane Derivatives II		
1	H (base)	157-158	$C_{29}H_{53}NO \cdot 0.5H_2O$
	H (HCl)	233-235	$C_{29}H_{54}ClNO \cdot 0.5H_2O$
2	n-C_3H_7 (HCl)[e]	171-173	$C_{32}H_{60}ClNO \cdot H_2O$
3	$(CH_2)_2C_6H_5$(HCl)[f]	161-163	$C_{37}H_{62}ClNO \cdot H_2O$
4	CH_2CO_2H (base)	232	$C_{31}H_{53}NO_3$
5	$(CH_2)_2N(CH_3)_2$ (2HCl)	254-255	$C_{33}H_{64}Cl_2N_2O \cdot 0.5H_2O$
6	$(CH_2)_2N(C_2H_5)_2$ (2HCl)	233-235	$C_{35}H_{68}Cl_2N_2O \cdot 0.5H_2O$
7	$(CH_2)_3N(CH_3)_2$ (2HCl)	259-261	$C_{34}H_{66}Cl_2N_2O \cdot 0.5H_2O$
	6β-Cholestane Derivatives IV		
8	$(CH_2)_2C_6H_5$ (HCl)	159-160	$C_{35}H_{58}ClNO \cdot H_2O$
9	CH_2CO_2H (base)	210	$C_{29}H_{51}NO_3$
10	$(CH_2)_2N(CH_3)_3$ (2HCl)	229-231	$C_{31}H_{60}Cl_2N_2O \cdot 0.5H_2O$
11	$(CH_2)_2N(C_2H_5)_2$ (2HCl)	217-219	$C_{33}H_{64}Cl_2N_2O \cdot 0.5H_2O$
12	$(CH_2)_3N(CH_3)_2$ (2HCl)	253-255	$C_{32}H_{62}Cl_2N_2O \cdot 0.5H_2O$

[a] Mps were taken with a Reichert microscope. [b] Bases were recrystd from aq EtOH; hydrochlorides and dihydrochlorides from EtOH-Et_2O (1:3). [c] Purity of all samples was confirmed by tlc (silica gel; spot detection with either Draggendorf's reagent or 1:1 Ac_2O-H_2SO_4. [d] All compds anal. satisfactorily for C, H, N, Cl. [e] Free base, mp 202-205°. [f] Free base, mp 168°.

for 6 days with the substance under test, in comparison with the untreated controls, and taking clofibrate (2-*p*-chlorophenoxy-2-methylpropionic acid Et ester) and 3,5,6-trihydroxystigmastane as reference substances. Results, recorded in Table II, showed that several of the amines and diamines produced a significant decrease in serum cholesterol levels; however, this effect was accompanied by significant reduction of the weight of the liver and increase in the weight of the adrenals. This suggests that the steroid amines and diamines investigated here induce hypocholesterolemia in normal rats *via* some interference in cholesterol and lipid metabolism rather than by preventing intestinal absorption of dietary cholesterol.

TABLE II

EFFECTS ON SERUM CHOLESTEROL

Compd[a]	Dose, mg/kg per day	Serum cholesterol, g/l.	Weight of liver, g/100 g of body wt rat	Weight of adrenals, mg/100 g of body wt rat
2	250	0.50	3.33	23
Control		0.70	3.93	16
3	250	0.63	2.42	15
Control		0.86	3.70	14
4	250	1.07	3.92	18
Control		0.99	3.84	16
5	125	0.75	3.27	16
	250	0.70	3.05	21
Control		0.78	2.88	16
6	125	1.06	3.62	18
	250	0.93	3.51	23
Control		0.99	3.79	19
7	125	0.87	3.40	16
	250	0.68	3.17	15
Control		0.78	2.88	16
8	250	0.77	3.25	19
Control		1.04	3.62	14
10	125	0.90	3.38	19
	250	0.74	2.97	32
Control		0.99	3.79	19
11	125	0.81	2.76	24
	250	0.55	2.81	31
Control		0.74	3.17	20
12	125	0.59	3.06	24
	250	0.52	2.68	33
Control		0.66	3.09	23
13[c]	250	0.87	3.84	14
Control		0.86	3.70	14
14[d]	250	0.72	3.44	16
Control		0.70	3.93	16
15[e]	125	0.75	4.18	16
	250	0.72	4.90	17
Control		0.88	3.64	14

[a] For identification of compds, see Table I. [b] All compds in the form of their hydrochloride or dihydrochloride except for **4**, **13**, **14**. [c] 3β-Hydroxy-6-oximino-5α-stigmastane. [d] 3β-Acetoxy-6β-amino-5α-stigmastane. [e] Clofibrate.

Experimental Section

A. Chemistry. 3β-Hydroxy-6β-amino-5α-stigmastane (II, R = H).—A soln of 2 g of the oxime of ketone I in 90 ml of glacial AcOH was reduced catalytically in the presence of 0.2 g of PtO_2 at 50° and at normal pressure. After the reduction was completed (48 hr) the soln was filtered, the solvent was vacuum distd, the product was dissolved in H_2O, and the amine was pptd with 2 *N* aq NaOH and taken up in PhH. The corresponding hydrochloride was prepared by treatment with HCl gas in Et_2O; overall yield, 70%.

3β-Hydroxy-6α-amino-5α-stigmastane.—A soln of 2 g of the oxime of ketone I in 250 ml of *n*-PrOH was quickly treated with 2 g of Na, and the mixt was maintained at boiling point until total disappearance of the Na. After cooling, Et_2O (250 ml) was added, and the product was shaken with 3000 ml of H_2O. The org layer was dried (Na_2SO_4) and HCl gas in Et_2O was added to form the hydrochloride; the *n*-PrOH was vacuum distd, and the cryst residue was washed with Et_2O and recrystd from aq EtOH to give the stigmastane hydrochloride in 65% yield, as colorless prisms, mp 259-261°. *Anal.* ($C_{29}H_{54}ClNO \cdot H_2O$) C, H, N. The free base crystd from aq EtOH as colorless prisms, mp 175-176°. *Anal.* ($C_{29}H_{53}NO \cdot 0.5H_2O$) C, H, N.

3β-Hydroxy-6α-(2-diethylaminoethyl)amino-5α-stigmastane.—A soln of 1 g of 3β-hydroxy-6α-amino-5α-stigmastane and 1 g of freshly distd 2-diethylaminochloroethane in 50 ml of anhyd C_6H_6 was refluxed for 24 hr with a few drops of Et_3N, the C_6H_6 was then distd off, the residue was taken up in 200 ml of Et_2O, and the Et_2O soln was extd with dil HCl. The aq layer was basified and the diamine was taken up in Et_2O, dried (Na_2SO_4), and converted into its dihydrochloride by means of HCl gas. This salt crystd from EtOH-Et_2O as colorless prisms; mp 216-218°, yield, 20%. *Anal.* ($C_{35}H_{68}Cl_2N_2O \cdot 2H_2O$) C, H, Cl.

3β-Hydroxy-6α-(2-diethylaminoethyl)amino-5α-cholestane was prepd in the same way; its dihydrochloride crystd from EtOH-Et_2O as colorless prisms, mp 209-210°. *Anal.* ($C_{33}H_{64}Cl_2N_2O \cdot 0.5H_2O$) C, H, Cl. A similar method was applied for the prepn of the 2 epimeric diamines (**6** and **11** in Tables I and II).

Reductive Amination of Ketones I and III.—A soln of ketone I or III (0.1 mole) and a large excess of the appropriate amine (0.25 mole) in 400 ml of abs EtOH contg 1 g of 10% Pd/C was hydrogenated at 60-90° under pressure (100 kg/cm²), with vigorous stirring. The reduction lasted *ca.* 24 hr; the soln obtd was filtered hot, the EtOH distd off, and the residue recrystd or converted into the mono- or dihydrochloride; yield, 65-70%.

The reaction could be performed in 2 steps: prepn of the Schiff base by refluxing a C_6H_6 soln of ketone I or III (0.1 mole), the primary amine (0.25 mole), and one drop of AcOH for 8 hr, under azeotropic elimination of H_2O in a Dean-Stark apparatus; the solvent was distd off, and the crude, viscous Schiff base was taken up in EtOH and catalytically hydrogenated as above; yield, 60-70%.

B. Pharmacology.—The animals used were male Wistar rats weighing *ca.* 200 g and fed a normal diet; the substances tested were administered orally (gavage) for a period of 6 days. The serum cholesterol was measured by means of the Zlatkis, *et al.*, colorimetric method[10] (Cl_3Fe-H_2SO_4; absorption band at 560 mμ after 30 min). The pharmacological assays were performed on batches of 20 rats per dose and per substance.

(10) A. Zlatkis, B. Zak, and A. J. Boyle, *J. Lab. Clin. Med.*, **41**, 486 (1953).