THE HYPOCHOLESTEREMIC EFFECT OF 3β-(β-DIMETHYLAMINOETHOXY)-ANDROST-5-EN-17-ONE AND ITS MECHANISM OF ACTION

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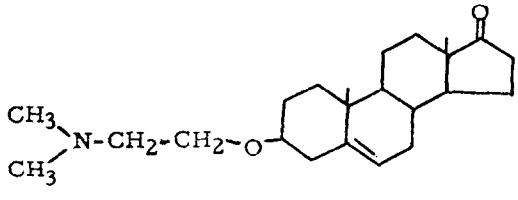
The great interest in compounds which lower blood cholesterol has prompted us to report on a new steroid which is a potent hypocholesteremic agent and appears to inhibit cholesterol biosynthesis by inhibiting the conversion of desmosterol to cholesterol.

Preparation

3β-(β-Dimethylaminoethoxy)-androst-5-en-17-one [m. p. 123-24°, $[\alpha]_D^{25}$ + 6.6°($CHCl_3$); hydrochloride m.p. 235-37°, $[\alpha]_D^{25}$ + 22° (CH_3OH)] was prepared by the alkylation of 17-ethylenedioxyandrost-5-en-3β-ol with β-dimethylaminoethylchloride and potassium t-butoxide in tetrahydrofuran solution. Both the free amine and its hydrochloride salt gave satisfactory elemental microanalyses.



Dose Response and Postmortem Studies

Male rats, C.F.E. strain, 125 g initial weight, obtained from Carworth Farms, New City, New York were divided into groups of 6 and fed the compound incorporated in the diet. After one and two weeks, tail blood samples were removed for serum sterol determination. At the end of 4 weeks, the animals were decapitated and various tissues examined.

The serum sterol concentration was markedly reduced after one week at a dietary dose level of 0.001% (Table I).

Table I

Dose Response to 3β-(β-dimethylaminoethoxy) androst-5-en-17-one

Dose		No. of Animals	Serum Sterol[a]		
			1 week	2 weeks	4 weeks
% in diet	mg/kg		mg %	mg %	mg %
Control	0	6	87 ± 2.9[b]	73 ± 3.3	72 ± 2.6
0.0005	.75	6	79 ± 3.7[c]	58 ± 3.3	56 ± 3.4
.001	1.5	6	66 ± 2.7	61 ± 2.9	45 ± 2.7
.003	4.5	6	63 ± 4.3	45 ± 2.4	32 ± 2.7
.01	15	6	54 ± 4.2	46 ± 4.3	24 ± 2.4
.03	45	6	56 ± 2.7	53 ± 4.8	26 ± 3.7

[a]Saponification and extraction by method of Trinder (1952) followed by colorimetric analysis using $FeCl_3$-H_2SO_4. (Zlatkis et al. 1953)

[b]Mean ± Standard Error

[c]This is the only group which is not significantly different from the control by Rank Test; $P > .05$ (Wilcoxon 1945)

The results of the postmortem studies are summarized in Table II. The values are the average of 6 determinations within each group. A level of 0.03% in the diet resulted in reduction of food intake and growth. Some adrenal and liver enlargement and liver lipid infiltration were observed. Marked adrenal sterol depletion occurred at all levels. Since sterols serve as precursors for adrenal corticoids, depletion of these sterols may account for adrenal enlargement. This would be analogous to thyroid hypertrophy during iodine depletion.

In an androgen assay[1] (rat, 4 days, subcutaneous route, ventral prostate weights) this compound was inactive at dose levels up to 2.5

[1]The assays were carried out by the Department of Metabolic Chemotherapy, Experimental Therapeutics Research Section of these Laboratories.

Table II

Effects of Various Levels of the Compound Given Orally for 4 Weeks

Dose % in Diet	Food Intake g/rat/day	Final Body wt. g/rat	Adrenal wt. mg.	Adrenal wt. / Body wt. mg/g	Adrenal[a] Sterol mg/g	Liver wt. g	Liver wt. / Body wt. g/g	Liver Sterol mg/g. [a]	Liver Fat[b] mg/g.
0 Control	21	285	39.5	0.139	29.7	12.8	0.045	2.2	67.7
0.0005	21	290	39.2	.134	18.8*	15.0	.052*	1.7*	71.6
.001	20	282	44.8	.159	13.0*	14.3	.051*	2.0	68.2
.003	21	287	43.2	.150	7.6*	15.9*	.055*	1.5*	74.4
.01	21	297	47.5*	.159*	6.4*	18.4*	.062*	1.4*	81.2*
.03	14*	188*	35.4	.188*	12.4*	14.4	.078*	1.8*	82.4*

[a]Determined on an aliquot of ethanol-ether extract by method used for serum.

[b]Method of Shipley et al. (1948).

*Significantly different from controls by Rank Test. $P < .05$ (Wilcoxon 1945).

mg/day, whereas dehydroisoandrosterone displayed a perceptible response at a 1 mg level.

Mechanism of Action

At the end of the 4 week feeding period, animals in the control and 0.01% treated groups were injected intraperitoneally with 1-C^{14} labeled acetate. Four hours later they were sacrificed. The livers were removed, saponified and the sterol fraction recovered as a petroleum ether (b.p. 30-60°) extract. A sample of liver sterol from each animal was subjected to gas liquid chromatography. The effluent fractions were trapped in tubes packed with glass wool and rinsed into counting vials with toluene phosphor. The vials were counted in a Packard 'Tri-Carb' liquid scintillation counter.

In contrast to the control livers which yielded only cholesterol, most of the sterol in the livers of the treated animals was desmosterol (Table III). Most of the C^{14} activity was in this fraction. This suggests that the administration of this compound inhibits the conversion of desmosterol to cholesterol. The gas chromatographic pattern of the liver sterols from the compound treated animals is essentially the same as that obtained from triparanol treated animals. Thus it would appear that this compound lowers tissue cholesterol by inhibiting the biosynthesis of cholesterol at the desmosterol stage as has been reported with triparanol (Avigan et al. 1960).

Table III

Sterol Content and C^{14} Activity of Rat Livers As Determined By Gas-Liquid Chromatography [a]

Treatment	Sterol [b]	% of total Sterols	% of total C^{14} in Sterols
Controls	Cholesterol	100	89.3
	Desmosterol	-	0
	Zymosterol	-	0
	Lanosterol	-	10.7
3β-(β-Dimethyl aminoethoxy)-androst-5-en-17-one	Cholesterol	1.5	7.5
	Desmosterol	98.5	76.6
	Zymosterol	-	12.1
	Lanosterol	trace	3.7
Triparanol[c]	Cholesterol	27.5	7.0
	Desmosterol	72.5	74.5
	Zymosterol	trace	9.6
	Lanosterol	trace	8.8

[a] Barber Colman Model 10, 3% SE-30 on Gas Chrom P, 100-140 mesh 6' x 1/4" ID, column temperature 240°, cell temperature 255°, Argon gas flow 105 ml/min. at 30 p.s.i.

[b] Identity of these sterols established by comparison with authentic samples.

- Indicates non-detectable quantities; trace, indicates less than 0.5% of total sterol.

[c] 1-[4-(diethylaminoethoxy) phenyl]-1-(p-tolyl)-2-(p-chlorophenyl) ethanol.

References

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