PREPARATION AND SYNANDROGENIC ACTIVITY OF 17β-HYDROXY-4,5-SECO-5-ANDROSTEN-3-ONE

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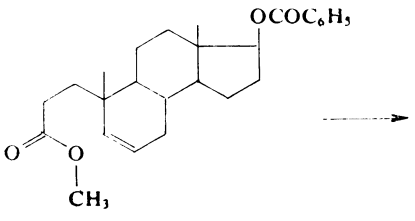
Acylation of the sodium salt of dimethyl sulfoxide with methyl 17β-benzoyloxy-A-nor-3,5-seco--5-androsten-3-oate (*I*) gave the corresponding sulfoxide *II* which was desulfurated with aluminum amalgam to 17β-hydroxy-4,5-seco-5-androsten-3-one (*IV*). *In vitro* and *in vivo* assays of compound *IV* have shown that in contrast to antiandrogenic 4,5-seco derivatives *V*—*VIII* it has a synandrogenic activity.

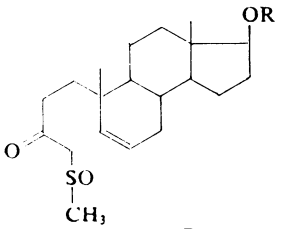
One of the possible modifications of the chemical structure of natural steroidal compounds, *i.e.* the opening of some of the skeleton rings, has been suggested by Nature itself (*cf.* vitamin D, toonacilin[1] or neriaside[2]). The testing of the physiological activity of synthetic steroids modified in this manner has shown that in a number of cases the intact steroidal skeleton is not indispensable for biological activity: for example, estrogenic activity was found in estradiol analogues with a severed bond between the carbon atoms 5 and 6, 9 and 11, 5 and 8, 8 and 9, 13 and 14 and 16 and 17 (see ref.[3-8]). The case of diethylstilbestrol shows that estrogenic activity is also displayed by substances structurally very remote from the original hormone. Unlike this the androgenic activity permits a much lesser degree of structure modification. Thus, for example, the typical androgen dihydrotestosterone (*i.e.* 17β--hydroxy-5α-androstan-3-one) was converted to an antiandrogenic substance, *V* (ref.[9]), by the interruption of the 4,5-bond. Antiandrogenic activity was also found in some 5,10-secoandrogens[10]. Allenic 5,10-secogestagens with double bonds in positions 4 and 5 inhibited the activity of 5α-steroid reductase both in rat epididimis and in human skin fibroblasts[11,12]. The binding of highly unsaturated secoanalogues o receptors was much stronger than the binding of natural hormones[13], and therefore we decided to introduce a double bond in the position 5 into the molecule of the antiandrogenic compound *IV*, hoping that the presence of this easily accessible double bond would increase the activity of the product.

We used testosterone benzoate (17β-benzoyloxy-4-androsten-3-one) as starting compound which was converted to the unsaturated ester *I* (ref.[14]) in a described manner. The reaction of the ester with the sodium salt of dimethyl sulfoxide afforded

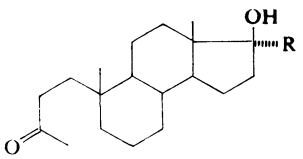
sulfoxide *II* which was characterized as acetate *III*. (Sulfoxides *II* and *III* represent a mixture of diasteroisomers differing in the configuration at the sulfur atom. No efforts to separate them were made.) Desulfuration of sulfoxide *II* with aluminum amalgam gave 17β-hydroxy-4,5-seco-5-androsten-3-one (*IV*) which showed in an *in vitro* screening test practically the same activity (Table I) as the saturated compound *V*. Therefore we assumed for the latter an antiandrogenic activity also in *in vivo* experiments. The increases in total weight and in the relative weights of organs in male mice were measured after administration of testosterone propionate (17β-propionyloxy-4-androsten-3-one), either alone or in combination with compound *IV*. The result of this bioassay (Table II) was that, in contrast to secoandrostane derivatives tested earlier, a distinct synandrogenic activity could be detected for compound *IV*, which became manifest not only by the weight increase of seminal vesciles but by the potentiation of the renotropic effect of testosterone propionate as demonstrated



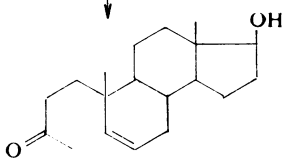
I



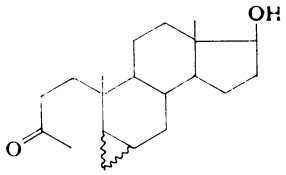
II, R = H
III, R = $COCH_3$



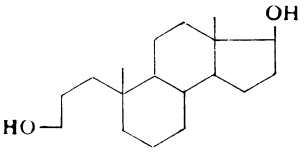
V, R = H
VI, R = CH_3



IV



VII, 5αH, 6αH
VIII, 5βH, 6βH



IX

by additional increase in the weight of the kidneys. Administration of compound *IV* decreased the weight of the adrenals additively to the adrenal effect of testosterone propionate.

TABLE I

Increases in total body weight and relative weights of organs (referred to 100 g of body weight) of castrated male rats after administration (for 3 weeks) of testosterone propionate in combination with 17β-hydroxy-4,5-seco-5-androsten-3-one (*IV*), in comparison with the group with testosterone propionate. The number of animals in the group $n = 8$

Increase in body weight g	Relative weights of the organs			
	seminal vesicles mg/100 g	spleen mg/100 g	kidneys g/100 g	adrenals mg/100 g
With the vehicle:				
2·1[a]	167·3[a]	276·1	1·27[a]	18·1
±1·9	± 53·9	± 58·4	±0·16	± 4·6
With testosterone propionate:				
7·7	672·3	288·7	1·48	15·0
±1·5	±123·3	± 71·4	±0·07	2·6
With testosterone propionate and compound *IV*:				
5·2[b]	811·2[b]	277·9	1·58	13·6
±2·6	102·9	56·6	±0·31	2·7

[a] $P < 0{\cdot}01$; [b] $P < 0{\cdot}05$ (relative to animal injected with testosterone propionate).

TABLE II

Competition of some seco-androstane derivatives with dihydrotestosterone in the bond to androgenic receptors from the cytosol of rat prostate *in vitro* (referred to compound *X*, dihydrotestosterone)

Compound	K_i nmol l^{-1}	%
IV	4·01	48·6
V	3·81	51·2
VI	5·08	38·4
IX	5·55	35·1
X	1·95	100·0

A comparison of the very similar structures of compounds *V* to *IX* (ref.[9]) offers itself for considerations on the relationship between the structure and biological activity of active substances. It was found that 4-nor-3,5-seco derivative *IX* is quite inactive, but all other substances of this group, which do not contain a C=C bond in position 5, displayed a higher or lower antiandrogenic activity, with the exception of the unsaturated substance *IV* which has an opposite activity. This effect of the double bond cannot be due to the possible greater rigidity of the B-ring in compound *IV*, and thus by the different conformation of the whole molecule, since in compounds *VII* and *VIII* the conformation of the ring B is very rigid. A possible explanation of the effect mentioned of the double bond should be sought rather in the higher planarity of the ring B, or in the fact that this C=C double bond is one of the binding sites for the coordination of compound *IV* to the receptor: the accessibility of the double bond in seco steroid *IV* is comparable to that of the multiple bonds in the molecules of biologically active 3-oxo-5,10-seco derivatives[10-13].

EXPERIMENTAL

The melting points were measured on a Kofler block and they are not corrected. Specific rotations and the infrared spectra were measured in chloroform solution, the ^{1}H NMR spectra in deuteriochloroform (on Tesla 60 MHz), using tetramethylsilane as internal reference. Methyl 17β-benzoyloxy-A-nor-3,5-seco-5-androsten-3-oate (*I*) was prepared according to ref.[14] from testosterone benzoate in a 65% yield.

17β-Hydroxy-4-methylthio-4,5-seco-5-androsten-3-one S-Oxide (*II*)

An emulsion of sodium hydride (50%, 0·5 g) was washed with three portions of light petroleum (5 ml) under argon and then heated with 15 ml of dimethyl sulfoxide at 65°C for 2 h. The mixture was cooled to room temperature and then mixed with a solution of the methyl ester *I* (0·4 g) in tetrahydrofuran (10 + 4 ml). After 4 h stirring at room temperature the excess of the sodium salt of dimethyl sulfoxide was decomposed by addition of solid ammonium chloride (about 0·5 g) and the product was partitioned between ethyl acetate and water. The organic fraction was washed with water till neutral, evaporated and the residue crystallized from a mixture of chloroform and ether, m.p. 86–90°C, $[\alpha]_D^{20}$ −6° (*c* 1·1). IR spectrum: 3 615, 1 052, 1 034, (OH), 1 711 (C=O), 1 657 (C=C) cm^{-1}. ^{1}H NMR spectrum 0·76 (s, 3 H, 18-H), 0·94 (s, 3 H, 19 H), 2·68 (s, 3 H, —S—CH_3), 3·66 (t, J = 8 Hz, 17-H), 3·77 (s, 2 H, 4-H), 5·24 (bd, J = 10 Hz, 5-H), and 5·65 (dd, J = 5 and 10 Hz, 6-H) ppm. Mass spectrum, *m/z*: 335 (M^+ − OH), 271 (b.p.). For $C_{20}H_{32}O_3S$ (352·5) calculated: 68·14% C, 0·14% H, 9·10% S; found: 68·22% C, 9·19% H, 8·89% S.

17β-Acetoxy-4-methylthio-4,5-seco-5-androsten-3-one S-Oxide (*III*)

Sulfoxide *II* (200 mg) was acetylated with acetic anhydride (0·5 ml) in pyridine (0·5 ml) at room temperature. After 20 h the mixture was decomposed with water, the product extracted with ethyl acetate, washed gradually with hydrochloric acid (5%), water, aqueous potassium hydrogen carbonate solution (7%) and water, dried over sodium sulfate and the solvent evaporated. The residue was crystallized from a mixture of acetone and ether. M.p. 103–105°C (90 mg), $[\alpha]_D^{20}$ −3°

(*c* 1·1). IR spectrum: 1 726, 1 259, 1 046 (CH_3COO), 1 712 (R—CO—R), 1 660 (C=C) cm^{-1}. ^1H NMR spectrum: 0·80 (s, 3 H, 18-H), 0·92 (s, 3 H, 19-H), 2·02 (s, 3 H, CH_3COO), 2·66 (s, 2 H, 4-H), 3·75 (s, 3 H, —SCH_3), 4·50 (t, J = 8 Hz, 1 H, 17-H), 5·22 (d, J = 10 Hz, 5-H) and 5·64 (dd, J = 10 and 4 Hz, 1 H, 6-H) ppm. For $C_{22}H_{34}O_4S$ (394·6) calculated: 66·97% C, 8·68% H, 8·12% C; found: 66·63% C, 8·54% H, 8·12% S.

17β-Hydroxy-4,5-seco-5-androsten-3-one (*IV*)

4 g of aluminum foil were cut into small squares (about 0·25 cm^2) which were shortly dipped (30 s) in a mercury-II chloride (1%) solution and rapidly washed with water, ethanol and ether. A solution of a mixture of diastereoisomeric sulfoxides *II* (0·45 g) in 50 ml of tetrahydrofuran and 5 ml of water was then added to the above aluminum amalgam. The mixture was stirred under reflux and nitrogen until the amalgam was dissolved (3 h). The solution was poured onto a column of anhydrous sodium sulfate (150 g) which was then washed with ether. All the steroidal product was eluted with 300 ml of the eluate. It was purified by thin-layer chromatography. Yield, 385 mg, $[\alpha]_D^{20}$ −9° (*c* 1·0). IR spectrum: 3 620, 1 053, 1 025 (OH), 1 720, 1 356 ($COCH_3$), 1 658, 3 080, 3 010 (C=C) cm^{-1}. ^1H NMR spectrum: 0·76 (s, 18-H), 0·92 (s, 19-H), 2·13 (s, 4-H), 3·65 (t, J = 8 Hz, 17-H), 5·22 (s, J = 10 Hz, 5-H), 5·62 (d., J = 10 Hz, 6-H) ppm. Mass spectrum, *m/z*: 290 (M^+). For $C_{19}H_{30}O_2$ (290·4) calculated: 78·57% C, 10·41% H; found: 78·20% C, 10·63% H.

17β-Hydroxy-4,5-secoandrostan-3-one (*V*)

100 mg of unsaturated ketone *IV* were hydrogenated in 5 ml of ethyl acetate using 5% palladium on charcoal as catalyst. The mixture was then purified by thin-layer chromatography on silica gel (50% of ether in toluene). The main product (70 mg) was identical with an authentic sample[16] (IR spectra).

Testing of Biological Activity

The activity screening was carried out identically as in the preceding paper[9], by simultaneous administration of testosterone propionate and the tested substance to castrated male mice. The males of the strain H (VELAZ Prague) weighing 35—40 g were fed with a standard laboratory diet and kept in an indirectly lighted room at 24 ± 1°C. The males were castrated 21 days before the beginning of the experiment and then kept in groups of 8 animals. The castrates were given either a vehicle (0·2 ml of olive oil) or testosterone propionate, or testosterone propionate in combination with the tested substance in 0·2 ml of olive oil. The injections were given subcutaneously every other day, over 3 weeks. Each animal was thus given 5 μmol of testosterone propionate, and those to which the combination was administered, got an additional 50 μmol of the tested substance. After the termination of the administration the animals were killed by ether anaesthesia, the seminal vesicles, kidneys, adrenals and spleen were removed and weighed (Table I). The weights of the organs were expressed in relative values (mg/100 g of body weight) and the results were evaluated statistically using Student's *t*-test.

Testing by Binding to Receptors for Androgens from Rat Prostate

The displacement of dihydrotestosterone (*X*, 17β-hydroxy-5α-androstan-3-one) from the bond to cytosol of the male rat prostates by the tested substance (Table II) was measured. The details are given in previous papers[9,15]. The prostate of male rats of 150—170 g body weight, castrated 24 h before killing, were homogenized and the cytosol fraction was obtained by ultracentrifuging

at 105 000 *g* for 1 h. Cytosol (0·75 ml, *i.e.* 14·9 mg of protein per one sample) was labelled with $1{\cdot}5 \cdot 10^{-3}$ mol/sample of [1,2,4,5,6,7-^{3}H]-17β-hydroxy-5α-androstan-3-one of spec. activity 4·8 TBq/mol (Radiochemical Centre, Amersham) at 0°C for 30 min. A solution (0·25 ml) of the tested competitor was added to the prelabelled cytosol, using 4 concentrations in the 10—640. 10^{-13} mol/ml range of a Tris-EDTA-glycerol buffer solution of pH 7·2. The samples were stirred and incubated at 0°C under shaking for 20 min. The separation of the free and the bound ligand was carried out by precipitation with ammonium sulfate (1 ml of 70% $(NH_4)_2SO_4$), as described elsewhere[15].

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