PREPARATION AND PHARMACOLOGICAL PROPERTIES OF 4,5-SECOPREGNANE-3,20-DIONE AND ITS DERIVATIVES*

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20β-Benzoyloxy-4-pregnen-3-one was oxidized to seco acid *VI* which was deoxygenated in the position 5 and converted to methyl ketone *XI*. This substance was oxidized directly to 4,5-seco-dihydroprogesterone and also converted to 17α-acetoxy derivative *XXI via* the corresponding monoketal *XV*. Compounds *XIV* and *XXI* do not display a gestagenic activity or an antiandrogenic activity either.

Recently we found[1] that 17β-hydroxy-4,5-secoandrostan-3-one[2] and its 5,6-cyclopropano analogues[3] *I–III* displayed antiandrogenic activity in assays *in vivo*, which could be useful in the therapy of androgen dependent diseases[4]. Since derivatives of progesterone or 17α-acetoxyprogesterone (for example *IV*, cyproterone acetate[4,5]) belong among the strongest antiandrogens used so far, we tried to modify the activity of our substances by introducing structural features of progesterone derivatives into them, *i.e.* to prepare substances *XIV* and *XXI*.

The starting substance was 20β-benzoyloxy-4-pregnen-3-one[6] (*V*) which was oxidized to the corresponding 3,5-seco acid *VI* with potassium permanganate and periodic acid. The methyl ester of this acid (*VII*) was converted to benzylthioenol ether *VIII* which was desulfurated with Raney-nickel in methanol. In the product, *IX*, a substance lacking a double bond in the position 5 could be detected by means of ^{1}H NMR spectroscopy. The conversion of the methoxycarbonyl group to the methyl keto group was carried out in two ways: *a*) aminolysis of ester *IX* in boiling pyrrolidine gave 20β-benzoyloxyamide *X* which on reaction with methyl lithium afforded 20β-hydroxy-4,5-secopregnan-3-one (*XI*); *b*) on acylation of the sodium salt of dimethyl sulfoxide with ester *IX* a mixture of sulfoxides *XII* and *XIII* was prepared, which were then desulfurated with aluminum amalgam to compound *XI*. For analysis the mixture was crystallized, giving thus pure (*R*)-sulfoxide *XII* with a characteristic[6] negative Cotton effect in the region of $n \rightarrow \pi$ transitions of the sulfoxide group.

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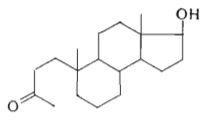
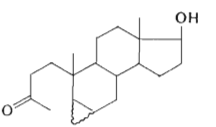
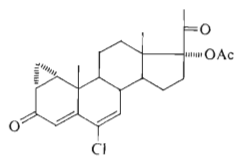
Compound *XI* represented a suitable intermediate for the preparation of compounds *XIV* and *XXI*. On oxidation, compound *XI* afforded 4,5-seco-pregnane--3,20-dione (*XIV*), a stereoselective introduction of an acetoxy group into position 17α required a preliminary protection of the 3-oxo group in compound *XI* in the form of dioxolane *XV*. Hydroxy ketal *XV* was oxidized and then treated with hydroxyl amine, under formation of oxime *XVII*. The reaction of the oxime with acetic an-



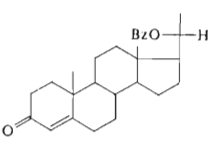
I



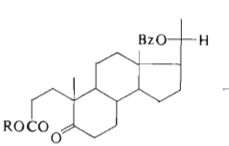
II, 5α, 6α-cyclopropano-
III, 5β, 6β-cyclopropano-



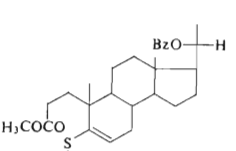
IV



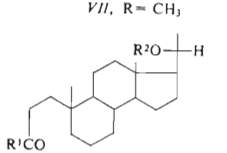
V



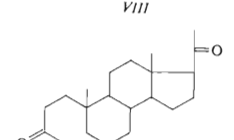
VI, R = H
VII, R = CH_3



VIII

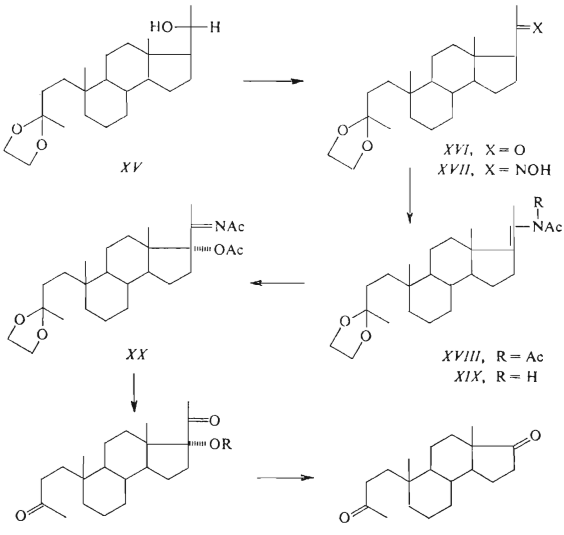


IX, $R^1 = OCH_3$, R^2 = Bz
X, R^1 = , R^2 = Bz
XI, $R^1 = CH_3$, R^2 = H
XII, R^1 = (R)—CH_3SOCH_2—, R^2 = H
XIII, R^1 = (S)—CH_3SOCH_2—, R^2 = H



XIV

hydride in pyridine gave N,N-diacetylimino derivative *XVIII*, without an attack of the dioxolane cycle taking place under the reaction conditions (Table I). Further steps of Barton's method[7], such as deacetylation on alumina to acetylamino derivative *XIX*, and the reaction with lead tetraacetate in benzene under formation of 17α-acetoxy-20-acetylimino derivative *XX*, also took place without an attack on the protecting ketal group. On acid hydrolysis of the imino bond the keto groups in the positions 3 and 20 were set free under formation of 17α-acetoxy-4,5-secopregnane-3,20-dione (*XXI*). The structure of this compound was confirmed after hydrolysis to 17α-hydroxy derivative *XXII* which was oxidized with chromium trioxide to the 4,5-secoandrostane-3,17-dione (*XXIII*) prepared earlier[2].



XV

XVI, X = O
XVII, X = NOH

XX

XVIII, R = Ac
XIX, R = H

XXI, R = Ac
XXII, R = H

XXIII

Bz = C_6H_5CO, Ac = CH_3CO

For the screening of the substances prepared the measurement of the binding of the steroids to receptors for androgens in rat prostate was used, and for progestins

the binding in rabbit uterus. Among the 4,5-secopregnane derivatives investigated, none displayed any more pronounced binding to progestin receptors in the cytosol of the rabbit uterus, which would warrant the expectation of a gestagenic effect *in vivo*. The analysis of the binding to androgen receptors in the cytosol of the rat prostate, which closely resembles the binding of testosterone or 17β-hydroxy-5α-androstan-3-one, showed in the case of active androgens or antiandrogens, that compound *XIV* competes distinctly with the binding of androgens in the receptor bond, while compound *XXI* did so less and compound *XI* only very little (Table II). In experiments with animals it was observed that none of the tested substances possessed an antigestagenic effect in the antifertility test. In bioassays on the syn- or antiandrogenic activity (Table III), measured by the change in the weight of the seminal vesicles, only the seco derivative *XIV* was significantly synandrogenic, while compounds *XI* and *XII* increased the weight of the seminal vesicles only negligibly. The administration of compound *XIV* also led to hyperplasia of adrenals. Hence,

TABLE I

Characteristic parameters of ^{1}H NMR spectra

Compound	4-H[a]	18-H[a]	19-H[a]	21-H[a]	Other signals
VI	—	0·77	1·10	1·31[b]	7·55[c], 8·08[c], 5·15[d]
VII	—	0·73	1·06	1·26[b]	5·13[d], 7·55[c], 8·08[c], 3·63[e]
VIII	—	0·66	1·01	1·26[b]	5·15[d], 3·65[e], 3·85[f], 5·58[g], 7·48[c], 8·08[c]
IX	—	0·65	0·81	1·25[b]	5·14[d], 3·64[e], 7·48[c], 8·08[c]
X	—	0·64	0·82	1·25[b]	5·06[d], 3·39[h], 7·50[c], 8·06[c]
XI	2·12	0·74	0·85	1·11[b]	3·70[d]
XII	3·77	0·74	0·86	1·12[b]	3·68[d], 2·65[i]
XIII	3·85	0·73	0·85	1·11[b]	3·67[d], 2·69[i]
XIV	2·10	0·59	0·85	2·15	—
XV	1·30	0·74	0·85	1·12	3·75[d], 3·90[j]
XVI	1·29	0·59	0·83	2·08	3·92[j]
XVII	1·30	0·61	0·84	1·87	3·91[j]
XVIII	1·28	0·84	0·87	1·87	2·29[k], 3·91[j]
XIX	1·31	0·87	0·87	1·98	2·36[k], 3·91[j]
XX	1·31	0·72	0·85	1·82	2·08[k], 2·13[k], 2·92[j]
XXI	2·09	0·60	0·84	2·14	2·00[k]
XXII	2·14	0·70	0·85	2·24	—
XXIII	2·15	0·86	0·89	—	—

[a] Singlet, unless stated otherwise; [b] doublet, $J = 6$ Hz; [c] multiplet of aromatic protons; [d] multiplet, $W_{1/2} = 19$ Hz (20-H); [e] singlet (OCH_3); [f] singlet (SCH_2); [g] multiplet, $W_{1/2} = 10$ Hz, (6-H); [h] doublet of doublets, $J = 5$ and 7 Hz, (pyrrolidine); [i] singlet, (SCH_3); [j] singlet, (dioxolane); [k] singlet, (acetyl).

the structural changes which led to the loosening of the rigidity and the planarity of the molecule did not contribute towards obtaining a substance of the required antiandrogenic and gestagenic biological activity.

EXPERIMENTAL

The melting points were measured on a Kofler block and they are not corrected. Optical rotations were measured in chloroform, the infrared spectra in tetrachloromethane. The ^{1}H NMR spectra

TABLE II

Binding to receptors for androgens in the cytosol of rat prostate

Compound	K_i nmol . l^{-1}	Relative binding[a] %
XIV	1·53	48·1
XXI	5·64	13·1
XI	21·20	3·5
17β-Hydroxy-5α-androstan-3-one	0·74	100·0

[a] 17β-Hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) 100%.

TABLE III

Testing of syn- or antiandrogenic activity on castrated male mice *in vivo*

Compound	Increase of body weight[a]	Relative weights of organs (mg/100 g body weight)[a]			
		seminal vesicles	adrenals	kidneys	spleen
Testosterone propionate (TP)	6·76 ± 1·38	416·2 ± 96·3	14·5 ± 3·0	1 590 ± 80	312·4 ± 45·4
Cyprosterone acetate ± TP	*0·04* ± 2·76	*254·8* ± 60·2	16·4 ± 3·8	1 598 ± 80	*236·6* ± 65·3
XIV ± TP	*4·59* ± 1·82	*569·8* ± 102·5	*22·0* ± 3·3	1 465 ± 140	*387·3* ± 58·8
XI ± TP	6·33 ± 2·59	504·3 ± 124·7	13·6 ± 3·4	1 544 ± 203	410·6 ± 108·1
XXI ± TP	5·78 ± 2·45	518·0 ± 114·5	17·1 ± 3·5	1 508 ± 210	357·7 ± 96·8

[a] Number of animals in the group; the values distinctly different (level of significance 5%) from the results in the group to which only TP was administered are printed in italics.

were measured in deuteriochloroform on a Tesla (60 MHz) apparatus, using tetramethylsilane as internal reference.

20β-Benzoyloxy-5-oxo-A-nor-3,5-secopregnan-3-oic Acid (*VI*)

An aqueous solution of potassium carbonate (3·5%, 160 ml), an aqueous solution of potassium permanganate (0·8%, 10 ml) and of potassium periodate (8%) were added to a solution of 14 g of 20β-benzoyloxy-4-pregnane-3-one in 600 ml of aqueous tert-butanol (90%). Further 400 ml of a sodium periodate solution were added over 1 h, under stirring. If necessary, a few drops of potassium permanganate were also added in order to keep a mild excess of this reagent. When the addition was terminated the mixture was stirred for another 2 h and then allowed to stand at 5°C for 20 h. The excess of the oxidant was eliminated by addition of an aqueous solution of sodium hydrogen sulfite and the main part of tert-butanol was eliminated by vacuum distillation on a rotatory evaporator. The mixture was cooled to 0°C and acidified with dilute sulfuric acid (5%). The product was extracted with ether, $[\alpha]_D^{20}$ +17° (*c* 1·1). IR spectrum (chloroform): 1 710 and 1 695 (infl.), 1 278 cm^{-1}. For $C_{27}H_{36}O_5$ (440·0) calculated: 73·60% C, 8·34% H; found: 73·36% C, 8·12% H.

Methyl 20β-benzoyloxy-5-oxo-A-nor-3,5-secopregnan-3-oate (*VII*)

A solution of 24 g of compound *VI* and 20 ml of concentrated hydrochloric acid in 375 ml of methanol was heated at 37°C for 22 h. The solution was concentrated *in vacuo* to 150 ml volume and diluted with ether. The ethereal solution was washed with an aqueous solution of potassium hydrogen carbonate and water, dried over anhydrous sodium sulfate and concentrated for crystallization from methanol, m.p. 144—145°C, $[\alpha]_D^{20}$ +18° (*c* 0·9). IR spectrum: 1 718, 1 275 (C_6H_5COO), 1 740, 1 435, 1 175 ($COOCH_3$) cm^{-1}. For $C_{28}H_{38}O_5$ (454·6) calculated: 73·98% C, 8·43% H; found: 73·78% C, 8·41% H.

Methyl 20β-benzoyloxy-5-benzylthio-A-nor-3,5-seco-5-pregnen-3-oate (*VIII*)

A solution of 25 g of ketone *VII* and 2 g of *p*-toluenesulfonic acid in 450 ml of benzene was refluxed and the water formed was bound with a molecular sieve in a Soxhlet extractor. After 44 g the solution was cooled, repeatedly washed with a 10% potassium hydroxide solution (totally 2 litres) and water, dried over sodium sulfate and concentrated in a vacuum. For analysis a sample (0·5 g) was chromatographed on silica gel (10% of ether in light petroleum), the pure compound (210 mg) was crystallized from methanol, m.p. 144—146°C, $[\alpha]_D^{20}$ −57° (*c* 1·5). IR spectrum (chloroform): 1 709, 1 278 (C_6H_5COO), 1 726, 1 439, 1 177 ($COCH_3$) cm^{-1}. For $C_{35}H_{44}O_4S$ (560·8) calculated: 74·96% C, 7·91% H, 5·72% S; found: 75·11% C, 8·04% H, 5·42% S.

Methyl 20β-Benzoyloxy-A-nor-3,5-secopregnan-3-oate (*IX*)

The crude product from the preceding reaction, corresponding to 24·8 g of substance *VII*, was stirred in boiling ethanol (300 ml) with an excess of Raney-nickel under nitrogen. After 5 h stirring the inorganic material was filtered off, washed with ethyl acetate and the filtrate concentrated in a vacuum. The residue was chromatographed on a silica gel column (250 g). The main product (14·8 g) was eluted with an ether–light petroleum mixture (1 : 10), m.p. of the product 136—138°C (methanol, at −20°C), $[\alpha]_D^{20}$ +35° (*c* 1·1). IR spectrum: 1 718, 1 275 (C_6H_5COO), 1 742, 1 438, 1 160 ($COOCH_3$) cm^{-1}. For $C_{28}H_{40}O_4$ (440·6) calculated: 76·32% C, 9·15% H; found: 76·16% C, 9·08% H.

N-(20β-Benzoyloxy-A-nor-3,5-secopregnan-3-oyl)pyrrolidine (*X*)

A solution of ester *IX* (5·3 g) in 50 ml of pyrrolidine was refluxed for 24 h under nitrogen and then evaporated in a vacuum. The residue was dissolved in benzene, washed with dilute hydrochloric acid and water, dried over sodium sulfate and concentrated. Crystallization from acetone gave 5·3 g of a compound with m.p. 187–189°C, $[\alpha]_D^{20}$ −8° (*c* 1·2). IR spectrum (chloroform): 1 625, 1 450 ($CONR_2$), 1 708, 1 278 (C_6H_5COO) cm^{-1}. For $C_{31}H_{45}NO_3$ (479·7) calculated: 77·72% C, 9·46% H, 2·92% N; found: 77·64% C, 9·73% H, 2·94% N.

20β-Hydroxy-4-methylthio-4,5-secopregnan-3-one S-oxide (*XII*)

A solution of the sodium salt of dimethyl sulfoxide was prepared according to ref.[3] from 0·5 g of sodium hydride and 15 ml dimethyl sulfoxide at 65°C. The reaction was carried out under argon and under stirring. A solution of 400 mg of ester *IX* in 12 ml of tetrahydrofuran was added to the above solution at 20°C and under stirring, which was continued for 3 h. Then the mixture was decomposed with solid ammonium chloride and partitioned between a saturated sodium chloride solution in water and ethyl acetate. The organic phase was washed with water till neutral, dried over sodium sulfate and concentrated in a vacuum. After 3 crystallizations from chloroform–ether mixture the ^1H NMR spectrum no longer displayed the presence of the minor component *XIII*. The m.p. of compound *XII* was 142–145°C (210 mg), $[\alpha]_D^{20}$ +11° (*c* 0·9). CD spectrum (methanol): $\Delta\varepsilon_{223} = -0{\cdot}63$. IR spectrum (chloroform): 3 620 (OH), 1 712 (C=O), 1 090, 1 080, 1 048, 1 033 (S–O and C–OH) cm^{-1}. For $C_{22}H_{38}O_3S$ (382·6) calculated; 69·06% C, 10·01% H, 8·38% S; found: 69·32% C, 9·99% H, 9·38% S.

20β-Hydroxy-4,5-secopregnan-3-one (*XI*)

a) 510 mg of crystalline sulfoxide *XII* were added to a suspension of freshly prepared aluminum amalgam (4 g of aluminum foil cut to strips etched for 30 s with 1% mercuric chloride solution, washed with water, ethanol and ether) in 60 ml of tetrahydrofuran and 10 ml of water. The mixture was stirred for 3 h at 65°C, then filtered through a column of sodium sulfate and the column washed with 300 ml of ether. The eluates were chromatographed on a silica gel column. With a mixture of 30% of ether in light petroleum 395 mg of compound *XI* were eluted, m.p. 56–59°C (heptane), $[\alpha]_D^{20}$ +5° (1·1). IR spectrum: 3 625 (OH), 1 720, 1 715, 1 356 ($COCH_3$) cm^{-1}. For $C_{21}H_{36}O_2.C_7H_{16}$ (420·7), calculated: 79·93% C, 12·46% H; found: 80·07% C, 11·93% H. The by-product, eluted with 40% of ether in light petroleum (45 mg) contains only hydroxy groups in the molecule (IR spectrum); on oxidation it is converted to 4,5-secopregnane-3,20-dione and therefore it is the product of subsequent reduction of compound *XI* (4,5-secopregnane-3ζ,20β-diol).

b) 4·9 g of amide *X* were added to a solution of methyllithium in ether (300 ml, 19 mg of methyllithium in 1 ml) and the mixture was refluxed under nitrogen. After 3 h the solution was poured carefully into a mixture of ice (400 g) and hydrochloric acid (100 ml), the product was extracted with ether, washed with an aqueous solution of potassium hydrogen carbonate and water, dried over sodium sulfate and concentrated. Chromatography of the residue in silica gel gave 1·7 g of *XI*.

4,5-Secopregnane-3,20-dione (*XIV*)

Hydroxy ketone *XI* (1·3 g) was oxidized according to Jones at 18°C for 10 min, the mixture was poured into an aqueous solution of potassium hydrogen carbonate, the product was extracted with ether, washed with water and dried. Crystallization of the product from methanol at −60°C afforded 0·43 g of dione *XIV*, m.p. 51–53°C and 93°C, $[\alpha]_D^{20}$ +29° (*c* 1·1). IR spectrum:

1 718, 1 709, 1 357 cm^{-1}. For $C_{21}H_{34}O_2$ (318·5) calculated: 79·19% C, 10·76% H; found: 79·11% C, 10·93% H.

3,3-Ethylenedioxy-4,5-secopregnan-20β-ol (*XV*)

A solution of 3·6 g of ketone and 70 mg of *p*-toluenesulfonic acid *XI* in 75 ml of benzene and 13 ml of ethylene glycol was refluxed under a Dean Stark separator for 8 h, the mixture was cooled, washed with an aqueous potassium hydroxide solution (10%, 20 ml) and water, dried by filtration through a sodium sulfate layer and concentrated in a vacuum. M.p. 105–107°C (toluene in heptane), $[\alpha]_D^{20}$ +8° (*c* 1·2). IR spectrum: 3 630 (OH), 1 100, 1 057, 857 (C—O) cm^{-1}. For $C_{23}H_{40}O_3$ (364·5) calculated: 75·77% C, 11·06% H; found: 75·80% C, 11·19% H.

3,3-Ethylenedioxy-4,5-secopregnan-20-one (*XVI*)

Chromium trioxide (2·3 g) was added in several portions into 20 ml of pyridine stirred at 0°C, followed after 10 min by a solution of hydroxy derivative *XV* (2 ml) in 5 ml of pyridine. The mixture was stirred for 1 h at 0°C and then 20 h at 20°C; after dilution with 200 ml of ether the mixture was filtered into 200 ml of aqueous sodium carbonate, shaken and the organic phase separated. The aqueous phase was extracted with a further portion of ether, the combined extracts were washed with three 25 ml portions of water, dried and concentrated. M.p. 77–78°C (light petroleum), $[\alpha]_D^{20}$ +89° (*c* 0·9). IR spectrum: 1 710, 1 356 ($COCH_3$), 1 255, 1 221, 1 058 (C—O) cm^{-1}. For $C_{23}H_{38}O_3$ (362·5) calculated: 76·10% C, 10·56% H; found: 76·31% C, 10·27% H.

20-Oximino-3,3-ethylenedioxy-4,5-secopregnane (*XVII*)

A mixture of 1·5 g of ketone *XVI* and 3 g of hydroxylamine hydrochloride was stirred with 20 ml of pyridine at room temperature for 1 h and the solution was allowed to stand for another 48 h. The product was precipitated by pouring it into and aqueous solution of potassium hydrogen carbonate and then extracted with ethyl acetate. The extract was washed with water, dried over sodium sulfate and concentrated. M.p. 134–136°C (light petroleum). IR spectrum: 3 610, 1 660 (NOH), 1 058, 1 048, 948, 861 (C—O) cm^{-1}. For $C_{23}H_{39}NO_3$ (377·6) calculated: 73·16% C, 10·41% H, 3·72% N; found: 72·20% C, 10·59% H, 3·42% N.

20-Diacetylamino-3,3-ethylenedioxy-4,5-seco-17(20)-pregnene (*XVIII*)

A solution of 2 g of oxime *XVII* in 120 ml of pyridine and 80 ml of acetic anhydride was refluxed under argon. After 48 h the mixture was cooled, the volatile components were evaporated in a vacuum, ether (600 ml) and an aqueous solution of potasium hydrogen carbonate (300 ml) were added to the residue and the mixture was shaken for 30 min. The emulsion was filtered through diatomaceous earth, the organic phase was separated, dried and concentrated for chromatography on silica gel (100 g). Using 20% of ether in toluene compound *XVIII* (1·9 g) was eluted, $R_F = 0{\cdot}45$ (on silica gel thin layer in 25% of ether in benzene: the starting oxime has $R_F = 0{\cdot}53$ in this system); $[\alpha]_D^{20}$ +14° (*c* 0·9). IR spectrum: 1 705 (CH_3COO), 1 369, 1 280, 1 243, 1 055, 1 043 (C—O) cm^{-1}. For $C_{27}H_{43}NO_4$ (445·6) calculated: 72·77% C, 9·73% H, 3·14% N; found: 72·30% C, 9·41% H, 3·19% N.

20-Acetylamino-3,3-ethylenedioxy-4,5-seco-17(20)-pregnene (*XIX*)

A solution of diacetylamino derivative *XVIII* (1·5 g) in 10 ml of toluene was put on a column of neutral alumina (act. II, 50 ml) and washed with another 10 ml of toluene. The column was

allowed to stand for 1 h and then eluted with 600 ml of ether. Yield of compound *XIX*, 1·35 g, $R_F = 0{\cdot}20$ (50% of ether in benzene); $[\alpha]_D^{20} + 19°$ (*c* 1·2). IR spectrum: 3 440 (NH), 1 697, 1 681 (inflexion), 1 480 (CONH), 1 375, 1 279, 1 244, 1 056, 1 057 (C—O) cm^{-1}. For $C_{25}H_{41}NO_3$ (403·6) calculated: 74·40% C, 10·23% H, 3·47% N; found: 74·56% C, 10·01% H, 3·40% N.

17α-Acetoxy-20-acetylimino-3,3-ethylenedioxy-4,5-secopregnane (*XX*)

From a solution of 1·2 g of compound *XIX* in 400 ml of benzene 100 ml of azeotropic mixture were distilled off. Lead tetraacetate (6 g) was then added to the residual solution. Lead tetraacetate had to be dried in vacuum at 55°C beforehand. The mixture was stirred at room temperature for 3 h, water was added (100 ml) and the mixture shaken. The emulsion was filtered through celite, the organic phase was washed with potassium hydrogen carbonate and dried over sodium sulfate. The residue was purified chromatographically on an alumina column (act. II), using ether for elution, which gave 840 mg of acetoxy derivative *XX*, $R_F = 0{\cdot}35$ (silica gel thin layer, 25% of ether in benzene), $[\alpha]_D^{20} - 19°$ (*c* 1·0). IR spectrum: 1 701, 1 680 (C=NCHOCH$_3$), 1 735, 1 247, 1 050 (OCOCH$_3$) cm^{-1}. For $C_{27}H_{43}NO_5$ (461·6) calculated: 70·25% C, 9·39% H; found: 69·81% C, 9·06% H.

17α-Acetoxy-4,5-secopregnane-3,20-dione (*XXI*)

Compound *XX* (0·5 g) was heated at 100°C under nitrogen in 100 ml of aqueous acetic acid (95%), the solvent was evaporated in a vacuum and the product was partitioned between toluene and an aqueous potassium hydrogen carbonate solution. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residue was put on a silica gel column and eluted with 20% of ethyl acetate in light petroleum, giving 250 mg of product *XXI*, m.p. 117—119°C (heptane), $[\alpha]_D^{20} + 19°$ (*c* 1·2). IR spectrum: 1 738, 1 248 (CH$_3$CO) cm^{-1}. For $C_{23}H_{36}O_4$ (376·5) calculated: 73·36% C, 9·64% H; found: 73·12% C, 9·51% H.

17α-Hydroxy-4,5-secopregnane-3,20-dione (*XXII*)

The polar fractions from the preceding chromatography (48 mg) were crystallized from a mixture of acetone and heptane, m.p. 115—117°C, $[\alpha]_D^{20} - 12°$. IR spectrum: 3 625 (OH), 1 720, 1 715, 1 356 (CH$_3$CO) cm^{-1}. Mass spectrum: 334 (M^+), 263 (M — residue of the A-ring), 316 (M—H_2O). For $C_{21}H_{34}O_3$ (334·5) calculated: 75·40% C, 10·25% H; found: 75·24% C, 10·15% H.

4,5-Secoandrostane-3,17-dione (*XXIII*)

A solution of 2 mg of hydroxy derivative *XXII* in 5 ml of acetone was mixed at room temperature with a few drops of Jones reagent. After 3 h standing the mixture was worked up. Chromatography of the product indicates that the oxidation gave diketone *XXIII*, the mass spectrum of the product was identical with that of an authentic specimen[2].

Binding to Receptors

Binding to the cytosol of rat prostate: As a screening method for the determination of synandrogenic or antiandrogenic activity the expelling of 17β-hydroxy-5α-androstan-3-one from the link to cytosol of rat prostate was tested. The prostates of rats (150—165 g body weight) castrated 24 h before killing were homogenized and the cytosol fraction was obtained by ultracentrifugation. The cytosol (0·75 ml) was labelled with $1{\cdot}5 \cdot 10^{-13}$ mol of [1,2,4,5,6,7-^{3}H]-17β-hydroxy-5α-androstan-3-one of 4·8 TBq $mmol^{-1}$ specific activity at 0°C for 1 h. A solution (0·25 ml)

of the tested competitors was then added to the cytosol (in 4 concentrations ranging from 10 to $640 . 10^{-10}$ mol) in Tris-EDTA-glycerol buffer. The samples were stirred and incubated at 0°C under shaking for 200 min. The separation of the free and the bound ligand was carried out by precipitation with ammonium sulfate[1,9]. The results are given in Table II.

Binding to cytosol of rabbit uterus: As a screening method for the determination of gestagenic activity the expelling of progesterone from the link to cytosol from the uteri of pregnant rabbit females[10] was used. Among the substances tested none displayed a sufficiently strong binding and therefore the testing on animals was restricted to the antifertility test alone. Only a weak linking of *XIV* was measurable, corresponding to less than 0·5% of the binding of progesterone.

Testing of Synandrogenic and Antiandrogenic Activity on Mice

The testing was carried out as in preceding studies[1,9], by simultaneous administration of testosterone propionate and the tested substance to castrated male mice. The male mice 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 ± 2 °C. The males were castrated 21 days before the beginning of the experiment, and kept in groups of 7. The castrates were administered either a vehicle (0·2 ml of olive oil) or testosterone propionate or testosterone propionate in combination with the tested substance. The dose in 0·2 ml of olive oil was administered subcutaneously over three weeks, every other day. Each animal obtained thus 1·7 mg (5·0 μmol) of testosterone propionate, and those to which the combination was administered also obtained 50 μmol of the tested substance. After the termination of the experiment the mice were killed with ether anaesthesia, weighed, and the seminal vesicles, kidneys, adrenals and spleens extracted. The weights of the organs were expressed in relative values (mg/100 g of body weight) and the results (Table III) were evaluated using the statistical Student's t-test.

Antifertility Test

The substances were dissolved in ethanol and added to three volumes of an aqueous medium containing sodium salt of carboxymethylcellulose (0·25 w/v) and Tween 80 (1% w/v) shortly before the *s.c.* administration. The control animals got only a vehicle.

Pregnant females of 150 g body weight were given 8·2, 2·0 and 20 mg of the tested substance on the 1st and the 8th day of pregnancy. The autopsy of the animals was done on the 15th day. In the case of all the substances tested a normal number of living embryos was found in the uterus.

Ovulation was induced in adult rabbit females (New Zealand White strain) by administration of 25 intern. units of hCG. Three animals got 30 mg of 3,5-secopregnan-3,20-dione in four consecutive days, beginning with the day of the injection of hCG. Three other animals obtained only the vehicle. The days after the last injection the animals were killed and the uterus taken out and fixed. Histological sections were then prepared. All the sections displayed a maximum endometrial proliferation, with a score 4 according to McPhail. No signs of antigestagenic activity could be observed in any of the substances.

EEFEREXNES

1. Stárka L., Hampl R., Kasal A., Kohout L.: J. Steroid Biochem. *17*, 331 (1982).
2. Kasal A.: This Journal *45*, 2541 (1980).
3. Kasal A., Kohout L.: This Journal *47*, 130 (1982).
4. Neumann F.: *Androgens and Antiandrogens*, p. 235. Springer, Berlin 1974.

5. Schering A. G.: Belg. 616 989 (1962); Chem. Abstr. *59*, 722 h (1963).
6. Turner R. B., Voitle D. M.: J. Amer. Chem. Soc. *73*, 2283 (1951).
7. Bartlett P. A.: J. Amer. Chem. Soc. *98*, 3305 (1976).
8. Boar R. B., Mc Ghie J. F., Robinson M., Barton D. H. R.: J. Chem. Soc., Perkin Trans. 1, *1975*, 1242.
9. Stárka L., Hampl R., Bičíková M., Černý V., Fajkoš J., Kasal A., Kočovský P., Kohout L., Velgová H.: J. Steroid Biochem. *13*, 455 (1980).
10. Philibert D., Ojasoo T., Raynaud J. P.: Endocrinology *101*, 1850 (1977).

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