SYNTHESIS OF POTENTIAL ANTIPROGESTINS II[1]

by

Bernardo Beyer[2], Lars Terenius, and

Raymond E. Counsell*

Laboratory of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, Michigan, 48109, U.S.A., and Department of Medicinal Pharmacology, University of Uppsala, Uppsala, Sweden.

Received 5-10-79

ABSTRACT

Alkylated derivatives of 17-acetoxyprogesterone were prepared in order to test the hypothesis that bulky groups in certain positions of the steroid molecule have the effect of transforming progestogens into antiprogestogens. These groups might exert binding influence outside the area occupied by progesterone itself. The compounds were tested for competitive affinity against tritiated progesterone and receptor from rabbit uterus cytosol. The low affinity of all derivatives makes it unlikely that they would be active as antiprogestational agents.

A large body of accumulating evidence tends to support a unitary theory for the mechanism of action of steroid hormones. According to this theory, the steroids elicit a sequence of events starting with the uptake of the steroid molecule into the target cell where the steroid binds to a specific cytoplasmic "receptor" protein. This receptor protein-steroid complex is then translocated to the nucleus where it binds to specific sites on the genome, thus inducing the transcription for a new, specific RNA. This RNA in turn, results in the ribosomal synthesis of new protein, which becomes the physiological expression for the specific steroid hormone.

As an approach to fertility control we considered the synthesis of agents that would compete with the normal hormones at their sites of action. An antiprogestational agent might compete with progesterone on the progestogen-receptor proteins in target organs such as the uterus and the oviduct.

*To whom inquiries should be addressed.

A recent review, by workers at the World Health Organization, states that "manipulation of receptor function offers great promise for the development of new contraceptive agents" (3). Attempts have been made to prepare potential alkylating agents of the receptor protein (4-7). Solo and Gardner (4,5) prepared diazoketone derivatives of 17-hydroxyprogesterone and concluded that no alkylation of the "Clauberg receptor" had taken place.

Initial efforts in this laboratory to synthesize antiprogestogens involved the addition of a dialkyaminoalkyl moiety to certain acylated derivatives of 17-hydroxyprogesterone (8). Other esters at the 17-position were also synthesized. All the derivatives had low affinity for the progestogen receptor protein, which makes it unlikely that they would be active as antiprogestational agents.

Our subsequent efforts were to synthesize antiprogestogens by the introduction of bulky groups at positions 1- and 7- of the progesterone molecule. It is known from structure-activity studies that it is possible to have full progestational activity in 1α- and 7α- substituted progestins (9). Some of these derivatives may exert binding influence outside the area occupied by progesterone itself. Incorporation of various functional groups in the 1α- and 7α- positions might have the potential of reinforcing such binding to sites outside the progesterone binding site, and in this way, produce receptor blockade. Other work from this laboratory (10) has shown that the introduction of substituents at the C-1 or C-7 positions of androstenedione led to agents exhibiting high activity as inhibitors of estrogen biosynthesis.

Scheme 1 shows the reactions used to prepared the desired products. Table 1 shows the physical and biological data for these compounds. The

dehydrogenation of 17-acetoxyprogesterone (I) to the $\Delta^{1,4}$-diene system was carried out with dichlorodicyanoquinone (DDQ) in dioxane by a procedure previously used for the 6-acetoxy analog (11). The dehydrogenation to the $\Delta^{4,6}$-diene system (IV) was performed with tetrachloroquinone (chloranil) as previously described in the patent literature (12). The addition of the mercaptans was carried out as previously described (10) using the mercaptans as solvents (a,b,e,f) whenever feasible (see Scheme I). For the others (c,d), dioxane was used as the solvent. In all cases the reactions were run at 50° for 48 hrs. usually under a nitrogen atmosphere. One of the mercaptans (p-(N,N-diethylamino)-thiophenol) had to be synthesized as previously reported (13). Addition at C-7 proceeded faster and in higher yields than at C-1. This was expected since the 7- position was known to be less hindered (14). The assignment of α- configuration to the 1- and 7- substituents is in accordance with the stereochemistry of other products obtained by similar reaction mechanisms (15,16).

The relative affinities shown in Table I clearly indicate that the introduction of bulky substituents at the 1- and 7- positions decrease binding affinity markedly. The only compound with relatively high affinity (Ve) has a butyl side chain instead of the bulky phenyl rings (Va, b, c, d) or more polar ester moiety (Vf). Substituents at the 1-position (IIIa, b) decrease affinity by approximately an order of magnitude more than substituents at the 7- position (Va, b). At the 7-position, introduction of a dialkylamino moiety on the phenyl ring (Vc) decreases affinity by an order of magnitude over the corresponding compound with an unsubstituted phenyl ring (Va).

The structure-activity studies at the 7- position are in agreement

Scheme I

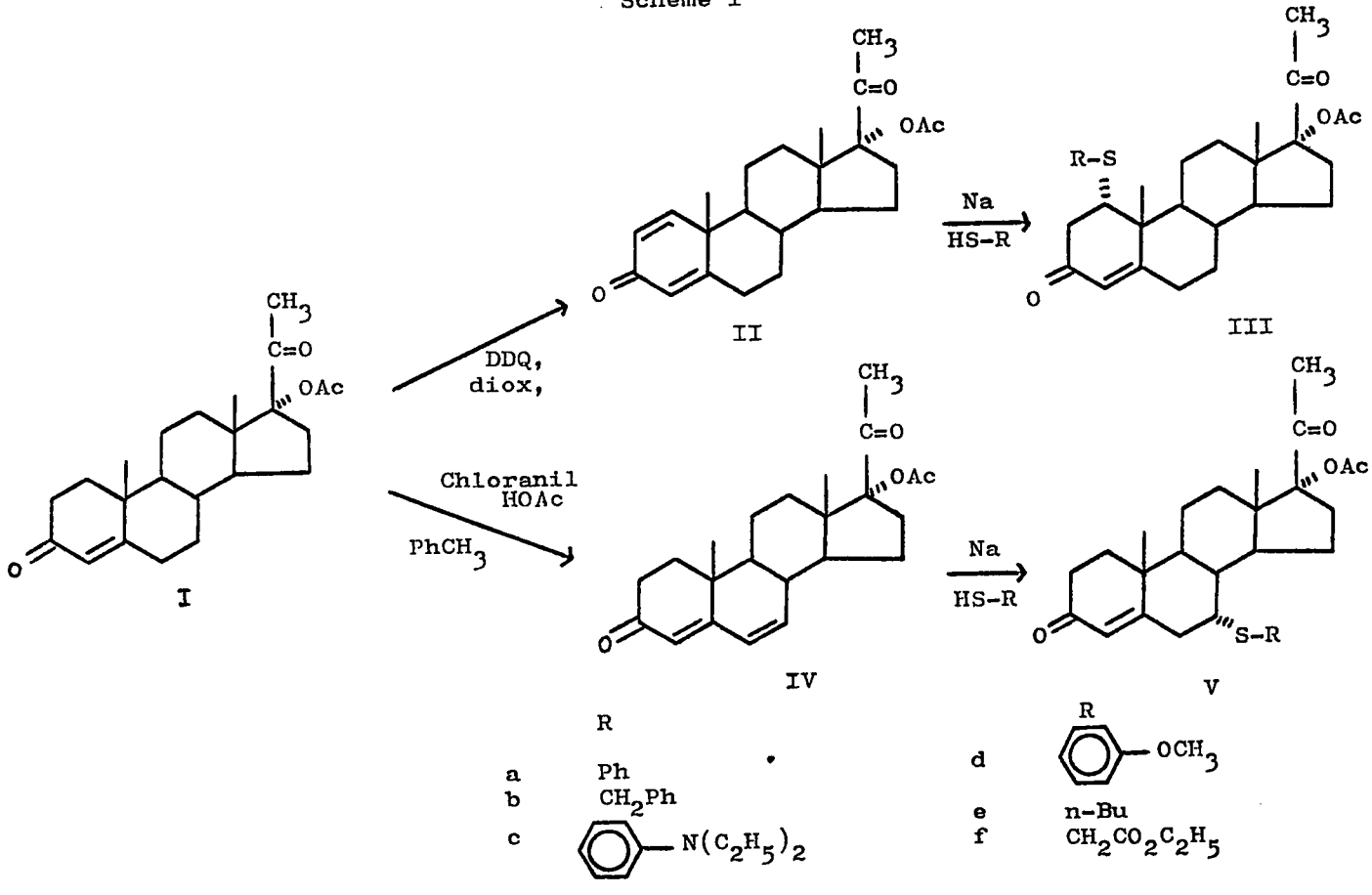
CH3
C=O
OAc
O
I
DDQ,
diox,
Chloranil
HOAc
PhCH3
II
IV
Na
HS-R
R-S
III
S-R
V
R
a Ph
b CH2Ph
c N(C2H5)2
d OCH3
e n-Bu
f CH2CO2C2H5

Table 1.
Physical and Biological Data on 1α- and 7α-
Substituted Derivatives of 17-Acetoxyprogesterone

Cpd.	Method	Yield(%)	M.P.	Solvent Recrystallization	Molecular Formula	Elemental Analysis Calc.	Elemental Analysis Found	R.A.[a]
I			243-44° [b]	-	$C_{23}H_{32}O_4$	-		0.40
IIIa	(A)	77	209-11°	Acetone/Hexane	$C_{29}H_{36}O_4S$	C: 72.47 H: 7.55	C: 72.54 H: 7.71	<0.002
IIIb	(A)	84	236-38°	Acetone	$C_{30}H_{38}O_4S$	C: 72.84 H: 7.74	C: 72.67 H: 7.84	0.003
Va	(A)	92	213-15°	Acetone	$C_{29}H_{36}O_4S$	C: 72.47 H: 7.55	C: 72.23 H: 7.77	0.016
Vb	(A)	81	206-08°	Acetone	$C_{30}H_{38}O_4S$	C: 72.84 H: 7.74	C: 72.60 H: 7.87	0.04
Vc	(B)	42	134-37°	Acetone/Hexane	$C_{33}H_{45}NO_4S$	C: 71.83 H: 8.22	C: 71.98 H: 7.97	0.0015
Vd	(B)	70	216-18°	Acetone	$C_{30}H_{38}O_5S$	C: 70.56 H: 7.50	C: 70.28 H: 7.60	0.002
Ve	(A)	69	148-51°	Acetone/Hexane	$C_{27}H_{40}O_4S$	C: 70.40 H: 8.75	C: 70.68 H: 8.60	0.10
Vf	(A)	62	150-52°	Acetone/Hexane	$C_{27}H_{38}O_6S$	C: 66.09 H: 7.81	C: 66.35 H: 7.84	0.0018

a. R.A. = relative receptor affinity (progesterone = 1; IC50 values for progesterone ranged from 1.5 to 3.5 x 10^{-9} M).

b. Purchased from Steraloids Inc., Pawling, N.Y.

with the results obtained by Lee et al. by QSAR studies (17). These showed the absence of a hydrophobic pocket at the 7- position of the progesterone molecule when binding to a progestogen receptor.

EXPERIMENTAL SECTION

The procedures given in this section are representative for each of the analogous compounds presented in Table I. The melting points which are corrected, were taken on a Thomas-Hoover (capillary tube) apparatus. Analyses were performed by Midwest Microlab, Ltd., Indianapolis, Indiana. Uv spectra were obtained with a Beckman DK-2A spectrophotometer. Optical rotations were obtained with a Perkin Elmer 141 polarimeter. Ir spectra were obtained with a Perkin Elmer 337 spectrophotometer. Nmr spectra were obtained with a A-60A spectrometer (Me_4Si or DSS). Spectra were consistent with structures shown in Table I

17-Acetoxypregna-1,4-diene-3,20-dione (II). A solution of 17-acetoxypregn-4-ene-3,20-dione (I) (1 g, 2.68 mmol) and 2,3-dichloro-5,6-dicyanobenzoquinone (0.92 g, 4.05 mmol) in dioxane (40 ml) was refluxed for 16 hr and then cooled. The precipitated hydroquinone was filtered and the solvent from the filtrate was removed under reduced pressure. The resultant dark solid was chromatographed over activated magnesium silicate. Elution with benzene-$CHC\ell_3$ afforded a white solid (0.61 g, 61%): mp 222-24° (lit[11] 233-35°).

17-Acetoxypregna-4,6-diene-3,20-dione (IV). A solution of 17-acetoxypregn-4-ene-3,20-dione (I) (10 g, 26.8 mmol) and tetrachloroquinone (6.9 g, 28.1 mmol) in glacial HOAc (80 ml) and toluene (20 ml) was refluxed for 30 min and then cooled. The precipitated hydroquinone was filtered and the solvent from the filtrate was removed under reduced pressure. Recrystallization from acetone afforded pure product: mp 223-28° (lit[12] 224-28°).

General methods for Michael additions to $\Delta^{1,4}$- and $\Delta^{4,6}$-ketopregnanes.

Method A. 17-Acetoxy-1α-phenylthiopregn-4-ene-3,20-dione (IIIa). To a solution of II (0.5 g, 1.35 mmol) in thiophenol (6 ml) was added Na metal (25 mg, 1.08 mmol). The mixture was stirred at 50° for 46 hr under N_2 and poured into ice-H_2O, acidified with dil HCℓ and extracted with $CHC\ell_3$. The organic layer was washed with H_2O and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford an oily residu which was chromatographed over silica gel. Elution with hexane-$CHC\ell_3$ 2:3 afforded a white solid. Recrystallization from acetone afforded pure product (0.50 g, 77%): mp 209-11°; $[\alpha]_D$ + 10.2° (c 0.5, MeOH); uv λ_{max} (EtOH) 243 nm (ε 15,500), 216 (16,300); ir 1735, 1710, 1670, 1610 cm^{-1}; pmr δ 0.68 (s, 3H, C_{18}-H's), 1.20 (s, C_{19}-H's), 2.06 (s, 3H, $COOCH_3$), 2.15 (s, 3H, C_{21}-H's), 3.56 (m, $C_{1\alpha}$-H), 5.77 (s, 1H, C_4-H), 7.00-7.73 (m, 5H, aromatic).
Anal. Calcd for $C_{29}H_{36}O_4S$: C, 72.47; H, 7.55. Found: C, 72.54; H, 7.71.

Method B. 17-Acetoxy-7α-(p-methoxyphenylthio)pregn-4-ene-3,20-dione (Vd). To a solution of IV (0.5 g, 1.35 mmol) in dioxane (5 ml) was added p-methoxybenzenethiol (2 g, 14.2 mmol) and Na metal (25 mg, 1.08 mmol). The mixture was stirred at 50° for 5 days and worked up as described for IIIa. Chromatography over silica gel and recrystallization from acetone afforded a white solid (0.48 g, 69%): mp 216-18°; $[\alpha]_D$ - 85.8° (c 0.5, MeOH); uv λ_{max} (EtOH) 231 (ε 22,000); ir 1735, 1710, 1670, 1590 cm^{-1}: pmr δ 0.70 (s, 3H, C_{18}-H's), 1.23 (s, 3H, C_{19}-H's), 2.11 (s, 3H, $COOCH_3$), 2.20 (s, 3H, C_{21}-H's), 3.43 (m, 1H, $C_{7\alpha}$-H), 3.90 (s, 3H, OCH_3), 5.88 (s, 1H, C_4-H), 6.77-7.78 (m, 4H, aromatic).
Anal. Calcd for $C_{30}H_{38}O_5S$: C, 70.56; H, 7.60. Found: C, 70.28; H, 7.60.

Competitive binding assay for progestogen-receptor affinity. Each substance was tested for competitive affinity against tritium-labelled progesterone-^{3}H (57 Ci/mmol, New England Nuclear, Boston, Mass.) and receptor from rabbit uterus cytosol (18). A series of test tubes were prepared with 0.5 ml buffer (10% glycerol in 0.01 M Tris, pH 7.4, with 0.015 M EDTA and 0.25 M sucrose). The appropriate amounts of unlabelled competitors and labelled progesterone (3×10^{-10} M) were dissolved in this buffer and 25 µl of uterine cytosol was added. The tubes were incubated for 16-18 hours at +4° C. ^{3}H-Progesterone binding was measured by absorption. Every substance was run in at least 4 different concentrations in 3-fold dilution steps. A standard curve with unlabelled progesterone, concentrations between 10^{-10}-10^{-8} M, was run at each experimental occasion. All substances were assayed at least twice. The percentage inhibition was plotted against log concentration of competitor and the point of 50% competition was used for potency comparison. In Table 1, the competitive potencies on a molar basis are given relative to progesterone (= 1).

ACKNOWLEDGEMENTS

This work was supported by the World Health Organization. B. Beyer was a recipient of a fellowship from the Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

REFERENCES

1. Taken in part from thesis research performed by B. Beyer in partial fulfillment of the requirements for the Doctor of Philosophy degree, University of Michigan, 1976.
2. Universidad Simon Bolivar, Departamento de Quimica, Apartado 80659, Caracas, Venezuela.
3. Aitken, R. J., and Harper, M. J. K., CONTRACEPTION 16, 227 (1977).
4. Solo, A. J., and Gardner, J. O., J. PHARM. SCI. 60, 1089 (1971).
5. Solo, A. J., and Gardner, J. O., STEROIDS 11, 37 (1968).
6. Clark, S. W., Sweet, F., and Warren, J. C., AM. J. OBSTET. GYNECOL. 121, 864 (1975).
7. Clark, S. W., Sweet, F., and Warren, J. C., BIOL. REPROD. 11, 519 (1974).

8. Beyer, B., Terenius, L., Brueggemeier, R. W., Ranade, V. V., and Counsell, R. E., STEROIDS 27, 123 (1976).
9. Junkmann, K., ARCH. EXP. PATHOL. PHARMACOL. 223, 244 (1954).
10. Brueggemeier, R. W., Floyd, E. E., and Counsell, R. E., J. MED. CHEM. 21, 1007 (1978).
11. Dusza, J. P., Joseph, J. P., and Bernstein, S., J. ORG. CHEM. 28, 92 (1963).
12. E. German Patent 41,938; C. A. 64, 14245g (1966).
13. Benn, M. H., Owen, L. N., and (in part) Creighton, A. M., J. CHEM. SOC. 2800 (1958).
14. Glen, A. T., and McLean, J., J. CHEM. SOC. 661 (1966).
15. Dodson, R. M., and Tweit, R. C., J. AMER. CHEM. SOC. 81, 1224 (1959).
16. Weinstein, A., Lindner, H. R., Friedlander, A., and Bauminger, S., STEROIDS 20, 789 (1972).
17. Lee, D. L., Kollman, P. A., Marsh, F. J., and Wolff, M. E., J. MED. CHEM. 20, 1139 (1977).
18. Terenius, L., STEROIDS 23, 909 (1974).