SYNTHESIS OF POTENTIAL ANTIPROGESTOGENS

by

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ABSTRACT

Acylated derivatives of 17α-hydroxyprogesterone were prepared in order to test the hypothesis that dialkylamino alkyl moieties have the effect of transforming progestogens into antiprogestogens. This approach has been successful with certain estrogens. Compounds with other functional groups were synthesized to determine whether these 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.

As an approach to fertility control, there is considerable interest in an agent that will antagonize the hormonal actions of progesterone. Progesterone is essential for the induction of implantation and for the maintenance of pregnancy. Conversely, interruption of this action would cause pregnancy to terminate.

To date, a true antiprogestogen has not been discovered. Such an agent might compete with progesterone for the binding sites on progestogen-receptor proteins in target organs such as the uterus and the oviduct. The progestogen-receptor protein has a key function in progesterone action and receptor blockade would prevent this action.

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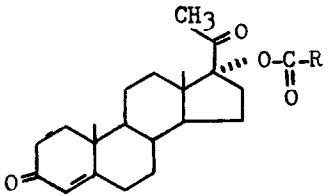
Attempts have been made to obtain derivatives of 17α-hydroprogesterone (17α-hydroxypregn-4-ene-3,20-dione) which covalently bind to the receptor protein (1,2). These contained a very reactive diazoketone moiety. All four diazoketones synthesized in this series failed to alkylate the receptor protein.

Initial efforts in this laboratory to synthesize antiprogestogens have employed the approach which successfully transformed estrogens into antiestrogens. Thus, the addition of a dialkylamino alkyl moiety to certain nonsteroidal estrogens provided antiestrogens such as clomiphene citrate. Such antiestrogens compete with estradiol for estrogen-receptor protein binding sites (3,4,5). The substituted amino moiety may bind to an anionic group inside or outside the site occupied by estradiol and thereby block interaction of the natural hormone with the receptor protein. It is known from structure-activity studies that it is possible to have full progestational activity in 17α-acyloxyprogesterone derivatives (6). Some of these derivatives may exert binding influence outside the area occupied by progesterone itself. Incorporation of various functional groups in the 17α-acyl position might have the potential of reinforcing such binding to sites outside the progesterone binding site, and in analogy with antiestrogens, produce receptor blockade.

Table 1 lists the compounds synthesized in this series along with the relative binding affinities (RA) to progestogen-receptor protein of rabbit uterus. The esters were made either by treatment of 17α-hydroxyprogesterone with the trifluoroacetic mixed anhydride (Method A) or with the appropriate anhydride in the presence of TsOH (Method B) (7). The dialkyl aminoacyloxy derivatives were obtained by treatment

Table 1. Physical and Biological Data on Esters of 17α-Hydroxyprogesterone



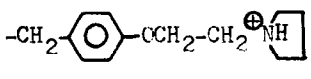
Compound No.	R	Method	Yield %	M.P.	Solvent of Recrystn.	Molecular Formula	Elemental Analysis	R.A.[a]
I	$-CH_3$			243-244°[b]		$C_{23}H_{32}O_4$		0.40
II	$-CF_3$	A	23	190.5-191°[c]	Acetone/H_2O	$C_{23}H_{29}F_3O_4$	C, H	< 0.005
III		A	5	211-213°	Ethyl Acetate	$C_{28}H_{34}O_4$	C, H	< 0.01
IVa	$-CH_2-C_6H_5$	B	32	202-203°[d]	Ethyl Acetate/ cyclohexanone	$C_{29}H_{36}O_4$		0.10
IVb	$-CH_2-C_6H_4-I$	A	37	194-196°	Ethyl Acetate	$C_{29}H_{35}IO_4$	C, H	0.04
V	$-CH_2-C_6H_4-OCH_2CH_2-Br$	A	62	148-150°	Ethanol	$C_{31}H_{39}BrO_5 \cdot 0.5\ H_2O$	C, H	0.15
VI	$-CH_2-C_6H_4-OCH_2-CH_2-\overset{\oplus}{N}H$ 	C	78	200-202°	Pet. ether/ ethanol	$C_{35}H_{48}ClNO_5$	C, H	< 0.01

Table 1, Contd.

Compound No.	R	Method	Yield %	M.P.	Solvent of Recrystn.	Molecular Formula	Elemental Analysis	R.A.
VII	$-CH_2-CH_2-CH_2-Cl$	B	46	134-136°	Acetone/H_2O	$C_{25}H_{35}ClO_4$	C, H	0.18
VIII	$-(CH_2)_4Br$	B	52	140-142°	Acetone/H_2O	$C_{26}H_{37}BrO_4$	C, H	0.35
IX	$-(CH_2)_5Br$	B	54	112-114°	Acetone/H_2O	$C_{27}H_{39}BrO_4$	C, H	0.47
X	$-(CH_2)_{10}Br$	B	58	48-50°	Acetone/H_2O	$C_{32}H_{49}BrO_4$	C, H	0.65
XI	$-(CH_2)_4{}^+NH$ Cl^-	D	82	164-167°[e]		$C_{30}H_{48}ClNO_4$	C, H	0.01
XII	$-(CH_2)_5{}^+NH$ Cl^-	D	80	169-171°[e]		$C_{31}H_{50}ClNO_4$	C, H	0.02
XIII	$-(CH_2)_{10}N$	D	80			$C_{36}H_{59}NO_4$	[f]	0.01
XIV	$-CH_2CH_2-CO_2CH_3$	A	37	100-101°[g]	Ether/ pet. ether	$C_{26}H_{36}O_6$	C, H	0.008
XV	$-CH_2CH_2-CO_2H$	A	75	178-181°[h]	Ether/ pet. ether	$C_{25}H_{34}O_6$		0.004

Table 1, Contd.

a. R.A. = relative receptor affinity (progesterone = 1; IC50 values for progesterone ranged from 1.5 to 3.0×10^{-9} M).

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

c. Reported mp 191-193° (13).

d. Reported mp 206-208° (14).

e. Purified by column chromatography. This compound resisted recrystallization from customary solvents.

f. Purified by column chromatography. This compound appeared to be unstable and satisfactory analysis could not be obtained. Spectral data (ir, nmr) supported the assigned structure and material appeared homogeneous on TLC.

g. Reported mp 112-114° (14).

h. Reported mp 173-175° (14).

of the corresponding bromoacyloxy compounds with the appropriate amine.

While progesterone and progesterone derivatives with a free 17α-hydroxyl have low receptor affinity, the esterification with fatty acids increases affinity markedly (8,9,10,11). Thus, the RA for acetate (I) and caproate were 0.4 and 0.8, respectively, while the free hydroxy derivative was only 0.01 (11). Addition of halogen in the acyl group reduces affinity markedly, the trifluoroacetate (II) being inactive. Increasing the chain length increases affinity despite the presence of halogen but even the bromocaproate ester (IX) (RA = 0.47) is less active than the corresponding unsubstituted ester (RA = 0.80). It is doubtful whether these acyl groups at the 17α-position actually contribute to binding, since these derivatives are all less potent than progesterone itself.

When basic side chains were introduced, there was a dramatic decrease in affinity (XI, XII, XIII). An ester function or a free carboxyl in the side chain was also very unfavorable. The benzoate (III) was inactive, probably for steric reasons, since the phenylacetates (IVa, b, VI) had significant receptor affinities. Again, introduction of a basic side-chain on the phenylacetate moiety (VI) led to inactivity.

These structure-affinity studies lead to the conclusion that the 17α-substituents project against a hydrophobic surface of the receptor protein and that any polar groups reduce affinity markedly. The low affinity of the substituted 17α-acyloxyprogesterones for progesterone receptor protein makes it unlikely that they would act as antiprogestational agents.

EXPERIMENTAL SECTION

The procedures given in this section are representative for each of the analogous compounds presented in Table 1. The melting points which are corrected, were taken on a Thomas-Hoover (capillary tube) apparatus. Analyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind. Ir spectra were obtained with a Perkin Elmer 337 spectrophotometer. Nmr spectra were obtained with a A-60A spectrometer (Me_4Si or DSS).

Method A - 17α-Hydroxypregn-4-ene-3,20-dione 17-p-Iodophenylacetate (IVb). Prepared according to the procedure of Solo and Gardner (1,2).

Method B - 17α-Hydroxypregn-4-ene-3,20-dione 17-(5-Bromopentanoate) (IX). A solution of p-toluenesulfonic acid (200 mg) in dry benzene (25 ml) was heated to gentle reflux and moisture removed with the aid of Dean-Stark water separator for 1 hr. 17α-Hydroxyprogesterone (200 mg) was added to the cooled solution followed by 5-bromopentanoic anhydride (The anhydrides were prepared by the procedure of Diczfalusy et al. (12).) (0.9 gm) dissolved in dry benzene (5 ml). The clear solution was then allowed to stand at room temperature for 2 days. After this period, the solution was poured into cold H_2O (50 ml) and the mixture stirred for several hours. It was then extracted into ether. The combined ether extracts were washed successively with H_2O, dilute HCl solution, 5% $NaHCO_3$ soln, and saturated NaCl soln. The organic phase was dried (Na_2SO_4) and the solvent completely removed under reduced pressure. The resulting oily residue was chromatographed over alumina. Elution with ethyl acetate and subsequent crystallization from aq. acetone afforded IX as colorless crystals.

Method C - 17α-Hydroxypregn-4-ene-3,20-dione 17-p-(2-Pyrrolidinethoxy)-phenylacetate Hydrochloride (VI). A solution containing V (400 mg) and a few crystals of KI in freshly distilled pyrrolidine (10 ml) was heated to gentle reflux with stirring for 2 days. Pyrrolidine was then removed under vacuum and residue was triturated with H_2O when solid separated. It was collected on filter and washed well with H_2O. Ir spectrum of this residue indicated formation of enamine, therefore, this solid was dissolved in 95% ethanol (20 ml) and heated to gentle reflux for 1.5 hr. After this period, solvent ethanol was completely removed under vacuum and residue treated with H_2O. It was then collected on a filter, dried and dissolved in dry ether. Addition of ethanolic HCl precipitated HCl salt which was collected and recrystallized from ethanol-petroleum ether (30°-60°) to afford VI as white solid.

Method D - 17α-Hydroxy-pregn-4-ene-3,20-dione 17-(5-Diethylamino)-pentanoate Hydrochloride (XI). To a solution containing VIII (200 mg) in anhydrous benzene (10 ml) was added diethylamine (1.4 ml) and the mixture was refluxed for 26 hr. The solvent was removed under reduced pressure and the residue was extracted with Et_2O and filtered. The organic layer was washed with H_2O, 5% $NaHCO_3$, H_2O and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford the free base

of XI (180 mg, 90%) as a thick oil. This oil was chromatographed over neutral alumina. Elution with hexane-$CHCl_3$ 1:1 afforded a clear oil. The oil was converted to the hydrochloride salt in the usual manner.

Competitive binding assay for progestogen-receptor affinity. Each substance was tested for competitive affinity against tritium-labelled progesterone-^3H (57 Ci/mmol, New England Nuclear, Boston, Mass.) and receptor from rabbit uterus cytosol (11). A series of test tubes were prepared with 0.5 ml buffer (10% glycerol in 0.01 M Tris, pH 7.4, with 0.0015 M EDTA and 0.25 M sucrose). The appropriate amounts of unlabelled competitors and labelled progesterone ($3x10^{-10}$ 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. ^3H-Progesterone binding was measured by charcoal 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).

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