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STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF SUBSTITUTED 17α-HYDROXY-9β, 10α-PROGESTERONES

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SUMMARY

The influence of substitution on carbon atom 6 of 1,2β-methylene or 16-methylene substituted 6-dehydro-17α-acetoxy-9β, 10α (=retro)-progesterones on progestational activity (Clauberg assay) was investigated. In particular 6-fluoro substitution led to high activities. A comparison with the corresponding 9α, 10β (=natural) steroids revealed a difference in effect of substitution. With the 9α, 10β compounds a good correlation was found between Clauberg potencies and *inter alia* LEMO. In contrast the Clauberg potencies of the 9β, 10α compounds correlated very well with FED on carbon atom 6. The endocrinological profile of representative compounds of the aforementioned 9β, 10α series was shown to be different from that of the corresponding 9α, 10β derivatives. A number of 1,2β-methylene or 16-methylene substituted 6-dehydro-17α-alkoxy-9β, 10α-progesterones were found to be orally active potent progestagens. In addition, these 17-ethers were shown to have a very prolonged progestational action when administered subcutaneously. This can—at least in part—be ascribed to the combination of a high acute efficiency with a low elution rate from the oily depot.

This contribution will be concerned with 17α-hydroxy-9β, 10α-progesterones. The 9β, 10α, also called retro-steroids, differ from the "normal" steroids by a change in configuration at the skeletal atoms C_9 and C_{10}. In the retro series, the hydrogen atom at C_9 and the methyl group at C_{10} are inverted (Fig. 1). This inversion at two atoms of the steroidal skeleton leads in fact to a change in the stereochemical configuration. This is illustrated in Fig. 2.

Two series of 6-dehydro-17-hydroxy-retroprogesterones will be discussed here, one substituted with a methylene bridge at the carbon atoms 1 and 2, the other with a methylene group at position 16.

The only variables in either series are the nature of the substituents at carbon atoms 6 and 17. As far as biological data are available, a comparison will be made with compounds in the normal series that are otherwise identical.

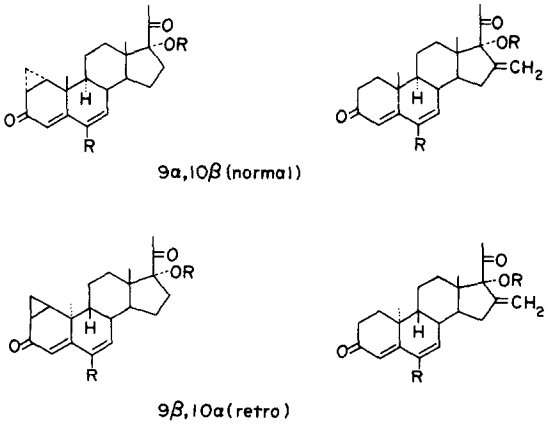


Fig. 1.

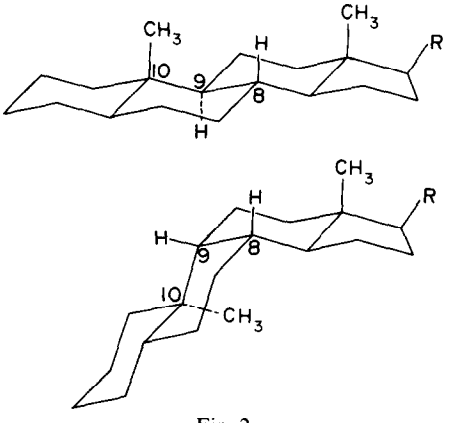
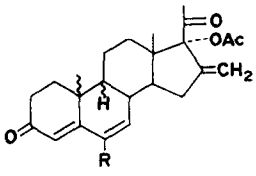


Fig. 2.

In Table 1, the activities after oral administration of some 17-acetoxy-16-methylene-9β, 10α-pregnanes as measured in the Clauberg test are given. Although in the original test dydrogesterone was used as a reference compound, the data have been recalculated in potencies relative to subcutaneously administered progesterone. If available, values of the isomeric compounds in the normal series have been included. For an account of these data and the methods used, reference should be made to the original publication [1]. Here it will suffice to say that, as far as data from the literature are concerned, determinations in our own laboratories are in full agreement. Differences within either series that will be discussed below lie certainly outside the limits of confidence. From Table 1 it is clear that the effect of substitution at position 6 in the retro series differs from that in the normal series. In the retro series the replacement of the hydrogen atom at position 6 by a methyl group lowers

Table 1. Progestational potency after oral administration in the Clauberg test (relative to progesterone sc)

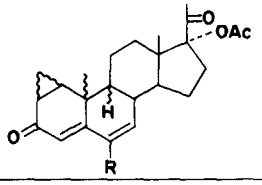


	9β, 10α (retro)	9α, 10β (normal)
R = H	50	13
CH_3	13	~30
Cl	280	55/75
F	610	50
Progesterone		1

the progestational activity. This is in contrast with what has been found generally in the normal series. A chlorine atom enhances activity, but a fluorine does so significantly more. In the normal series, the substitution by a chlorine or a fluorine atom leads to almost equally potent compounds. Apparently the fluorine substituted "retro" is a very potent progestagen indeed.

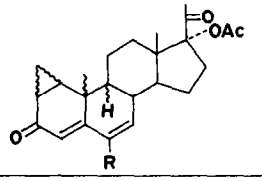
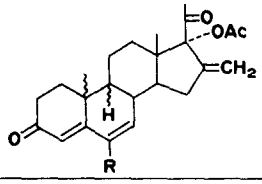
In the series of 17-acetoxy-1,2β-methylene-9β, 10α-pregnanes an identical effect of substitution is seen (Table 2) [2]. Whereas, in comparison with the unsubstituted compound, methyl substitution reduces the activity, a chlorine atom increases it, and a fluorine atom has a significantly greater effect. In the normal series a fluorine atom enhances the activity, also, but a chlorine atom has an even greater effect.

Table 2. Progestational potency after oral administration in the Clauberg test (relative to progesterone sc)



	9β, 10α (retro) (1, 2β – CH_2)	9α, 10β (normal) (1, 2α – CH_2)
R = H	30	4
CH_3	7	
Cl	110	110
F	600	35
Progesterone		1

Table 3. Inhibition constants for the progestagen receptors of rabbit uterus

	R	K_i (M)	
		9β,10α (retro)	9α,10β (normal)
	H	1.8×10^{-10}	
	CH_3	1.1×10^{-9}	
	Cl	4.2×10^{-12}	8.5×10^{-10}
	F	8.7×10^{-13}	
	H	4.4×10^{-10}	
	Cl	2.1×10^{-10}	
	F	3.9×10^{-11}	
Progesterone			4.1×10^{-10}
Chlormadinone acetate			2.1×10^{-10}

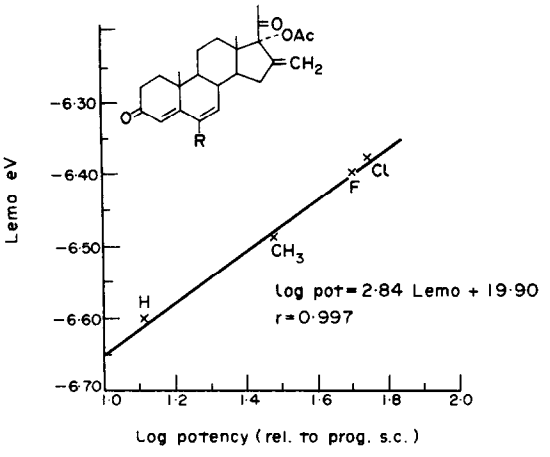


Fig. 3.

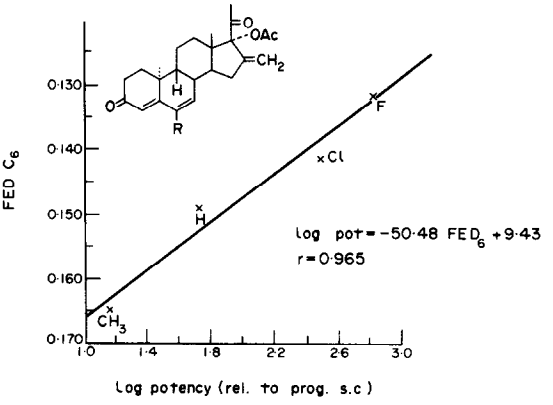


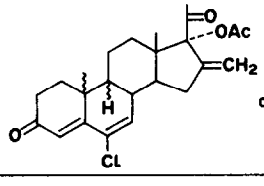
Fig. 4.

By kind permission of Dr. Terenius of the Biomedical Centre of the University of Uppsala some previously unpublished affinities for progestagen receptors of the rabbit uterus can now be reported (Table 3) [3]. In particular, for the 1,2β-methylene substituted retro series, the similarity with the data from the Clauberg test is striking. A methyl group lowers affinity, a chlorine atom enhances it, and fluorine substitution is better still. Noteworthy is the high affinity of the chlorine and fluorine substituted retro compounds in comparison with cyproterone acetate, the 6-chloro substituted counterpart in the normal series. In the 16-methylene substituted retrosteroids the affinities for the receptor are apparently lower; the effect of substitution is less pronounced.

It was thought worthwhile to correlate, if possible, the differences in biological effects of substitution in the normal and the retro series with differences in the physical-chemical properties of these molecules. To this end, a quantumchemical calculation of relevant electronic parameters was made by Mr. Tipker of our laboratories, with the help of a computer program which was based essentially on the extended Hückel method and elaborated by Dr. Hoogenstraaten, of Philips Research Laboratories. A special program was designed for the computation of the input of atomic numbers and coordinates [4]. The outcome of these calculations revealed no significant differences between the normal and retro series for parameters such as net atomic charge, atomic polarizability, frontier electron density (FED), and frontier hole density (FHD), or for parameters such as the energies of the highest occupied molecular orbitals (HOMO) and lowest empty molecular orbitals (LEMO), which reflect respectively the ionization energy and electron affinity of the whole molecule. However, if the biological effects are matched to these parameters, it becomes clear that the potency in the Clauberg test, as measured in the normal series, can be correlated very well with the parameters of net atomic charge and atomic polarizability of several carbon atoms and LEMO, which, as already mentioned, is a measure of the electron affinity of the total molecule. This is illustrated in Fig. 3., where the correlation of LEMO with the Clauberg potencies is given. Equally good correlations were obtained with the net atomic charge at carbon atom 7 and with FHD of carbon atoms 1 and 5. No correlation was found with the FED of any carbon atom. In the retro series, however, a good correlation was found only with the FED at carbon atom 6 but not with the other parameters mentioned. This is illustrated in Fig. 4.

With these data in hand, one is tempted to speculate—and admittedly it is no more than speculation —that in the retro series, because of the changed

Table 4. Endocrinological profile of 6-chloro-16-methylene-9α, 10β (normal) and 9β, 10α (retro) pregnane derivatives

	Clauberg Rabbit oral; rel. potency Progesterone s.c. = 1	Maintenance of pregnancy Rat oral; ED_{50} (μg/kg/day)	Inhibition of ovarian compensatory hypertrophy Hemi ♀ Rat s.c.; rel. potency Progesterone s.c. = 1
9α, 10β (normal)	75[a]	»5000[b]	17[c]*
9β, 10α (retro)	280	600	420

[a] Čekan Z., *et al.*: *Steroids* **8** (1966) 205.
[b] Seda M., *et al.*: *Experientia* **23** (1967) 664.
[c] Jelínek J. M., *et al.*: *Steroids* **11** (1968) 565.

stereo-chemistry, a different fit is obtained with the receptor, resulting in a shorter distance from carbon atom 6 to some important electrophilic centre in that receptor. Accordingly, the FED at carbon atom 6 now becomes a dominating factor, which leads to a different effect of substitution at position 6 and apparently to a high affinity. In the normal series there is no evidence, from these calculations, of a binding with such an electrophilic centre; on the contrary, there is some evidence of binding with nucleophilic sites on the receptor. A difference in fit for the retro and the normal series with steroid metabolizing enzymes has been reported previously [5].

So far, the results of the Clauberg assay of both series have been discussed. It might be interesting to consider next a few important differences in endocrinological profile between the 1,2-methylene and 16-methylene substituted compounds of the retro and the normal series. The comparison is limited by the amount of data available in the literature as far as the normal series is concerned. The methods used in obtaining the experimental data mentioned in the following tables are well known and generally described in the literature (6*a*: inhibition of ovulation in rabbits and rats; *b*: maintenance of pregnancy in rats; *c*: maintenance of pregnancy in rabbits; *d*: inhib. ov. compensatory hypertrophy; *e*: anti-androgenic activities). Results are expressed as ED_{50} in μg/kg or as potencies relative to progesterone given subcutaneously. Where data from the literature have been recalculated to such data, this is indicated by an asterisk. Values found in the Clauberg test are given for the sake of comparison.

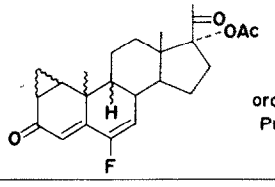
In Table 4 some comparative data are given for the 6-chloro-16-methylene substituted normal and retro compounds. The "retro" has apparently a relatively high central effect, as can be deduced from the results in the test of the inhibition of ovarian compensatory hypertrophy in the hemi-spayed rat. Furthermore it is clear that the retro compound is highly active in the maintenance of pregnancy in the rat, whereas the normal compound is virtually inactive. In this test, only the progestagen was given, without addition of an estrogen; it might perhaps be mentioned that none of the retro compounds discussed here have any estrogenic activity.

In Table 5 the 6-fluoro-1,2-methylene compounds are compared. Again the retro isomer is characterized by a strong central effect, as can be deduced from a comparison of the values found on assessing the inhibition of ovulation in the rat. In maintaining pregnancy in the rabbit the retro compound was highly active, too, whereas the normal isomer was virtually inactive.

In Table 6 data are given for the anti-androgenic activities of the 6-chloro-1,2-methylene derivatives. The compound in the normal series was cyproterone acetate, the well known, potent anti-androgen. With the retro compound, no such activity was found at the dosages tested.

In conclusion it can be stated that in several important aspects, the endocrinological profile of the retro

Table 5. Endocrinological profile of 6-fluoro-1,2-methylene-9α, 10β (normal) and 9β, 10α (retro) pregnane derivatives

	Clauberg Rabbit oral; rel. potency Progesterone s.c.=1	Maintenance of pregnancy Rabbit oral ; ED_{50} (μg/kg/day)	Inhibition of ovulation Rat s.c.; ED_{50} (μg/kg)
1,2α-CH_2; 9α, 10β (normal)	35[a]	2000[b]	»15·000[b]
1,2β-CH_2; 9β, 10α (retro)	600	15	120

[a] Wiechert R. and Neumann F.: *Arzneim.-Forsch.* **15** (1965) 244.
[b] Handbuch Exp. Pharmakologie, XXII, Teil 1, Die Gestagene, p. 871 Ed. Junkmann, Springer-Verlag, Berlin, Heidelberg und New York (1968).

Table 6. Comparison of anti-androgenic effects of 6-chloro-1,2-methylene-9α, 10β (normal) and 9β, 10α (retro) pregnane derivatives

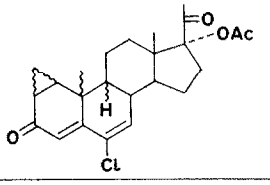
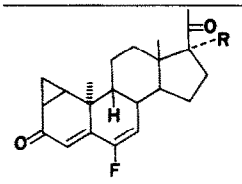
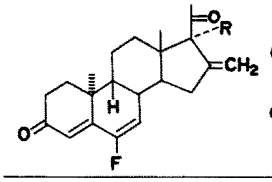
	Clauberg Rabbit oral; rel. potency Progesterone s.c.=1	Anti-androgenic effect ♂ Mouse s.c.; ED_{50} (ug/kg/day)
1,2α-CH_2; 9α, 10β (normal)	110	6200
1,2β-CH_2; 9β, 10α (retro)	110	»40.000

Table 7. Endocrinological profile of 6-fluoro-17-acetoxy and 6-fluoro-17-ethoxy-9β, 10α-pregnane derivatives

		Clauberg oral; rel. potency Progesterone s.c.=1	Maintenance of pregnancy oral; ED_{50} (μg/kg/day)		Inhibition of ovulation oral; ED_{50} (μg/kg/day)		Inhibition of ovarian compensatory hypertrophy s.c.; ED_{100} (μg/kg/day)
	R	Rabbit	Rat	Rabbit	Rat	Rabbit	Hemi ♀ rat
	OAc	600	2000	15	200	0·5	120
	OEt	60	330	3	70	2·3	14
	OAc	610	290	15	150	0·4	12
	OEt	35	570	6	220	1·4	43

compounds differs considerably from that of the normal isomers.

Another class of 1,2β-methylene or 16-methylene retropregnanes has been obtained by exchange of the 17-acetoxy group for a 17-alkoxy group. In particular, the potency of the 17-methoxy and 17-ethoxy derivatives is noteworthy. In several of the tests of progestagenic activity or of the influence on the pituitary the effect of these ethers was greater than that of the corresponding 17-acetoxy derivatives, though in some respects the effect was smaller. This is demonstrated for the 6-fluoro-17-ethoxy derivatives in Table 7.

Besides a high, acute efficacy, these ethers display a remarkably prolonged action. This was demonstrated in a specially adapted anti-estrogenic test in spayed rats. Ethynylestradiol added to the drinking water maintained these rats in constant estrus, as shown by vaginal smears. The compound under test was administered once in several dose levels subcutaneously and the number of days that the animals were out of estrus was determined. A microcrystalline preparation of dydrogesterone, commercially available as Duphaston Depot, was used as a reference substance, and the activity was expressed relative to this drug. The results are given in Table 8. With the 17-acetates, virtually no prolonged action was found. The 17-methoxy as well as the 17-ethoxy ethers are highly active. The effect of substitution on carbon atom 6 can be seen in Table 9. For the sake of comparison the value for Depo Provera, a long acting commercially available preparation of medroxyprogesterone acetate has been included.

Some preliminary experiments performed in our laboratories suggest that the isomeric ethers of the normal series lead neither to the high, acute nor to the prolonged activity.

In our search for an explanation of this remarkably

Table 8. Acute and prolonged progestational activity of 17-alkoxy and 17-acetoxy-6-fluoro-9β, 10α-pregnane derivatives

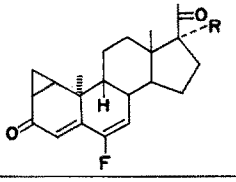
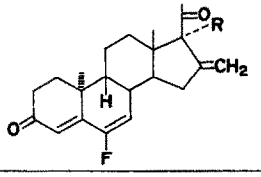
		Clauberg Rabbit oral; rel. potency Progesterone s.c. = 1	Prolonged action ♀ Rat s.c.; rel. potency Duphaston depot=1
	R		
	OMe	30	80
	OEt	60	300
	OAc	600	<10
	OMe	70	130
	OEt	35	390
	OAc	610	<10

Table 9. Acute and prolonged progestational activity of 6-substituted-17-ethoxy-9β, 10α-pregnane derivatives

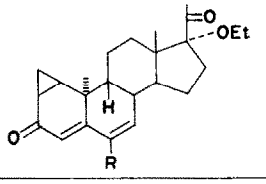
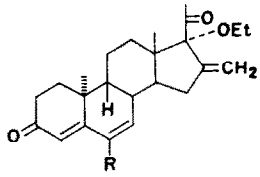
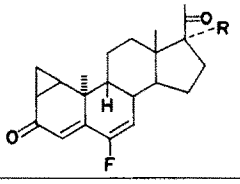
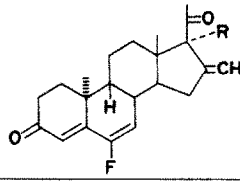
	R	Clauberg Rabbit oral; rel. potency Progesterone s. c.=1	Prolonged action ♂ Rat ♀ s.c.; rel. potency Duphaston depot=1
	H	20	220
	CL	60	130
	F	60	300
	H	50	160
	CL		60
	F	35	390
Depo Provera			7

Table 10. Elution rates of arachis oil into pig plasma

	R	Clauberg Rabbit oral; rel. potency Progesterone s.c.=1	Prolonged action ♂ Rat ♀ s.c.; rel. potency Duphaston depot = 1	Elution rates $t_{\frac{1}{2}}$ (hr)
	OMe	30	80	7·34
	OEt	60	300	20·15
	OAc	600	<10	~1·78[a]
	OMe	70	130	13·98
	OEt	35	390	13·65
	OAc	610	<10	4·74

[a] Estimated value, compound has very low solubility.

prolonged action of these ethers, the elution rates from arachis oil into pig plasma have been determined according to a method described by van der Vies[7]. These experiments were performed in our laboratories by Mr. de Lange. The results, given in Table 10, make it clear that the ethers have much lower elution rates or much greater elution halflife times than the corresponding acetates. In our opinion, the high acute efficacy, together with the beneficial elution rate, can explain to a large extent the highly prolonged action of the 17-retro ethers.

In conclusion it can be said that within the series of 1,2β-methylene or 16-methylene substituted 6-dehydro-17-acetoxyretroprogesterones, highly potent progestagens were found. The effect of substitution at carbon atom 6 of these retro compounds differs from that of the normal isomers. Moreover, in several important aspects there is a difference in endocrinological profile. The 17-alkoxyretro derivatives are, on oral administration, potent progestagens but in addition have, on subcutaneous administration, a remarkably prolonged activity.

REFERENCES

1. Hartog J., Wittelaar J. I. G. M., Morsink L. and de Wachter A. M.: *J. med. Chem.* **15** (1972) 1292–1297.
2. Halkes S. J., Hartog J., Morsink L. and de Wachter A. M.: *J. med. Chem.* **15** (1972) 1288–1292.
3. Terenius L.: (private communication).
4. Verloop A., Hoogenstraaten W. and Tipker J.: *Drug Design* (Edited by E. J. Ariens). Academic Press, New York, Vol. 6, in press.
5. Walop J. N. and de Lange N.: *Biochem. biophys. Acta* **130** (1966) 249–252.

6*a*. Claassen V., Morsink L. and de Wachter A. M.: *Acta endocr., Copenh.* **67** (1971) 551–562.

b. (unpublished observation).

c. Jost A.: *Acta endocr., Copenh.* **43** (1963) 539–544.
d. Jelínek J. M., Seda M. and Marhan O.: *Steroids* **11** (1968) 565–570.
e. Neumann F.: In *Handbuch Exp. Pharmakologie*, XXII/I, *die Gestagene*. Springer-Verlag, Berlin, Heidelberg, New York, 1968, p. 762.
7. van der Vies J.: *Acta endocr., Copenh.* **64** (1970) 656–669.