44. Steroidal, Aldosterone Antagonists: Increased Selectivity of 9α,11-Epoxy Derivatives[1])[2])

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Dedicated to Dr. *Georg Anner* on the occasion of his 80th birthday

(5.XII.96)

In the search for aldosterone antagonists with an optimal activity profile, twelve 9α,11-epoxy-steroids were prepared and compared with their 9α, 11α-unsubstituted analogues in terms of steroid receptor binding *in vitro* and electrolyte excretion *in vivo*. Substitution of the parent structures by an epoxy group at positions 9α,11 resulted in marginal effects on mineralocorticoid receptor binding and electrolyte excretion, but greatly reduced androgen and gestagen receptor binding. This finding is reflected in the largely lacking unwanted anti-androgenic and gestagenic side effects in animal models of the three most interesting 9α,11-epoxy-spirolactones **4**(CGP 33033), **18**(CGP 29245), and **25**(CGP 30083).

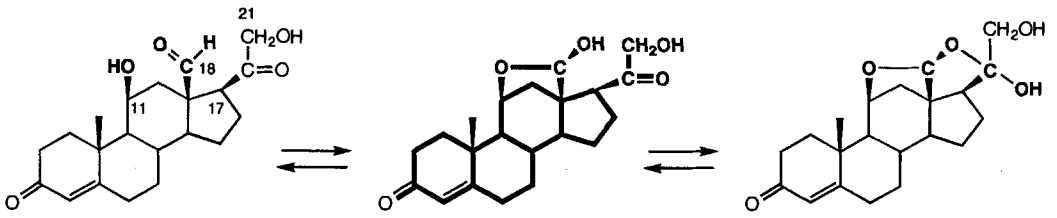
Introduction. – Aldosterone (see *Fig. 1*) is the most potent natural mineralocorticoid, playing an important role in regulating the electrolyte composition of body fluids by sodium retention and promotion of potassium excretion. An excess of this hormone is observed in such conditions as edema, congestive heart failure, essential hypertension, and cirrhosis of the liver. Spironolactone (*Aldactone®*), on the contrary, is a competitive antagonist of aldosterone at the receptor level [2] and has been used for many years as a therapeutic agent for the treatment of the above mentioned disorders. Its clinical usefulness as an aldosterone antagonist is often limited by adverse endocrine effects such as cycle disturbances (gestagenic), hirsutism (androgenic), gynecomastia (antiandrogenic), and loss of libido (antiandrogenic).

A primary goal in our search for novel aldosterone antagonists was, therefore, the identification of structures which would be devoid of such unwanted side effects. It is generally accepted that these sexual disturbances are mediated mainly by an interaction of the aldosterone antagonist with gestagen and androgen receptors [3–5]. Using the structure of deoxycorticosterone (DOC; *Fig. 1*), and, in a later phase of the project, that of spironolactone as a starting point, we have studied the effect of structural modifications of some hundreds of steroids on the binding to corticoid and sex-hormone receptors. The introduction of OH groups as potential sites of H-bonds, of O- or C-bridges, and of halogen atoms, but especially the introduction of additional double bonds,

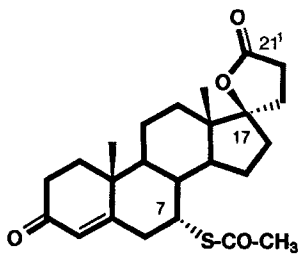
[1]) Presented in part by J. K. on September 15, 1991, at the 14th Conference on Isoprenoids in Tabor (Czech Republic) and on October 18, 1991, at the Meeting of the Swiss Chemical Society in Bern (Switzerland). Patents, [1 a].

[2]) *Cf.* also [1 b].

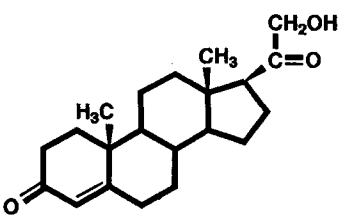
[3]) Present address: Leimgrubenweg 21, CH-4102 Binningen.



Aldosterone



Spironolactone



Deoxycorticosterone (DOC)

Fig. 1

cyclopropyl, or epoxy groups at selected positions of the steroid skeleton (see *Fig. 2*) permitted a subtle manipulation of the conformation of various parent compounds. As a consequence, flattening of the molecular shape or changes to more convex or to more concave structures (β-face) as well as distances between potential anchorage points in the molecule were controlled.

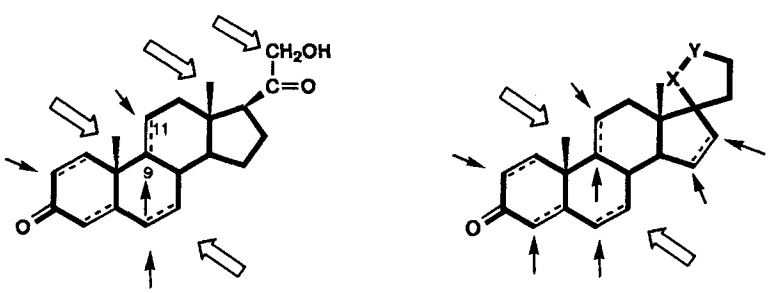


Fig. 2

The present communication is restricted to the discussion of the influence of a double bond and of an oxirane function at position 9(11) on the spatial arrangement of these steroids, on the binding of selected molecules to the mineralocorticoid, androgen and gestagen receptors, and, in some instances, on their *in vivo* activities.

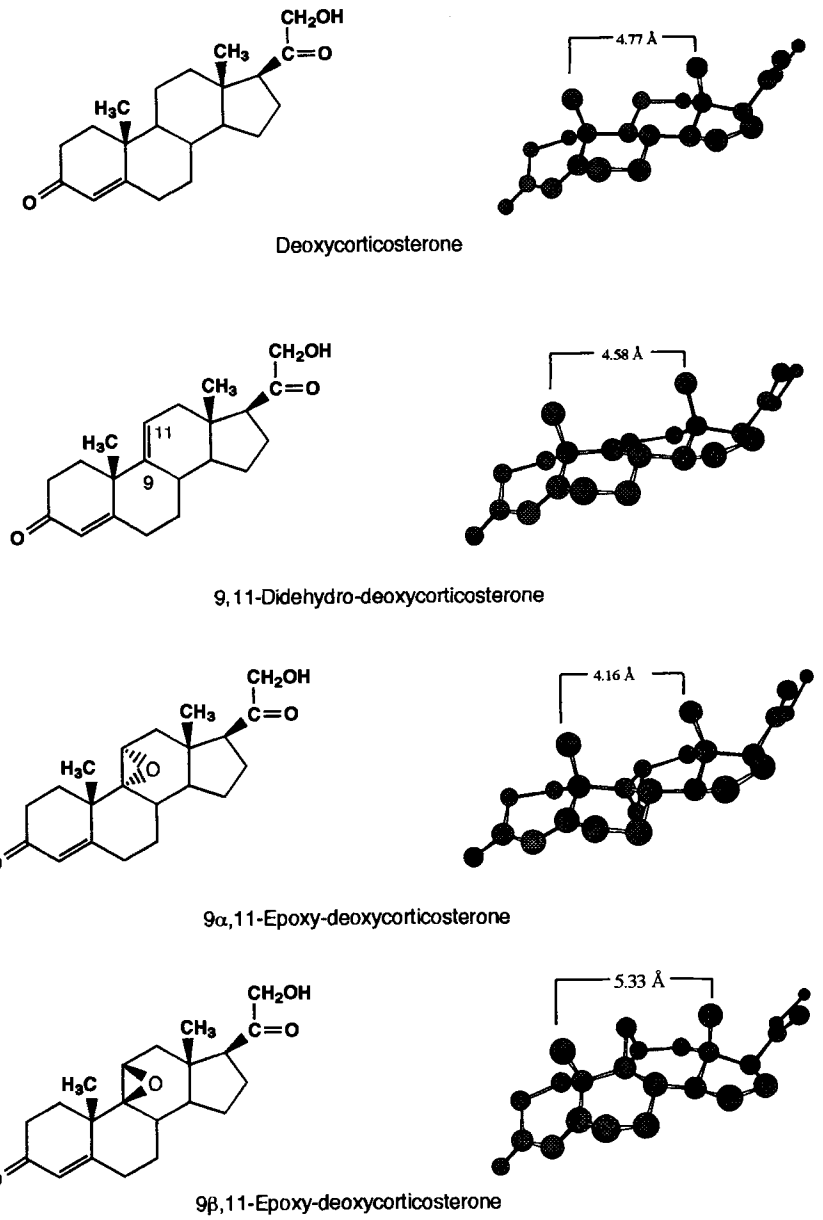


Fig. 3. *Conformations of corticosterone derivatives as determined by molecular modelling*[4])

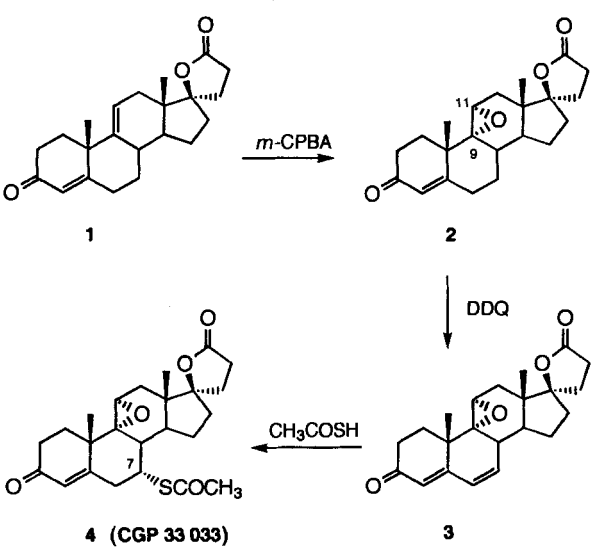
[4]) The various conformations were generated using the Conceptor software, an in-house MM programm written by *N. C. Cohen*, similar to Script [6]. We would like to thank Dr. *Pascal Furet* for the calculations (see *Fig. 3*) and for his valuable suggestions.

As a crude estimate for the bending of the steroid backbone served the distance between the two angular Me groups (C(18) and C(19)), deduced from energy-minimized computer models[4])[5]). The flatness of the BCD-ring portion of deoxycorticosterone (DOC) is expressed by the distance of *ca.* 4.8 Å between the two Me groups. In the known 9,11-didehydro-DOCA [7] (see *Fig. 3*), the additional C=C bond reduces that distance to *ca.* 4.6 Å and, hence, induces a more concave structure. A similar, but even stronger effect is exerted by an oxirane function in the same position on the α-side of the molecule (C(18)–C(19) = 4.16 Å). A 9β,11-epoxide, on the contrary, forces the two Me groups apart, to reach a distance of *ca.* 5.3 Å. The molecule adopts a convex form.

Chemistry. – In the above context, we decided to study the influence of an oxirane ring at position 9α,11α of spironolactone-like compounds. Reactions leading to the novel 9α,11-epoxy steroids **4**, **11** and **12**, **16**–**18**, **25**–**27**, and **30** are summarized in *Schemes 1*–*5*.

For the synthesis of the 9α,11-epoxy analogue **4** of spironolactone, the isolated C=C bond of 9,11-didehydrospirolactone **1** [8] was epoxidized by 3-chloroperbenzoic acid (*m*-CPBA; 3-$ClC_6H_4CO_3H$) in CH_2Cl_2 to afford the known α-epoxide **2** [9] (*Scheme 1*). After treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (= 4,5-dichlorocyclohexa-1,4-diene-1,2-dicarbonitrile; DDQ) in dioxane/HCl (→ dienone **3** [9]), 1,6-addition of thioacetic acid to the conjugated system generated the desired 7α-(acetylthio) derivative **4**. Using an alternative sequence of reactions, involving epoxidation as the last step, no significant amounts of **4** were obtained!

Scheme 1

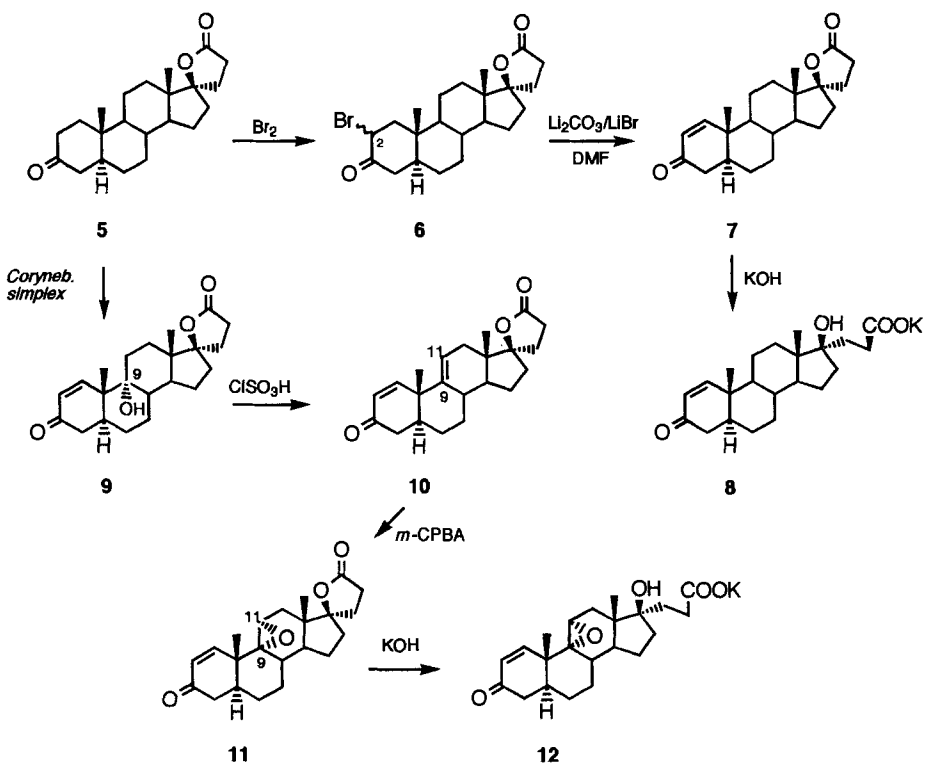


1 **2** **3** **4 (CGP 33 033)**

[5]) For crude estimations of the most stable conformations and for the visualization of the 3D structures, the CSC Chem3D Pro and CSC ChemDraw Pro programs were used.

For the synthesis of the two representatives **11** and **12** of the 1,2-didehydro-5α series and for the reference compounds **7** and **8** (*via* **6**), the saturated 3-ketone **5** [10] served as starting material (*Scheme 2*). The crucial step for the introduction of the 1,2,9,11-tetradehydro moiety consisted of two consecutive microbiological transformations performed as a one-pot reaction under the influence of *Corynebacterium simplex*, namely, dehydrogenation of **5** at positions 1,2 and hydroxylation at C(9) to give **9**[6]). Various methods (*e.g.* treatment with piperidine sulfurtrifluoride/$CHCl_3$, pyridine/$POCl_3$, or trimethylpyridine/($MeSO_2Cl + SO_2$) DMF) were evaluated for the dehydration of **9**. The best procedure, however, consisted of a short treatment of **9** with chlorosulfonic acid in CH_2Cl_2 at $-4°$, yielding **10** in *ca.* 56% yield after chromatography and crystallization. Epoxidation of **10** by 3-$ClC_6H_4CO_3H$ in CH_2Cl_2 generated **11**, which was finally saponified to the potassium salt **12**.

Scheme 2

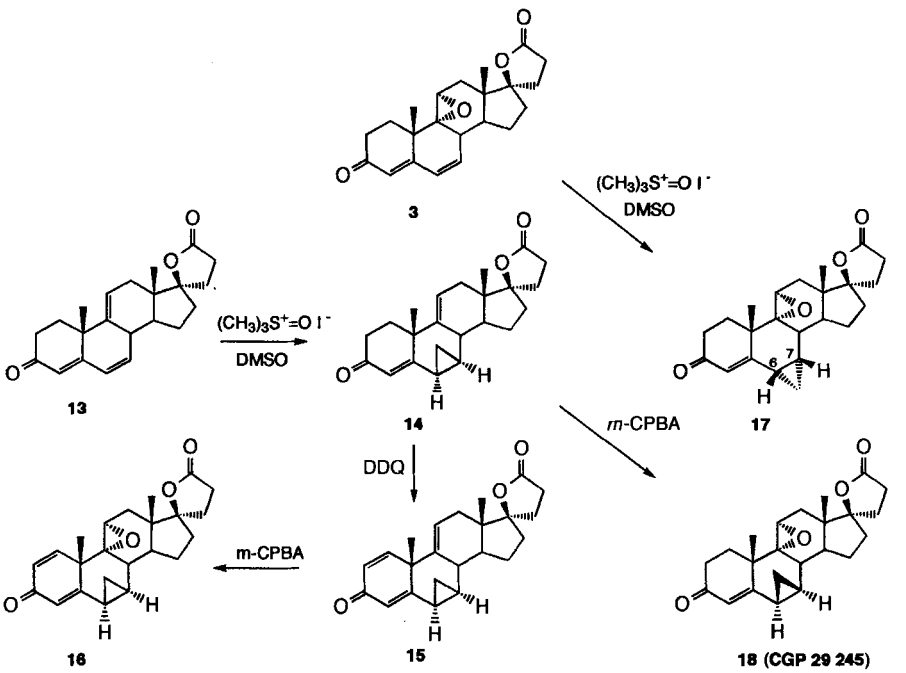


5 **6** **7** **9** **10** **8** **11** **12**

[6]) The dehydrogenation step is reversible. The isolable 1,2-didehydro intermediate was shown to be hydroxylated selectively at position 9α. The microbiological transformations were kindly performed and developed by the late Mr. *John Auden* in our Biotechnology Department.

The cyclopropane derivatives **15**–**18** were obtained starting from the known trienone **13** and epoxy-dienone **3** [9] (*Scheme 3*). Treatment of **13** by trimethylsulfoxonium iodide in DMSO afforded the 6β,7-methylene-lactone **14**, which was epoxidized to **18**. Dehydrogenation of **14** and selective epoxidation of the isolated C=C bond of **15** generated the desired compound **16**. Cyclopropanation of epoxide **3**, however, followed a different stereochemical course: under the influence of the homoallylic O-function at the 9α position, addition of the CH_2 group to the C(6)=C(7) bond proceded preferentially from the α-side, thus permitting the isolation of compound **17** (a stereoisomer of **18**).

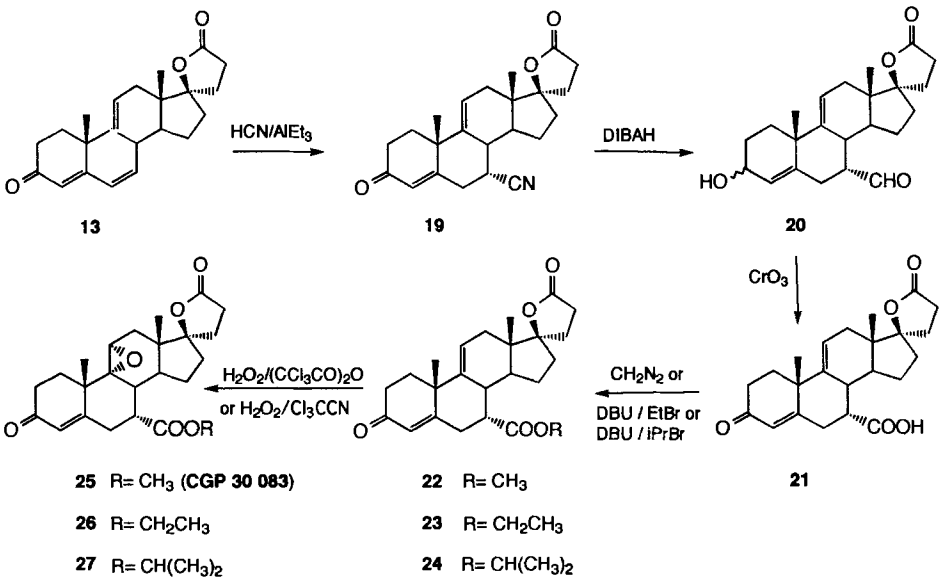
Scheme 3



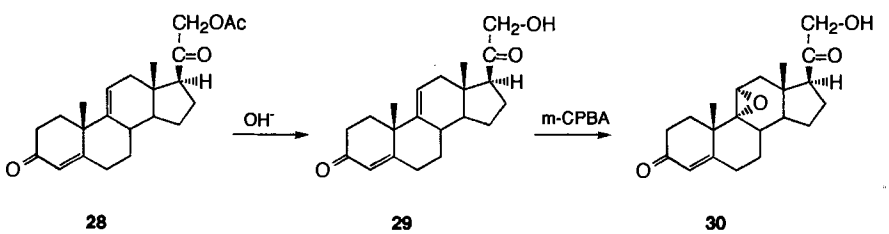
For the synthesis of the three 9α,11-epoxy-lactones, **25**–**27**, substituted at C(7) by an ester function, the above trienone **13** was used again as starting material (*Scheme 4*). Upon treatment of **13** with HCN/$AlEt_3$ in benzene, the 7α-cyano derivative **19** was formed. For the next step – the diisobutylaluminium hydride (DIBAH) reduction to aldehyde **20** – the crude product **19** could be used directly. Oxidation of **20** by CrO_3/H_2SO_4 in acetone to the corresponding acid **21** and subsequent 1,8-diazabicyclo[5.4.0]undec-7-ene(DBU)-catalyzed alkylation of the crude reaction mixture yielded the alkyl esters **22**–**24**. In a final step, the epoxidation of the C(9)=C(11) bond of these compounds was performed by hydrogen peroxide in the presence of $(CCl_3CO)_2O$ or CCl_3CN.

The 9α,11-epoxy analogue **30** of deoxycorticosterone (*Scheme 5*) was synthesized from the 9(11)-unsaturated precursor **29**, which in turn was obtained from the known 21-acetate **28** [7] by epoxidation with 3-$ClC_6H_4CO_3H$.

Scheme 4



Scheme 5



X-Ray Crystal-Structure Analysis of Compound 25. – To obtain more information about the detailed structure of **25**, *e.g.*, the conformation of the side chain and of the spirolactone ring, an X-ray analysis was performed. Compound **25** was recrystallized from CH_2Cl_2/Et_2O. The colorless, transparent needles became opaque upon standing (in air) due to evaporation of the included solvent molecules and deterioration of the crystal lattice. The monocrystal used for the analysis was, therefore, fixed by *Araldite rapid*® on a *Lindemann* glas tube and covered by the same adhesive. Crystal data[7]) and the structure are given in *Table 1* and *Fig. 4*, respectively.

Cell constants were determined by a least-square fit to the Θ values of 25 independent reflexions. Data were reduced, and *Lorentz*, polarization, and decomposition corrections were applied. The structure was solved by direct methods using the program SHELXS-86 [11] [12]. H-Atom positions were calculated assuming normal geometry. Lists of fractional atomic coordinates, isotropic thermal parameters, and bond lengths and angles have been deposited at the *Cambridge Crystallographic Data Centre* as Supplementary Publication No. CCDC-10/35. Copies of the data can be obtained, free of charge, on application to the Director of the *CCDC*, 12 Union Road, Cambridge CB2 1EZ, UK (fax: + 44(0)1 223 336033 or email: teched@chemcrys.cam.ac.uk)

[7]) For anal. data, *cf. Exper. Part.*

Table 1. *Crystal Data of Compound* **25**

Formula	$C_{24}H_{30}O_6$	Scan mode	$\theta/2\theta$
Mol. wt.	414.49	Scan range (2θ)	6–134
Crystal system	orthorhombic	No. of measured reflections	2567
Space group	$P2_12_12_1$	No. of observed reflections	
a [Å]	8.247(1)	($I > 2\sigma(I)$)	2035
b [Å]	12.903(2)	Refinement method	full matrix on F
c [Å]	23.422(3)	No. of parameters	271
V [Å^3]	2492.5(7)	R	0.093
Z	4	Rw	0.106
Crystal size [mm]	0.51 × 0.12 × 0.12	No. of reflections used	2035
Diffractometer	*Philips PW1100*	Weighting scheme	$1/\sigma^2(F)$
Radiation (graphite monochromated)	CuK_α	Treatment of H-atoms	calculated, not refined
Wavelength [Å]	1.54178	Max/min density in final difference map [eÅ^{-3}]	1.231/ – 1.153

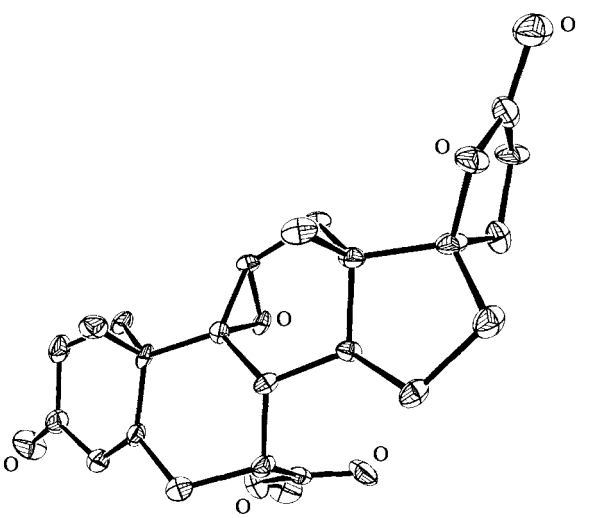


Fig. 4. *X-Ray crystal structure of* **25**. Displacement ellipsoids at the 20% probability level.

Biology. – *Figs. 5* and *6* show the effect of 9α,11-epoxy substitution on the relative binding affinities of ten known 9,11-unsubstituted spirolactones (pairs **A**–**K**), of one K-salt (pair **L**), and of deoxycorticosterone (DOC, an agonist; *cf.* **M**) to the mineralocorticoid (MR), androgen (AR), and progesterone receptors (PR). Nine pairs of compounds, including the K-salts (pair **L**) and DOC (pair **M**), showed only a slight change in MR binding, ranging from a 2.25-fold decrease (pair **C**) to a 1.5-fold increase (pair **D**). A rather more pronounced effect (7.5-fold decrease) was observed with three 7α-COOR substituted spirolactones (pairs **H**, **J**, and **K**). In contrast, the epoxy group caused a considerable reduction in binding to AR and PR: this ranged from 3-(pair **A**) to 100-fold (pair **C**) for AR binding and 10-(pair **A** and **G**) to 100-fold (pair **C**) for PR binding.

The minimal effect of the 9α,11-epoxy group on MR binding was reflected in the anti-mineralocorticoid activities of these compounds (*Table 2*): none of the epoxy-substituted aldosterone antagonists showed a difference greater than ± 2.5-fold compared

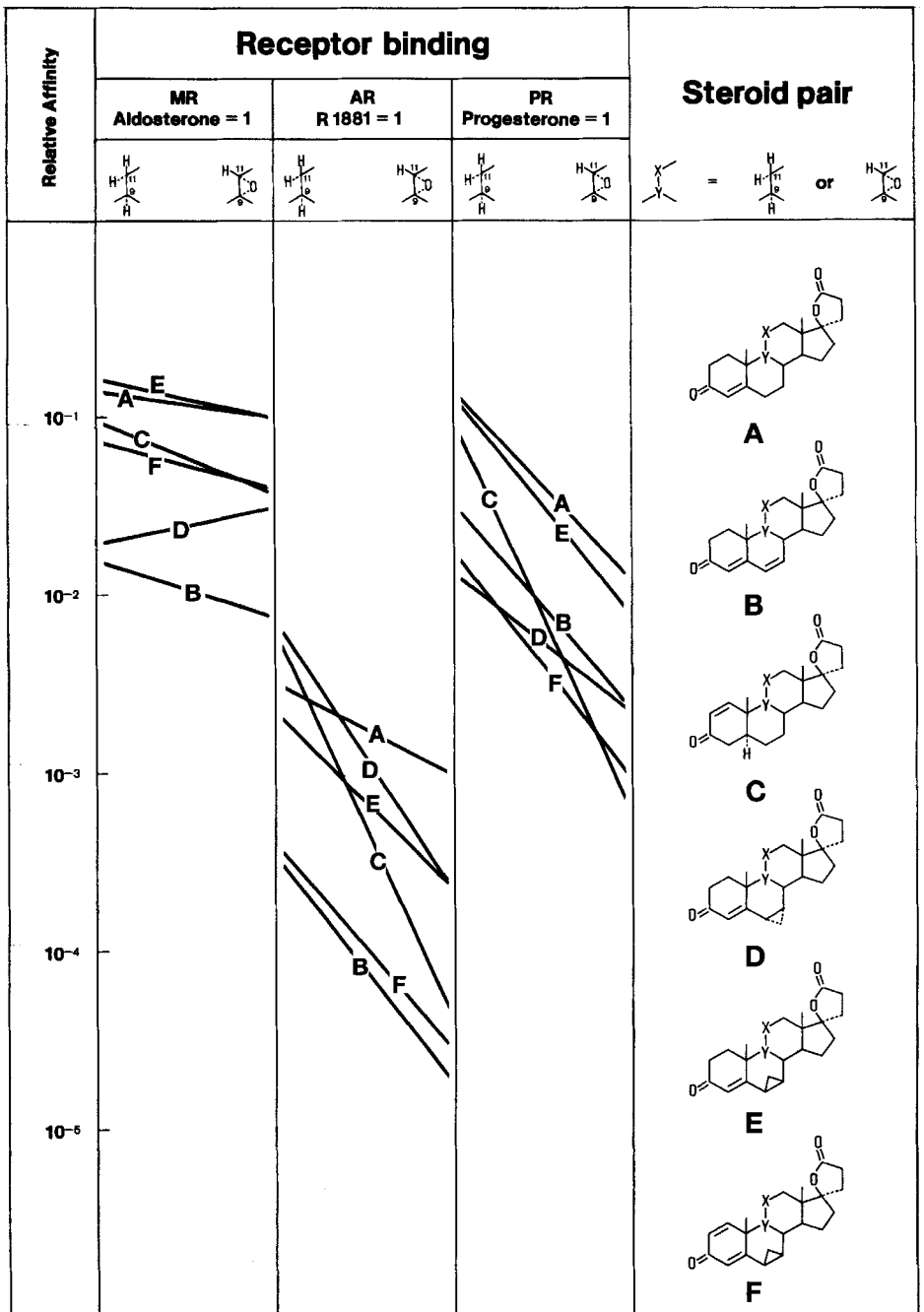


Fig. 5. *Effect of 9α,11-epoxy substitution on the affinity of spirolactones* (pairs **A**–**F**) *for the mineralocorticoid receptor* (MR), *androgen receptor* (AR), *and the progesterone receptor* (PR). Affinities are expressed relative to aldosterone (MR), to R-1881 (AR) and progesterone (PR). The effect of 9α,11-epoxy substitution is visualized by plotting the affinities of the epoxy derivatives at the right side of each column and that of the unsubstituted compound on the left, and connecting the points by a straight line.

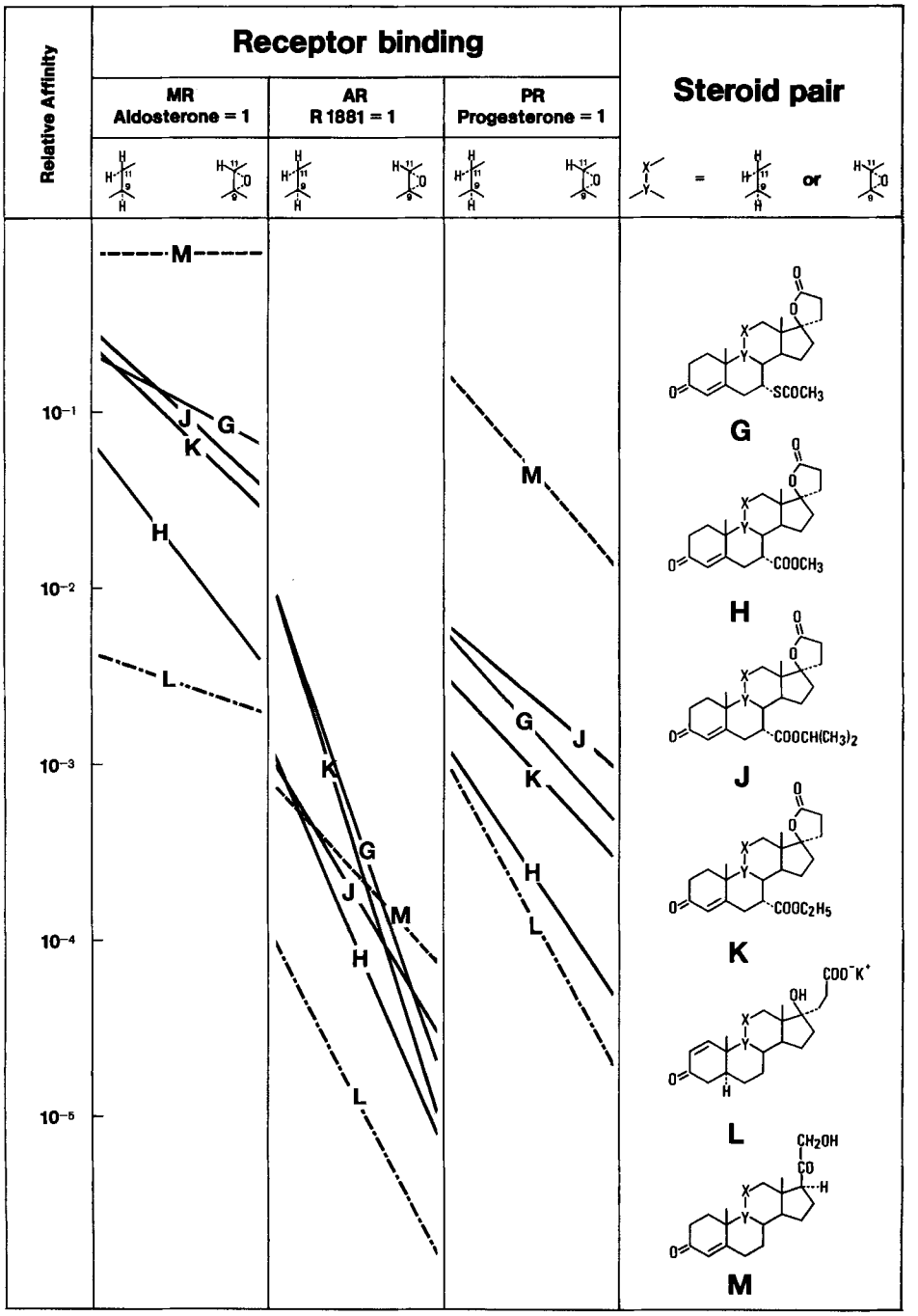


Fig. 6. *Effect of 9α,11-epoxy substitution on the affinity of spironolactone* (pair **G**), *three 7α-COOR substituted spirolactones* (pairs **H**, **J** and **K**; ——), *one K salt* (pair **L**; – · – ·), *and one agonist* (pair **M**; – – –). For notations, see *Fig. 6*.

Table 2. *Effect of the 9α,11-Epoxy Function on Electrolyte Excretion* in vivo*: Anti-mineralocorticoid and Mineralocorticoid Activities.* See also *Exper. Part.*

Pair (see *Figs. 5* and *6*)	9α,11-Epoxy derivative		9,11-Unsubstituted analogue	
	Compound	*Kagawa* test (spironolactone activity: 1[a])	Compound	*Kagawa* test (spironolactone activity: 1[a])
A	**2**	inactive at 60 mg/kg	[10]	inactive at 60 mg/kg
B	**3**	0.5	[13]	0.5
C	**11**	1	**7**	1
D	**17**	1	[14]	1
E	**18**	2	[14]	2
F	**16**	0.2	[15]	0.2
G	**4**	0.5	spironolactone	1
H	**25**	2	[16]	2
J	**27**	1	[16]	1
K	**26**	1	[16]	1
L	**12**	1	**8**	1
		Mineralocort. activity (aldosterone activity: 1[b])		
M	**30**	1	DOC	0,03

[a]) The anti-mineralocorticoid activity (*Kagawa* test) is expressed as relative patency compared to spironolactone (ED_{50} = 5 mg/kg *p.o.*).

[b]) The mineralocorticoid activity is expressed as relative patency compared to aldosterone (ED_{50} = 3 μg/kg *s.c.*).

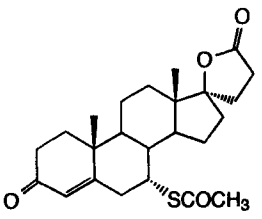
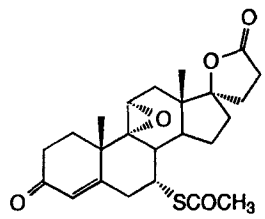
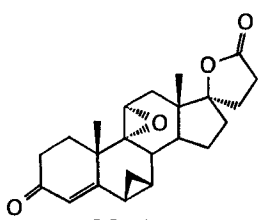
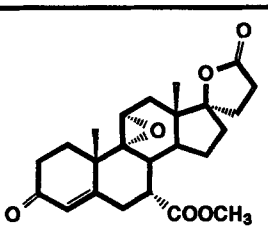
to their parent compounds. The three 7α-COOR substituted spirolactones **25**–**27** were no exception in this case. However, substitution of the agonist DOC (see **30**; pair **M**) resulted in a 30-fold increase in mineralocorticoid potency.

Receptor binding *in vitro* and potency *in vivo* may not correlate *quantitatively* (*e.g.* pair **A**). The lacking correlation is easily interpreted on pharmacokinetic grounds and does not justify doubts about the relevance of the MR as the main mediator of mineralocorticoid activity. This is demonstrated by the good correlation found between receptor occupancy *in vivo* and (anti)-mineralocorticoid potency [1b] [17].

The most interesting 9α,11-epoxy compounds (**4**, **18**, and **25**) were selected for the assessment of the unwanted sexual endocrine effects *in vivo* in comparison to spironolactone [1] [18] [19] (*Table 3*). In this case, *in vitro* binding results were found to be largely reflected *in vivo*. Regarding the *gestagenic* effect in rabbits (*McGinty* test), *e.g.*, both spironolactone and **18**, which bind rather strongly to PR (*cf.* pairs **G** and **E** in *Figs. 6* and *5*, resp.) were found to be equipotent and showed considerable activity at 100 mg/kg, whereas **4** and **25** were *inactive* at this concentration. Concerning *anti-androgenicity*, spironolactone was shown to be anti-androgenic and, in addition, inhibited ovulation in rats, but *none* of these effects could be demonstrated even at excessive doses for the 9α,11-epoxy derivatives (**4**, **18**, and **25**) [1] [18] [19].

Discussion. – An explanation for the observed negative selectivity for the androgen (AR) and to a lesser degree for the progesterone (PR) receptor induced by the introduc-

Table 3. In vivo *Activity of selected 9α,11-Epoxy-spirolactones*: ED_{50} [mg/kg *p.o.*]. ϕ: inactive at [mg/kg].

Compound	Anti-mineral. *(Kagawa)*	Gestagenic *(McGinty)*	Antiandrogenic Prostate	Sem.ves.
 Spironolactone	5	100	35	20
 4 (CGP 33 033)	5-10	ϕ(100)	ϕ(180)	ϕ(180)
 18 (CGP 29 245)	3	100	ϕ(180)	60
 25 (CGP 30 083)	3	ϕ(100)	ϕ(180)	ϕ(180)

tion of a 9α,11-epoxy function into steroid structures[8]) can be sought at the molecular level. As mentioned in the introduction, the epoxide at position 9α,11 of steroids (*e.g.* of DOC) forces the molecule in a more concave form (see *Fig. 3*). If we compare *e.g.* the

[8]) This effect seems to be rather general as it is observed even in the case of strong androgens like 17α-methyltestosterone. The corresponding 9α,11-epoxy analogue shows, *e.g.* a 30- to 100-fold decrease of binding to the AR compared to the parent hormone, retaining, however, the low but definite affinity for the mineralocorticoid (MR) receptor.

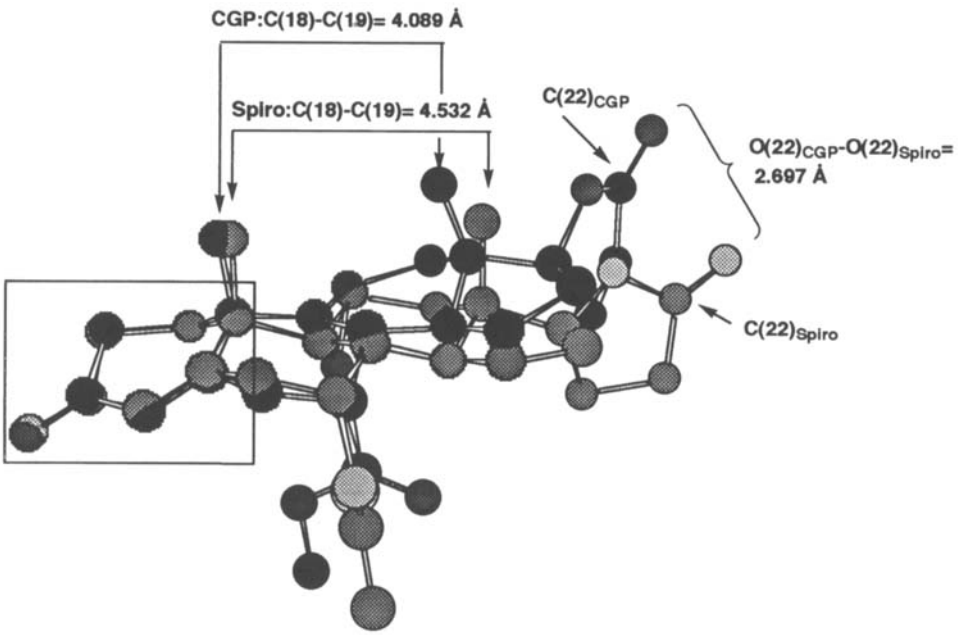


Fig. 7. *Superposition of ring A of spironolactone and of* **25** (CGP 30083)

X-ray structures of spironolactone[9]) [20] and **25** (*Fig. 4*) and superpose the A- and B-rings of the two molecules, the rest of the epoxide derivative **25** is bent upward. As a consequence, the lactone carbonyl O-atoms become separated by *ca.* 2.7 Å (*Fig. 7*).

Duax and *Griffin* [21–23] have suggested that in steroid hormones '...the receptor binding is primarily controlled by the interaction of the steroid A ring with the receptor, and the nature and degree of the hormonal response is primarily controlled by the stereochemical features of the D-ring region of the molecule...'. In androgens/antiandrogens, however, the steroid D-ring (with the 17β-hydroxy group) plays a critical role in initiating receptor binding ('D-ring binding/A-ring acting model'). Equally important appears to be a planar structure of the molecule. Superposing the structure of spironolactone and **25**, respectively, to that of testosterone (*Fig. 8*), the former molecule appears to be well suited for the binding to AR[10]). On the contrary, the concave form of the 9α,11-epoxide **25** deviates strongly from an ideal androgen receptor substrate. This fact could, at least partially, explain the absence of an (anti)androgenic activity in that compound and its analogues. In the case of the mineralocorticoid receptor, however, the interaction with the rings B, C, and D seems to be less important, although, the presence of the 17-spirolactone ring is essential for the binding of *Aldactone* and for its antagonistic activity [21] [24–26].

In conclusion, it is shown that introduction of a 9α,11-epoxy function into steroids results in a considerable loss of AR (and PR) binding, whereas loss of binding to MR is minimal. Anti-mineralocorticoid activity *in vivo* is not diminished. As the discovery of

[9]) Based on an in-house X-ray structure analysis (unpublished results of Mrs. *G. Rihs* and *H. R. Walter*).

[10]) The lack of a H-donating 17-hydroxy group in spironolactone might explain the relatively low affinity of the compound to the receptor, even if a molecule of H_2O could participate in the binding process.

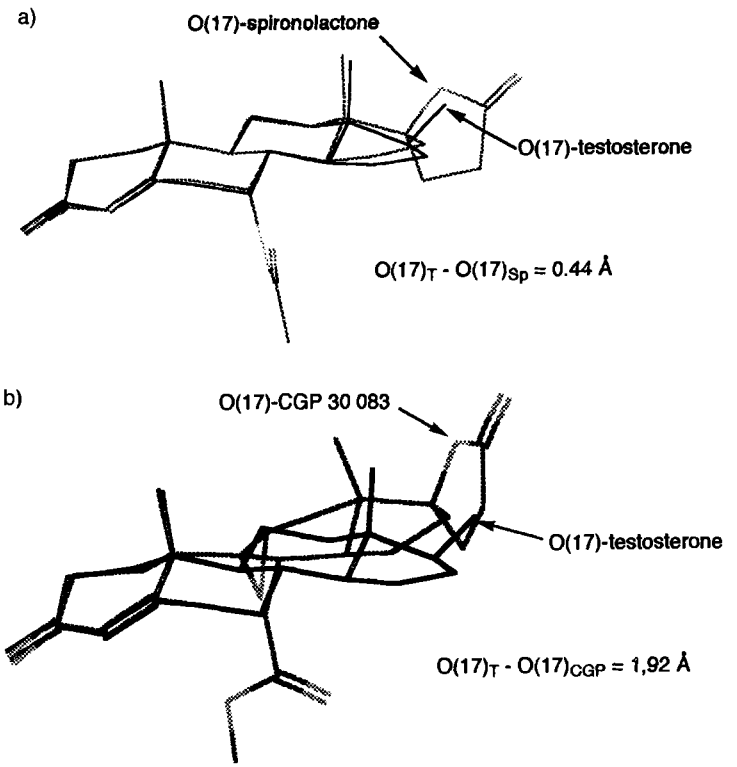


Fig. 8. *Superposition of the X-ray-structure* a) *spironolactone and* b) **25** (CGP 30083) *with that of testosterone*

a K-sparing steroidal aldosterone antagonist with fewer sexual endocrine effects is of high therapeutic relevance, the 9α,11-epoxy compound **25** is currently being evaluated in human pharmacological tests.

Experimental Part

1. *Chemistry. General.* UV Spectra: λ_{max} (ε) in nm. NMR Spectra: *Varian-HA100-D* or *Bruker-AM-360* spectrometer (^{1}H at 100 or 360 Hz, ^{13}C at 90.55 MHz); in $CDCl_3$ or D_2O; δ in ppm vs. $SiMe_4$ *J* in Hz. MS: *Varian-CH-7* spectrometer. Elemental and spectral analyses were performed in our specialized laboratories.

9α,11-Epoxy-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**2**). To a soln. of **1** [8] (4.0 g, 11.7 mmol) in CH_2Cl_2 (80 ml) 3-$ClC_6H_4CO_3H$ (85%; 4.00 g, 19.7 mmol) was added. The mixture was allowed to stand at 20° for 1 h, then diluted with an equal volume of CH_2Cl_2, washed successively with a soln. of KI (10%), NaS_2O_3 and ice-cold NaOH (dil.), dried and evaporated. Crystallization from CH_2Cl_2/Et_2O yielded pure **2** (3.12 g, 78%). M.p. 268–270°. $[\alpha]_D$ = + 17 (*c* = 0.49, $CHCl_3$). IR (CH_2Cl_2): 1700, 1670, 1620, 1160. ^{1}H-NMR: 1.02 (*s*, Me–C(13)); 1.46 (*s*, Me–C(10)); 3.32 (*d*, *J* = 4, H–C(11)); 5.86 (*s*, H–C(4)). Anal. calc. for $C_{22}H_{28}O_4$ (356.47): C 74.13, H 7.92; found: C 73.63, H 7.86.

9α,11-Epoxy-3-oxo-17α-pregna-4,6-diene-21,17-carbolactone (**3**). To a soln. of **2** (3.12 g, 8.76 mmol) in dioxane (50 ml), DDQ (2.18 g, 9.6 mmol) and 0.5N HCl in dioxane (135 ml) were added. The mixture was stirred at 20° for 90 min. The dark-colored soln. was diluted with CH_2Cl_2 and filtered through neutral Al_2O_3 (act. II; 150 g). Evaporation and recrystallization of the crude product from CH_2Cl_2/MeOH/Et_2O furnished pure **3** (1.66 g, 53%). M.p. 303–305°. $[\alpha]_D$ = – 165 (*c* = 0.411, $CHCl_3$). IR (CH_2Cl_2): 1770, 1670, 1655, 1620, 1590. ^{1}H-NMR: 1.07 (*s*, Me–C(13)); 1.44 (*s*, Me–C(10)); 3.35 (*d*, *J* = 4, H–C(11)); 5.83 (*s*, H–C(4)); 6.08 (*dd*, *J* = 10, 3, H–C(7)); 6.3 (*dd*, *J* = 10, 3, H–C(6)). Anal. calc. for $C_{22}H_{26}O_4$ (354.45): C 74.55, H 7.39; found: C 74.60, H 7.52.

7α-(Acetylthio)-9α,11-epoxy-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**4**). A soln. of **3** (10.62 g, 30.0 mmol) in MeOH (300 ml) and freshly distilled thioacetic acid (15 ml) was refluxed for 3.5 h under Ar, diluted with H_2O, (15 ml) concentrated to *ca.* 1/3 of the volume by destillation at 1 atm, and cooled. The crude crystallized product (10.2 g) was filtered, washed, dried, and recrystallized (CH_2Cl_2/MeOH) yielding **4** (9.3 g, 71.9%). M.p. 250–256° (dec.). $[\alpha]_D = -73.5$ ($c = 0.938$, $CHCl_3$). UV: 293 (19600). IR (CH_2Cl_2): 3053, 2968, 1770, 1679, 1621, 1420, 1377, 1354, 1190, 1159, 1010, 971. ^{1}H-NMR: 1.02 (*s*, Me–C(13)); 1.47 (*s*, Me–C(10)); 2.32 (*s*, MeCOS); 3.13 (*d*, $J = 2$, H–C(11)); 4.07 (*m*, H–C(7)); 5.83 (br. *s*, H–C(4)). MS: 430 (M^+), 415 ([M – Me]$^+$), 412 ([$M - H_2O$]$^+$), 388 ([$M - CH_2CO$]$^+$), 355 ([388 – SH]$^+$; Ml 325), 354 ([M – MeCOSH]$^+$). Anal. calc. for $C_{24}H_{30}O_5S$ (430.56): C 66.96, H 7.02, S 7.45; found: C 66.85, H 6.84, S 7.49.

3-Oxo-5α,17α-pregn-1-ene-21,17-carbolactone (**7**). At r.t., 3-oxo-5α-17α-pregnane-21,17-carbolactone (**5**) [10] (47.5 g, 138.1 mmol) was added to a stirred 0.35M Br_2 soln. in dioxane (495 ml, 173 mmol). After 5 min at r.t., the soln. was diluted with dioxane (145 ml) and dimethylformamide (400 ml), and after the addition of Li_2CO_3 (19 g) and LiBr (19 g), boiled under reflux for 2 h. The mixture was cooled, poured on 9.5 l of ice-water, acidified by 2N HCl (950 ml), and stirred for 30 min. The precipitated product was filtered off, washed with H_2O, dried, and then dissolved in CH_2Cl_2 and the soln. filtered through neutral Al_2O_3 (act. II, 950 g). The crude product obtained after evaporation was chromatographed on silica gel (toluene/AcOEt 95:5). The uniform fractions were recrystallized from CH_2Cl_2/Et_2O yielding pure **7** (19.3 g, 40.7%). M.p. 164–166°. $[\alpha]_D = +25$ ($c = 0.668$, $CHCl_3$). UV: 228 (9240). IR (CH_2Cl_2): 1770, 1675, 1180, 1120. ^{1}H-NMR: 0.98 (*s*, Me–C(13)); 1.03 (*s*, Me–C(10)); 5.90 (*d*, $J = 10$, H–C(2)); 7.15 (*d*, $J = 10$, H–C(1)). Anal. calc. for $C_{22}H_{32}O_3$ (344.50): C 77.16, H 8.83; found: 76.95, H 8.81.

Potassium 17-Hydroxy-3-oxo-5α,17α-pregn-1-ene-21-carboxylate (**8**). A suspension of **7** (0.5 g, 1.46 mmol) in EtOH (10 ml) was treated with 0.146N KOH soln. (9.51 ml, 1.39 mmol) and boiled under reflux for 6 h. The clear soln. was concentrated to *ca.* 10 ml, diluted with H_2O, and extracted twice with $CHCl_3$. The aq. phase was frozen and lyophilized, yielding amorphous **8** (470 mg, 81%). IR (nujol): 3400, 1585, 1570, 1450. Anal. calc. for $C_{22}H_{31}KO_4$ (398.59): K 9.81; found: K 8.67 (the compound contains 8.72% of H_2O!).

Schematic Presentation of the General Procedure for the Microbial Transformation of **5**[11]. BHl slant of *Corynebacterium simplex* 28°, 24 h) → 2-l flask, 500 ml NL 145[12]) (22 h, 28°, 120 rpm) → 2% *v*/*v* → 30-l fermenter (NL 145[12]), 12 h, 1 l/l/min air, 28°, 1 bar pressure, 750 rpm) → 2.5% *v*/*v* → 30-l fermenter (NL 146[13]), 1 l/l/air, 28°, 1 bar pressure, 750 rpm). After 18 h, a soln. of **5** (7.5 g, 21.8 mmol) in MeOH (375 ml) is added directly to the culture broth and the transformation process then proceeds for 24 h under the same conditions. When the reaction is completed, the pH of the mixture is immediately adjusted to 2.0 by addition of H_2SO_4, and recovery follows.

9α-Hydroxy-3-oxo-5α,17α-pregn-1-ene-21,17-carbolactone (**9**). To a crude transformation soln. (370 l) of **5** (84.0 g, 0.244 mol), AcOEt (330 l) was added. The mixture was stirred for 15 min, the two phases were separated (sludge separator BRPX-207) and washed with H_2O (10 l) and AcOEt (10 l). The AcOEt extract (300 l) was subsequently concentrated (*in vacuo*) to 0.3 l and allowed to stand at 0° for 1 h. The precipitate was filtered and washed with a small amount of ice-cold AcOEt to yield yellowish crystals of crude **9** (49.19 g, 56%). The mother liquor was evaporated and the residue distributed between MeOH (80%, 350 ml) and heptane (500 ml). Evaporation of the heptane phase yielded 95 g of an oily mixture. A brownish, partially crystalline residue (27.0 g) obtained from the MeOH phase was dissolved in AcOEt (50 ml) and allowed to crystallize at 0°, to yield a 2nd portion of **9** (4.18 g, 4.75%). The two crystalline fractions were purified by chromatography on alumina (act. II, CH_2Cl_2, $CHCl_3$) and twice on silica gel (CH_2Cl_2/AcOEt/MeOH 90:9:1) and furnished nearly pure **9** (36.3 g, 41,8%; containing *ca.* 5% of the corresponding saturated compound), which was used without further purification in the following transformations. A sample was twice recrystallized from CH_2Cl_2/Et_2O. M.p. 237–239°. $[\alpha]_D = +8$ ($c = 0.707$, $CHCl_3$). UV: 225 (11000). IR (CH_2Cl_2): 3600, 1770, 1675, 1190. ^{1}H-NMR: 0.99 (*s*, Me–C(13)); 1.15 (*s*, Me–(10)); 5.95 (*d*, $J = 10$, H–C(2)); 7.15 (*d*, $J = 10$, H–C(1)). ^{13}C-NMR: 156.5 (C(1)); 129.6 (C(2)); 199.4 (C(3)); 40.8 (C(4)); 37.0 (C(5)); 27.3 (C(6)); 24.9 (C(7)); 38.1 (C(8)); 74.9 (C(9)); 44.6 (C(10)); 27.3 (C(11)); 27.3 (C(12)); 45.9 (C(13)); 42.2 (C(14)); 22.7 (C(15)); 35.7 (C(16)); 95.9 (C(17)); 14.9 (C(18)); 13.8 (C(19)); 31.6 (C(20)); 29.4 (C(21)); 176.8 (C(21^1)). Anal calc. for $C_{22}H_{30}O_4$ (358.48): C 73.71, H 8.40; found: 73.83, H 8.33.

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[11]) We would like to thank Mr. *Alan Smith* (Research Development Department) for the compilation of this process.

[12]) NL 145 Formula: peptone C (5.0 g/l), corn-steep powder (2.5 g/l), and cerelose (5.0 g/l); before sterilization, the pH is adjusted with KOH to 7.0.

[13]) NL 146 Formula: yeast extract (*Difco*); (1 g/l); before sterilization the pH is corrected to 6.0 with H_2SO_4.

From the more polar fractions of the chromatograms, *3β,9α-dihydroxy-5α,17α-pregnane-21,17-carbolactone* (1.03 g) was isolated. M.p. 320–324° (from CH_2Cl_2/MeOH/Et_2O). IR (nujol): 3460, 3410, 1750, 1735(sh), 1195. ^{1}H-NMR: 0.96(*s*, Me–C(13) or Me–C(10)); 0.94(*s*, Me–C(13) or Me–C(10)); 3.60(*m*, H–C(3)). Anal. calc. for $C_{22}H_{34}O_4$ (362.51): C 72.89, H 9.45; found: C 72.49, H 9.16.

In addition, *9α-hydroxy-3-oxo-5α,17α-pregnane-21,17-carbolactone* (2.06 g) was obtained after recrystallization (CH_2Cl_2/Et_2O) of the corresponding fractions. M.p. 220–222°. IR (CH_2Cl_2): 3605, 1767, 1710, 1190. ^{1}H-NMR: 0.97(*s*, Me–C(13)); 1.17(*s*, Me–C(10)). Anal. calc. for $C_{22}H_{32}O_4$ (360.49): C 73.30, H 8.95, O 17.75; found: C 73.61, H 8.88, O 17.78.

3-Oxo-5α,17α-pregna-1,9(11)-diene-21,17-carbolactone (**10**). To a precooled (4°) soln. of **9** (9.76 g, 27.3 mmol) in CH_2Cl_2 (390 ml), chlorosulfonic acid (5.08 ml, 76.2 mmol) was added within 30 s. The mixture was stirred for another 15 min at 4°, diluted with Et_2O (1000 ml), washed subsequently with ice-cold dil. NaOH soln., dil. HCl soln., and H_2O, dried, and evaporated. Chromatography (silica gel, toluene/AcOEt (95:5) and crystallization (CH_2Cl_2/Et_2O) yielded pure **10** (5.15 g, 55.6%). M.p. 218–219°. $[\alpha]_D = -60$ ($c = 0.352$, $CHCl_3$). IR (CH_2Cl_2): 1765, 1680, 1190. ^{1}H-NMR: 0.90(*s*, Me–C(13)); 1.17(*s*, Me–C(10)); 5.57(*d*, $J = 6$, H–C(11)); 5.92(*d*, $J = 10$, H–C(2)); 71.5(*d*, $J = 10$, H–C(1)). Anal. calc. for $C_{22}H_{28}O_3$ (340.47): C 77.61, H 8.29; found: C 77.51, H 8.20.

9α,11-Epoxy-5α,17α-pregn-1-ene-21,17-carbolactone (**11**). A soln. of **10** (0.41 g, 1.2 mmol) and 3-$ClC_6H_4CO_3H$ (85%; 0.36 g, 1.77 mmol) in CH_2Cl_2 (8.5 ml) was left for 5 h at 4°, then diluted with CH_2Cl_2, washed subsequently with an excess of aq. KI and $Na_2S_2O_3$ soln., ice-cold dil. NaOH soln., and H_2O, dried, and evaporated. The crude crystalline product was twice recrystallized from CH_2Cl_2/Et_2O: pure **11** (300 mg, 69.9%). M.p. 275–277°. $[\alpha]_D = -27$ ($c = 0.402$, $CHCl_3$). IR (CH_2Cl_2): 1770, 1680, 1020. ^{1}H-NMR: 1.04(*s*, Me–C(13)); 1.34(*s*, Me–C(10)); 3.60(*d*, $J = 4$, H–C(11)); 5.90(*d*, $J = 10$, H–C(2)); 6.72(*d*, $J = 10$, H–C(1)). Anal. calc. for $C_{22}H_{28}O_4$ (356.47): C 74.13, H 7.92; found: C 74.22, H 7.87.

Potassium 9α,11-Epoxy-17-hydroxy-3-ono-5α,17α-pregn-1-ene-21-carboxylate (**12**). A mixture of **11** (2.14 g, 6.0 mmol), THF (110 ml), 0.84N aq. KOH (6.44 ml, 5.41 mmol), and H_2O (21.5 ml) was stirred for 18 h at 50°. After cooling the soln. to r.t., addition of H_2O (75 ml), and evaporation of THF, H_2O (215 ml) was added. After an additional 30 min, the precipitate was sucked off, the filtrate extracted with Et_2O, and the aq. phase frozen and lyophilized to furnish **12** (1.8 g, 72.7%). Amorphous powder. IR (nujol): 4320, 3200, 1690, 1675, 1570. ^{1}H-NMR (D_2O): 0.90(*s*, Me–C(13)); 1.32(*s*, Me–C(10)); 3.89(*d*, $J = 4$, H–C(11)); 5.95(*d*, $J = 11$, H–C(2)); 7.05(*d*, $J = 11$, H–C(1)). Anal. calc. for $C_{22}H_{29}KO_5$ (412.57): K 8.72; found: K 8.56 (the compound contains 2 mol of H_2O).

6α,7α-Dihydro-3-oxo-3'H-cyclopropa[6,7]-17α-pregna-4,9(11)-diene-21,17-carbolactone (**14**). To a soln. of trimethylsulfoxonium iodide (30.4 g, 138 mmol) in DMSO (100 ml) was added a 72% suspension of NaH in mineral oil (4.37 g, 131 mmol) and the mixture stirred for 1 h at r.t. After addition of **13** [9] (7.8 g, 23.1 mmol) and rinsing with DMSO (8 ml), stirring at r.t. was continued for an additional 2 h. The mixture was poured on ice-water, acidified with 2N HCl, and extracted with AcOEt. The org. phase was shaken with sat. NaCl soln., ice-cold aq. NaOH soln., and again with sat. NaCl soln., dried (Na_2SO_4) and evaporated. Chromatography (silica gel (50-fold amount), toluene/AcOEt 85:15) and recrystallization from CH_2Cl_2/AcOEt gave pure **14** (4.1 g, 50.5%). M.p. 174–178°. $[\alpha]_D = -316$ ($c = 0.443$, $CHCl_3$). IR (CH_2Cl_2): 1770, 1670, 1600, 1190. ^{1}NMR: 0.93(*s*, Me–C(13)); 1.28(*s*, Me–C(10)); 5.51(*m*, H–C(11)); 6.00(*s*, H–C(4)). Anal. calc. for $C_{23}H_{28}O_3$ (352.48): C 78.38, H 8.01; found: C 78.26, H 7.95.

6α,7α-Dihydro-3-oxo-3'H-cyclopropa[6,7]-17α-pregna-1,4,9(11)-triene-21,17-carbolactone (**15**). A soln. of **14** (320 mg, 0.91 mmol) and DDQ (320 mg, 1.4 mmol) in dioxane (5 ml) was stirred for 17 h at 100° and subsequently evaporated. The obtained residue was dissolved in CH_2Cl_2 and filtered through a column of neutral Al_2O_3 (act. II), yielding amorphous **15**. The compound was used in the following step without further purification.

9α,11-Epoxy-6α,7α-dihydro-3-oxo-3'H-cyclopropa[6,7]-17α-pregna-1,4-diene-21,17-carbolactone (**16**). A soln. of **15** (170 mg, 0.48 mmol) in CH_2Cl_2 (7 ml) was treated for 20 h at r.t. with 3-$ClC_6H_4CO_3H$ (85%; 140 mg, 0.69 mmol). Following dilution with additional amounts of CH_2Cl_2, consecutive washing with dil. ice-cold aq. NaOH soln. and H_2O, the soln. was dried and evaporated. The crude product was purified by prep. TLC (2 1 m long plates, toluene/Et_2O) and recrystallization from CH_2Cl_2/Et_2O): **16** (62 mg, 34.9%). M.p. 295–296°. $[\alpha]_D = -264$ ($c = 0.425$, $CHCl_3$). IR (CH_2Cl_2): 1770, 1660, 1626, 1600, 1160. ^{1}H-NMR: 1.00(*s*, Me–C(13)), 1.38(*s*, Me–C(10)); 3.47(*d*, $J = 4$, H–C(11)); 6.28(*dd*, $J = 8, 1.5$, H–C(2)); 6.42(*d*, $J = 1.5$, H–C(4)); 6.55(*d*, $J = 8$, H–C(1)). MS: 367 ($[M + H]^+$). Anal. calc. for $C_{23}H_{26}O_4$ (366.46): C 75.38, H 7.15; found: C 74.68, H 7.02.

9α,11-Epoxy-6β,7β-dihydro-3-oxo-3'H-cyclopropa[6,7]-17α-pregn-4-ene-21,17-carbolactone (**17**). To a soln. of trimethylsulfoxonium iodide (5.7 g, 25.9 mmol) in DMSO (19 ml), a 72% suspension of NaH in mineral oil (0.82 g, 24.6 mmol) was added and the mixture stirred for 1 h at r.t. After addition of **3** (1.46 g, 4.12 mmol) and

rinsing with DMSO (*ca.* 1.5 ml) stirring at r.t. was continued for an additional 3.5 h. The mixture was poured on ice-water, acidified with 2N HCl, and extracted with AcOEt. The org. phase was shaken with sat. NaCl soln., ice-cold aq. NaOH soln. and again with sat. NaCl soln., dried (Na_2SO_4), and evaporated. Chromatography (silica gel (100-fold weight of the residue, toluene/AcOEt 95:5) and recrystallization from CH_2Cl_2/Et_2O yielded pure **17** (205 mg, 13.5%). $[\alpha]_D = +24$ ($c = 0.405$, $CHCl_3$). UV 257 (18800). IR (CH_2Cl_2): 1770, 1655, 1610. ^{1}H-NMR: 1.00 (*s*, Me–C(13)); 1.41 (*s*, Me–C(10)); 3.06 (*d*, $J = 5.7$, H–C(11)); 6.00 (*s*, H–C(4)). ^{13}C-NMR: 33.1 (C(1)); 31.6 (C(2)); 197.0 (C(3)); 128.0 (C(4)); 169.5 (C(5)); 17.1 (C(6)); 14.7 (C(7)); 31.2 (C(8)); 63.6 (C(9)); 38.0 (C(10)); 49.4 (C(11)); 27.2 (C(12)); 43.7 (C(13)); 39.3 (C(14)); 21.9 (C(15)); 35.3 (C(16)); 94.7 (C(17)); 16.2 (C(18)); 22.0 (C(19)); 31.3 (C(20)); 29.0 (C(21)); 176.2 (C(21^1)); 10.7 (CH_2(3′)). Anal. calc. for $C_{23}H_{28}O_4$ (368.48): C 74.97, H 7.66; found: C 74.87, H 7.53.

9α,11-Epoxy-6α,7α-dihydro-3-oxo-3′H-cyclopropa[6,7]-17α-pregn-4-ene-21,17-carbolactone (**18**). A soln. of **14** (15.7 g, 44.6 mmol) in $CHCl_3$ (630 ml) was treated with 3-$ClC_6H_4CO_3H$ (80%; 11.3 g, 49.3 mmol) and kept for 2 h at r.t. The mixture was successively washed with dil. KI and $Na_2S_2O_3$ soln., ice-cold 2N NaOH and H_2O, dried and evaporated. Chromatography (silica gel (100-fold amount) toluene/AcOEt 80:20) and recrystallization (CH_2Cl_2/Et_2O) afforded pure **18** (11.8 g, 75%). M.p. 299–301°. $[\alpha]_D = -293$ ($c = 0.556$, $CHCl_3$). UV: 260 (18800). IR (CH_2Cl_2): 1770, 1660, 1600, 1190. ^{1}H-NMR: 0.97 (*s*, Me–C(13)); 1.34 (*s*, Me–C(10)); 3.27 (*d*, $J = 4$, H–C(11)); 6.11 (*s*, H–C(4)). ^{13}C-NMR: 33.2 (C(1)); 29.8 (C(2)); 196.9 (C(3)); 128.3 (C(4)); 167.0 (C(5)); 18.7 (C(6)); 18.4 (C(7)); 35.7 (C(8)); 67.4 (C(9)); 38.8 (C(10)); 54.0 (C(11)); 31.4 (C(12)); 44.1 (C(13)); 41.5 (C(14)); 22.3 (C(15)); 35.3 (C(16)); 94.5 (C(17)); 16.6 (C(18)); 21.9 (C(19)); 31.2 (C(20)); 29.1 (C(21)); 176.1 (C(22^1)), 19.4 (CH_2(3′)). Anal. calc. for $C_{23}H_{28}O_4$ (368.48): C 74.97, H 7.66; found: C 74.95, H 7.85.

7α-Cyano-3-oxo-17α-pregna-4,9(11)-diene-21,17-carbolactone (**19**). To 270 ml (*ca.* 0.6 mol) of a stirred ice-cold soln. of HCN (66.7 g) in benzene (1 l), 530 ml (*ca.* 1.03 mol) of $AlEt_3$ (180 ml in 480 ml of benzene) were added dropwise with 2 h. Stirring at r.t. was continued for additional 20 h. The thus obtained soln. of diethylaluminium cyanide was added to **13** (80 g, 236 mmol) in THF (1600 ml). The mixture was boiled under reflux for 30 min, cooled (ice/MeOH), poured under Ar on crushed ice (4 kg) and 1N NaOH (1600 ml), diluted with sat. $NaKC_4H_4O_6$ soln. (*Seignette* salt; 5000 ml) and extracted with CH_2Cl_2. The org. phase was washed with H_2O, ice-cold 2N HCl, and H_2O, dried, and evaporated. The brownish amorphous residue was filtered through silica gel yielding amorphous **19** (82 g), which was used without further purification in the next step. A small sample was rechromatographed and crystallized (CH_2Cl_2/Et_2O): pure **19**. M.p. 241–243°. $[\alpha]_D = +28$ ($c = 0.560$, $CHCl_3$). IR (CH_2Cl_2): 2240, 1770, 1675, 1620. ^{1}H-NMR: 0.98 (*s*, Me–C(13)); 1.39 (*s*, Me–C(10)); 3.14 (*m*, H–C(7)); 5.80 (*m*, H–C(11)); 5.90 (*s*, H–C(4)). Anal. calc. for $C_{23}H_{27}NO_3$ (365.48): C 75.59, H 7.41, N 3.83; found: C 75.34, H 7.41, N 4.02.

7α-Carboxy-3-oxo-7α-pregna-4,9(11)-diene-21,17-carbolactone (**21**). A well stirred, cooled (5°) soln. of **19** (6.8 g, 18.6 mmol) in 1,2-dimethoxyethane (475 ml), was treated with *ca.* 20% DIBAH in toluene (95 ml, 114 mmol). Stirring was continued for 2 h (ice-cooling), then (at 0–5°) successively 4N HCl (95 ml) and H_2O (680 ml) were added. The mixture was extracted twice with CH_2Cl_2 and the org. phase dried and evaporated. The isolated mixture of isomeric hydroxyaldehydes **20** (6.9 g) was dissolved in acetone and treated at 5–10° with 8N CrO_3 in aq. H_2SO_4 (32 ml) and stirred for 30 min at 5°. After successive addition of EtOH (3.2 ml) and sat. NaCl soln. (420 ml), the mixture was extracted with AcOEt and washed with ice-cold sat. $NaHCO_3$ soln. The stirred ice-cold alkaline aq. phase was acidified with conc. HCl soln., extracted with $CHCl_3$, dried, and evaporated. The obtained crude amorphous acid **21** (6.6 g) was used in the following experiments without further purification.

7α-(Methocycarbonyl)-3-oxo-17α-pregna-4,9(11)-diene-21,17-carbolactone (**22**). To a soln. of crude **21** (3.5 g, *ca.* 9.5 mmol) in CH_2Cl_2 (35 ml) a soln. of CH_2N_2 in Et_2O was added dropwise. After the evolution of N_2 had ceased, the soln. was stirred for another 10 min and then evaporated. The residue was purified by filtrating its soln. in CH_2Cl_2 through a short column of neutral Al_2O_3 (act. II) and crystallization (CH_2Cl_2/Et_2O): **22** (2.7 g, 68.8% rel. to **19**). M.p. 205–206°. $[\alpha]_D = \pm 0$ ($c = 0.434$, $CHCl_3$). IR (CH_2Cl_2): 1765, 1735, 1670, 1620. ^{1}H-NMR: 0.96 (*s*, Me–C(13)); 1.41 (*s*, Me–C(10)); 3.59 (*s*, COOMe); 5.66 (*m*, H–C(11)); 5.73 (*s*, H–C(4)). ^{13}C-NMR: 35.8 (C(1)); 33.8 (C(2)); 198.4 (C(3)); 125.8 (C(4)); 166.4 (C(5)); 33.0 (C(6)); 43.9 (C(7)); 40.4 (C(8)); 142.4 (C(9)); 40.6 (C(10)); 119.0 (C(11)); 34.2 (C(12)); 44.5 (C(13)); 43.1 (C(14)); 23.3 (C(15)); 35.5 (C(16)); 95.0 (C(17)); 14.1 (C(18)); 27.2 (C(19)); 31.5 (C(20)); 29.2 (C(21)); 176.3 (C(21^1)); 172.6 (*C*OOMe); 51.4 (COO*Me*). Anal. calc. for $C_{24}H_{40}O_5$ (398.50): C 72.34, H 7.59; found: C 72.18, H 7.28.

7α-(Ethoxycarbonyl)-3-oxo-17α-pregna-4,9(11)-diene-21,17-carbolactone (**23**). To a suspension of **21** (7.2 g, 19.6 mmol) in dry benzene (45 ml), DBU (2.88 ml, 19.3 mmol) and EtBr (5.6 ml, 75 mmol) were added. The mixture was refluxed for 3 h, cooled to r.t., diluted with NaCl soln., and extracted with AcOEt. The org. phase was successively washed with ice-cold dil. HCl soln., NaOH, and sat. NaCl soln., dried, and evaporated. Chromatography of the brown residue (silica gel (50-fold amount), CH_2Cl_2/acetone 93:7) and recrystallization from

CH_2Cl_2/MeOH (with small amount of hexane) gave pure **23** (3.6 g, 48.3%). M.p. 128–129°. $[\alpha]_D = -4$ ($c = 0.898$, $CHCl_3$). IR (CH_2Cl_2): 1770, 1725, 1665, 1620. ^{1}H-NMR: 0.98 (*s*, Me–C(13)); 1.17 (*t*, $J = 7$, *Me*CH_2O); 1.41 (*s*, Me–C(10)); 4.10 (*q*, $J = 7$, MeCH_2O); 5.60 (*m*, H–C(11)); 5.75 (*s*, H–C(4)). Anal. calc. for $C_{25}H_{32}O_5$ (412.53): C 72.79, H 7.82; found: C 72.82, H 7.88.

7α-(Isopropoxycarbonyl)-3-oxo-17α-pregna-4,9(11)-diene-21,17-carbolactone (**24**). As described for **23**, with **21** (13 g, 35.3 mmol), benzene (80 ml), DBU (5.2 ml, 34.8 mmol), and i-PrBr (5.6 ml, 75 mmol): **24** (8.35 g, 60.8%). M.p. 138–139°. $[\alpha]_D = -12$ ($c = 0.816$, $CHCl_3$). IR (CH_2Cl_2): 1770, 1720, 1670, 1620. ^{1}H-NMR: 1.14 (*d*, $J = 6$, *Me*$_2$CH); 1.40 (*s*, Me–C(10)); 4.92 (*sept.*, $J = 6$, Me$_2$C*H*); 5.60 (*m*, H–C(11)); 5.76 (*s*, H–C(4)). Anal. calc. for $C_{26}H_{34}O_5$ (426.56): C 73.21, H 8.03; found: C 73.18, H 8.10.

9α,11-Epoxy-7α-(methoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**25**). To an ice-cooled soln. of **22** (10.0 g, 25.1 mmol in CH_2Cl_2 (100 ml)), 30% H_2O_2 soln. (10 ml, 89 mmol) and, within 90 min (CCl_3COl_2O 4.8 ml) in CH_2Cl_2 (12 ml) were added keeping the temp. below 4°. After addition of CH_2Cl_2 (200 ml), the mixture was washed with H_2O, 5% $Na_2S_2O_3$ soln., and ice-cold dil. aq. $NaHCO_3$ soln., dried, and evaporated. The residue was recrystallized at −15° (CH_2Cl_2/i-PrOH) yielding **25** (7.45 g, 71.6%). M.p. 240–242°[14]. $[\alpha]_D = +5$ ($c = 0.437$, $CHCl_3$). IR: 1770, 1740(sh), 1730, 1670, 1620. UV 240 (16800). ^{1}H-NMR: 1.06 (*s*, Me–C(13)); 1.52 (*s*, Me–C(10)); 3.15 (*d*, $J = 4$, H–C(11)); 3.66 (*s*, COOMe); 5.98 (*m*, H–C(4)). ^{13}C-NMR: 33.2(C(1)); 27.1(C(2)); 198.0(C(3)); 127.2(C(4)); 164.9(C(5)); 34.9(C(6)); 38.9(C(7)); 37.4(C(8)); 65.4(C(9)); 39.8(C(10)); 51.6(C(11)); 31.1(C(12)); 44.0(C(13)); 41.4(C(14)); 22.2(C(15)); 35.1(C(16)); 94.6(C(17)); 16.3(C(18)); 22.4(C(19)); 31.0(C(20)); 29.0(C(21)); 176.2(C(21^1)); 172.6(*C*OOMe); 51.6(COO*Me*). MS: 414(M^+), 399([M − Me]$^+$), 396([M − H_2O]$^+$), 355([M − COOMe]$^+$), 337([355 − H_2O]$^+$). Anal. calc. for $C_{24}H_{30}O_6$ (414.50): C 69.55, H 7.30; found: C 69.44, H 7.32.

9α,11-Epoxy-7α-(ethoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**26**). A soln. of **23** (1.9 g, 4.6 mmol) in CH_2Cl_2 (20 ml) was reacted under stirring at r.t. for 6 h with K_2HPO_4 (965 mg, 5.5 mmol), CCl_3CN (2.9 ml, 28 mmol), and 30% H_2O_2 soln. (8.9 ml, 79 mmol). Usual workup yielded an amorphous crude product which was chromatographed (silica gel (50-fold amount), CH_2Cl_2/acetone 95:5) and crystallized from CH_2Cl_2/Et_2O: pure **26** (1.05 g, 53.3%). M.p. 177–179°. $[\alpha]_D = +2$ ($c = 0.584$, $CHCl_3$). IR (CH_2Cl_2): 1770, 1730, 1670, 1620. ^{1}H-NMR: 1.03 (*s*, Me–C(13)); 1.23 (*t*, $J = 7$, *Me*CH_2O); 1.50 (*s*, Me–C(10)); 3.11 (*d*, $J = 4$, H–C(11)); 4.11 (*m*, MeCH_2O); 5.92 (*s*, H–C(4)). Anal. calc. for $C_{25}H_{32}O_6$ (428.53): C 70.07, H 7.53; found: C 69.99 H 7.64.

9α,11-Epoxy-7α-(isopropoxycarbonyl)-3-oxo-17α-pregn-4-ene-21,17-carbolactone (**27**). After the addition of K_2HPO_4 (4.68 g, 26.9 mmol), CCl_3CN (14 ml, 136 mmol), and 30% H_2O_2 soln. (42.1 ml, 375 mmol) to a cooled soln. of **24** (9.36 g, 22.8 mmol) in CH_2Cl_2 (95 ml), the mixture was stirred for 5 h at r.t. (control of the initial exothermic reaction). The mixture was diluted with CH_2Cl_2, washed with H_2O, Na_2SO_3 soln. and dil. $NaHCO_3$ soln., dried, and evaporated. Chromatography (neutral silical gel (50-fold amount), CH_2Cl_2/acetone 94:6) and crystallization from CH_2Cl_2/Et_2O gave pure **27** (4.86 g, 50%). M.p. 177–179°. $[\alpha]_D = -5$ ($c = 0.872$, $CHCl_3$). IR (CH_2Cl_2): 1770, 1730, 1670, 1620. ^{1}H-NMR: 1.03 (*s*, Me–C(13)); 1.22 (*d*, $J = 6$, *Me*$_2$CH); 1.50 (*s*, Me–C(10)); 3.13 (*d*, $J = 4$, H–C(11)); 5.00 (*sept.*, $J = 6$ Me$_2$C*H*); 5.95 (*s*, H–C(4)). ^{13}C-NMR: 33.2(C(1)); 31.0(C(2)); 197.8(C(3)); 127.3(C(4)); 165.2(C(5)); 35.2(C(6)); 38.8(C(7)); 37.3(C(8)); 65.6(C(9)); 39.9(C(10)); 51.4(C(11)); 27.2(C(12)); 44.0(C(13)); 41.5(C(14)); 22.0(C(15)); 35.2(C(16)); 94.6(C(17)); 16.3(C(18)); 21.6(C(19)); 31.3(C(20)); 29.0(C(21)); 176.2(C(21^1)); 171.6(*C*OOCHMe$_2$); 68.3(Me$_2$*C*H); 22.4, 22.2(*Me*$_2$CH). Anal. calc. for $C_{26}H_{34}O_6$ (442.56): C 70.57, H 7.74; found: C 70.41, H 7.76.

21-Hydroxypregna-4,9(11)-diene-3,20-dione (**29**). A suspension of **28** [7] (0.62 g, 1.67 mmol) in MeOH (40 ml) was treated with 3.1 ml (3.14 mmol) of a soln. of $NaHCO_3$ (276 mg in 3.24 ml H_2O). After stirring for 4 h at r.t., the soln. was neutralized by addition of AcOH (0.2 ml) and H_2O (30 ml). MeOH was eliminated by distillation, the mixture refrigerated, and the precipitate filtered, washed with H_2O, and crystallized from CH_2Cl_2/Et_2O: pure **29** (410 mg, 74.5%). M.p. 138–140°. $[\alpha]_D = +155$ ($c = 0.449$, $CHCl_3$). IR (CH_2Cl_2): 3520, 1710, 1670, 1620. Anal. calc. for $C_{21}H_{28}O_3$ (328.46): C 76.79, H 8.59; found: C 76.80, H 8.60.

9α,11-Epoxy-21-hydroxypregn-4-ene-3,20-dione (**30**). To a cooled soln. of **29** (200 mg, 0.61 mmol) in CH_2Cl_2 (4 ml), 3-Cl$C_6H_4CO_3H$ (85%; 180 mg, 0.88 mmol) was added. The mixture was kept at 4° for 5 h, then diluted with CH_2Cl_2, washed with dil. KI and $Na_2S_2O_3$ soln., ice-cold dil. aq. NaOH soln., and H_2O, dried, and evaporated. Two crystallizations from CH_2Cl_2/Et_2O yielded **30** (94 mg, 44.7%). M.p. 146–148°. $[\alpha]_D = +135$ ($c = 0.431$, $CHCl_3$). IR (CH_2Cl_2): 3500, 1710, 1675, 1620, 1075. ^{1}H-NMR: 0.71 (*s*, Me–C(13)); 1.45 (*s*, Me–C(10)); 3.25 (*d*, $J = 5$, H–C(11)); 4.20 (*d*, $J = 4$, 2H–C(2)); 5.82 (*s*, H–C(4)). Anal. calc. for $C_{21}H_{28}O_4$ (344.45): C 73.23, H 8.19; found: C 73.09, H 8.10.

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[14]) Crystallization from CH_2Cl_2/Et_2O gave better, but rather instable, transparent orthorhombic crystals (containing Et_2O), which were used for the X-ray analysis.

2. *Biology (Methods). Receptor Binding.* [^{3}H]Aldosterone (49 Ci/mmol), [^{3}H]methyltrienolone[15]) ([^{3}H]-R 1881, 87 Ci/mmol) and [^{3}H]progesterone (57 Ci/mmol) were purchased from *New England Nuclear*. Unlabeled R 1881 was obtained from *Roussel-Uclaf*. All other unlabeled steroids were prepared in our laboratories. Methods for measuring MR, AR, and PR binding have been reported [1]. Bound and free steroids were separated by the dextran-coated charcoal (DCC) technique [27]. Competition curves for the unlabeled standards of the corresponding [^{3}H]-ligand were run in each assay. The level of nonspecific binding was determined from incubations with 100-fold excess of unlabeled standard ligand. This value was subtracted from the total binding. The rel. binding affinity of each test compound was determined by comparing the concentration of test compounds required to produce 50% inhibition (IC_{50}) with that of the standard (IC_{50} (standard)): rel. binding affinity = IC_{50} (standard)/IC_{50}. In no case, the s.e. for the rel. binding affinity exceeded 40% of the mean and was usually $< 20\%$.

Electrolyte Excretion in vivo. Anti-mineralocorticoid activity was determined according to a method based on that of *Kagawa* [1] [10] [28] [29]. Male *Ivanovas* rats (Iva:SDIV) were used 4–12 days after bilateral adrenalectomy, during which time they received 1% NaCl in their drinking water. On the day before the test, the animal received twice 0.85% NaCl (20 ml/kg *p.o.*). Overnight (for a total of *ca.* 16 h) they were deprived of food and given access to tap water. Test compounds were given *p.o.* in water containing 2.5% carboxymethylcellulose 30 min before aldosterone (1 μg/kg/*s.c.*) and 0.85% NaCl (20 ml/kg *p.o.*). Urine was collected over the next 4 h. Urinary Na and K were determined using a flame photometer (*Ingold IL 243*). The dose required to inhibit the Na/K ratio lowering effect of aldosterone by 50% (ED_{50}) was used to determine relative potencies of test compounds. ED_{50}s of test compounds were compared to that of spironolactone (5 mg/kg *p.o.*).

The same procedure was used to measure mineralocorticoid activity, except that aldosterone was given only to control animals, and the test compounds were given subcutaneously. ED_{50}s of test compounds were compared to that of aldosterone (3 μg/kg *s.c.*).

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