93566-38-2; **10B**, 93566-39-3; EtCHO, 123-38-6; $BrCH_2CH{=}CH_2$, 106-95-6; *p*-$ClCOC_6H_4Cl$, 122-01-0; 2,3-$Cl_2C_6H_3NO_2$, 3209-22-1; 2-$CH_2{=}C(CH_3)CH_2C_6H_4OH$, 20944-88-1; 2-$CH_2{=}C(CH_3)$-$CH_2C_6H_4OK$, 93566-55-3; 2-Cl,6-$O_2NC_6H_3OC_6H_4$-2-$CH_2C(CH_3)$-${=}CH_2$, 93566-45-1; 2-Cl,6-$O_2NC_6H_3OC_6H_4$-2-$CH_2COCH_3$, 93566-46-2; 2-$CH_2{=}CHCH_2C_6H_4OH$, 1745-81-9; 2-$CH_2{=}$ $CHCH_2C_6H_4OK$, 79015-70-6; 2-Cl,3-$O_2NC_6H_3CH_3$, 3970-40-9; 2-$CH_3$,6-$O_2NC_6H_3OC_6H_4$-2-$CH_2CH{=}CH_2$, 93566-50-8; 2-$H_2N$,6-$CH_3C_6H_3OC_6H_4$-2-$CH_2CH{=}CH_2$, 93566-51-9; 2-AcNH,6-$CH_3C_6H_3OC_6H_4$-2-$CH_2CH{=}CH_2$, 93566-52-0; 2-AcNMe,6-$CH_3C_6H_3OC_6H_4$-2-$CH_2CH{=}CH_2$, 93566-53-1; fenclofenac, 34645-84-6; 6,8-dichlorodibenz[*b,f*]oxepin-10(11*H*)-one, 93566-40-6.

# Mineralocorticoid Properties of Potential Metabolites of 18-Hydroxydeoxycorticosterone and 18-Hydroxyprogesterone

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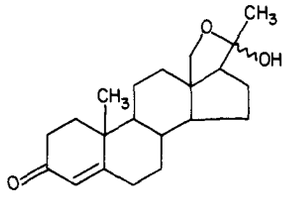
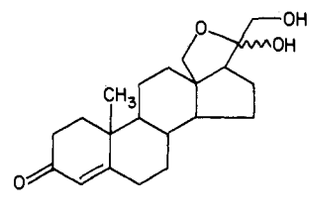
The high secretion rate of 18-hydroxydeoxycorticosterone in hypertensives and the steroids implication as a mineralocorticoid has led to the synthesis of potential di-, tetra-, and hexahydro metabolites of it and 18-hydroxyprogesterone. These potential metabolites have been synthesized by reduction of the double bond and the 3- and 20-ketones, singly or in combination. They have been evaluated for pro- and antimineralocorticoid activity and their affinity for the renal aldosterone receptor. All except one of the potential metabolites either lack or have reduced mineralocorticoid activity and aldosterone receptor binding affinity. The exception is the 3-ketopregn-4-ene-18,20-diol which has high receptor affinity but functions as an aldosterone antagonist.

The physiological role of 18-hydroxydeoxycorticosterone (**4G**) (18-OH-DOC) has been a controversial issue for a number of years. It has mineralocorticoid activity both in vivo[1] and in vitro[2] and, unlike the classical mineralocorticoids, is produced primarily in the zona fasciculata of the adrenal cortex[3] and is ACTH dependent.[4] However its presence in high concentration makes it a mineralocorticoid of potentially great significance.[5] Although the significance of 18-OH-DOC in human essential hypertension is debatable,[6] the parent compound or a metabolite may play a role in sensitizing the kidney to other hypertensinogenic hormones.[7]




**4E**

(18-hydroxyprogesterone)




**4G**

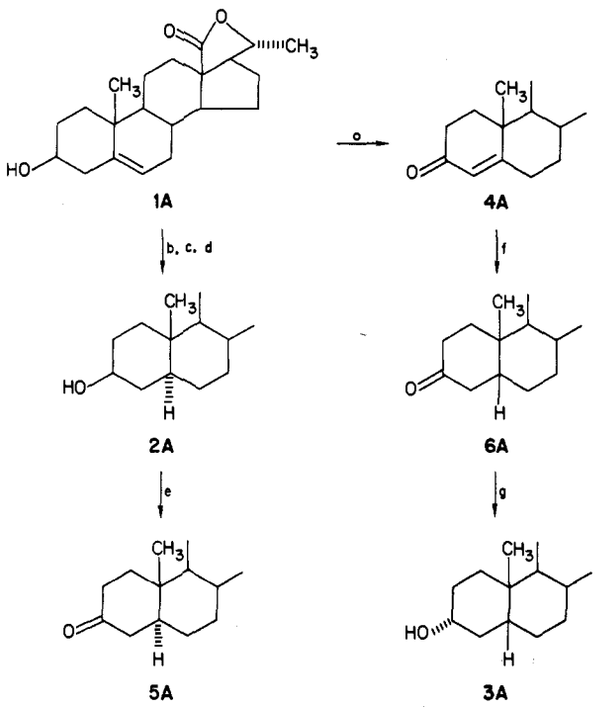
(18-hydroxydeoxycorticosterone)

Both oxidized and reduced metabolites of 18-OH-DOC have been isolated. Melby[8] isolated 16,18-dihydroxydeoxycorticosterone from human adrenal incubations.[9] Although inactive as a mineralocorticoid, this metabolite appeared to potentiate the action of aldosterone,[10] although this activity has not been confirmed.[11] Reduced metabolites of 18-OH-DOC have been observed after incubation with the adrenals and liver of adult rats. These metabolites are all tetrahydro derivatives derived from reduction of the A-ring enone, primarily the 3$\beta$-hydroxy-5$\alpha$-pregnane and 3$\alpha$-hydroxy-5$\beta$-pregnane derivatives, although the 3$\alpha$-hydroxy-5$\alpha$-pregnane has been tentatively identified.[12] This reduction pattern indicates that the metabolism of 18-OH-DOC is similar to that of progesterone[13] and would be expected to proceed through the saturated 3-ketones. Additionally, the reported isolation of the reduced 20-ketone metabolite of aldosterone indicates that this group is potentially capable of reduction in the 18-OH-DOC series.[14] At the inception of this work, none of the potential dihydro, tetrahydro, and hexahydrometabolites had been adequately characterized. During the course of this study, the 3$\alpha$-hydroxy-5$\beta$-pregnane (**2G**) was described[15a] and, after its completion, the preparation from 18-OH-DOC of a variety of AB-ring potential metabolites was published.[15b] To the best of our knowledge, no published report exists on the biological activity of the metabolites of 18-OH-DOC. As part of our continuing interest in 18-oxygenated steroids,[16] we have prepared a series of di-, tetra-, and hexahydro derivatives of both 18-hydroxyprogesterone (18-OH-PROG, **4E**) and 18-OH-DOC and evaluated them for

(1) Kagawa, C. M.; Pappo, R. *Proc. Soc. Exp. Biol. Med.* **1962**, *109*, 982.
(2) Feldman, D.; Funder, J. W. *Endocrinology* **1973**, *92*, 1389.
(3) Lucis, O. J.; Dyrenfurth, I.; Venning, E. H. *Can. J. Biochem.* **1961**, *39*, 901.
(4) Peron, F. G. *Endocrinology* **1961**, *60*, 39.
(5) Birmingham, M. K.; Ward, P. J. *J. Biol. Chem.* **1961**, *236*, 1661.
(6) Ulick, S. *Am. J. Cardiol.* **1976**, *38*, 814.
(7) Agrin, R. J.; Dale, S. L.; Holbrook, M.; LaRossa, J. T.; Melby, J. C. *J. Clin. Endocrinol. Metab.* **1978**, *47*, 877.
(8) Dale, S. L.; Melby, J. C. *Steroids* **1973**, *21*, 617.
(9) Kirk, D. N.; Sea Melo, M. L. *J. Chem. Soc., Perkin Trans 1* **1982**, 723.
(10) Melby, J. D.; Dale, S. L. *J. Steroid Biochem.* **1975**, *6*, 761.
(11) Fuller, P. J.; Pressley, L.; Adam, W. R.; Funder, J. W. *J. Steroid Biochem.* **1976**, *7*, 387.
(12) Prost, M.; Bournot, P.; Maume, B. F. *Steroids* **1975**, *25*, 177.
(13) Fieser, L. F.; Fieser, M. *Steroids* **1959**, 571.
(14) Aldercreutz, H. *Scand. J. Clin. Lab. Invest.* **1977**, *37* (147), 103.
(15) (a) Hossain, M.; Kirk, D. N. *Steroids* **1979**, *34*, 677. (b) Harnick, M.; Aharonowitz, Y.; Lamed, R. *Tetrahedron* **1982**, *38*, 3173.
(16) Chinn, L. J.; Desai, B. N.; Lenz, G. R. *J. Chem. Res. Synop.* **1976**, 284. Lenz, G. R. *J. Chem. Soc., Chem. Commun.* **1978**, 241. Lenz, G. R.; Dorn, C. R. *J. Org. Chem.* **1983**, *48*, 2696.

0022-2623/85/1828-0233$01.50/0 

Scheme I. Preparation of the AB-Ring Substitution Patterns for the Reduction Products of the 3-Keto 4-Ene Group[a]



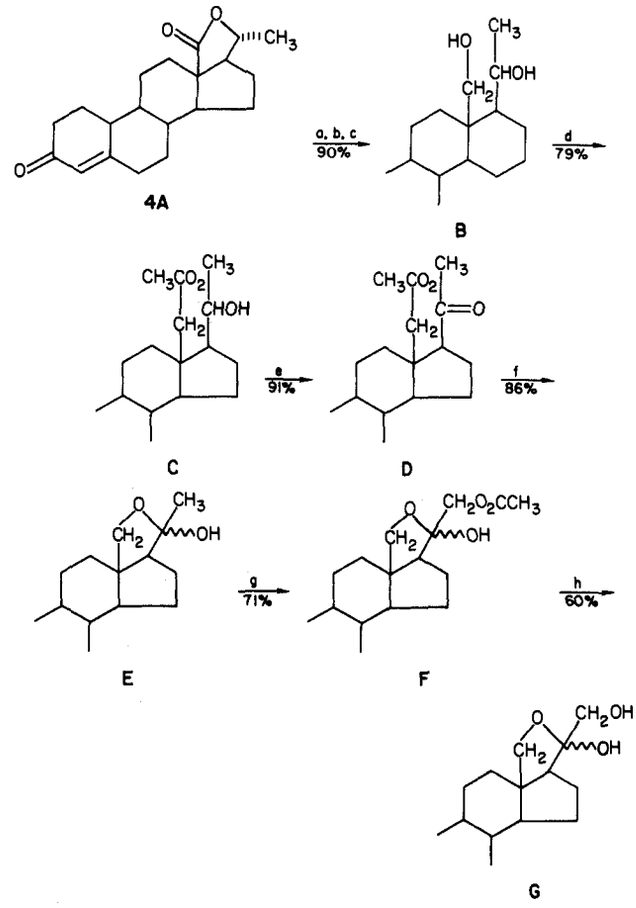

[a] Regents: (a) Modified Oppenauer, (b) acetic anhydride in pyridine, (c) $H_2$, Pd/C, (d) sodium methoxide in methanol, (e) Jones reagent, (f) $H_2$, Pd/C in pyridine solution, (g) lithium tri-*tert*-butoxyaluminum hydride.

their pro- and antimineralocorticoid effects, as well as for their aldosterone receptor binding affinities.

**Chemistry.** A synthesis of both 18-hydroxyprogesterone and deoxycorticosterone, starting from the steroidal alkaloid conessine, was reported by Pappo in 1959.[17] A subsequent preparation of 18-hydroxyprogesterone[18] started from readily available progesterone and used the hypoiodite reaction to introduce the requisite 18-hydroxyl group.[19] A significant advance occurred when it was discovered that a 21-hydroxyl group could be readily introduced into 18-hydroxyprogesterone derivatives.[20] Although these methods allowed access to individual 18-oxygenated derivatives, they were not considered by us as suitable for the preparation of a series of reduced potential metabolites. We wanted to use a common intermediate which would allow us to adjust the level of oxidation in the AB rings and subsequently to generate the 18,21-dihydroxy 20-ketone functionality. The dihydroxy ketone had to be introduced at the end of the synthetic sequence because the 18-hydroxy 20-ketone exists in the hemiacetal form and is prone to irreversible dimer formation.[21]

The preparation of the various AB-ring oxidation states from a common intermediate is illustrated in Scheme I. The readily available 18,20-lactone **1A**[22] was converted into

Scheme II. Synthesis of 18-Hydroxyprogesterone and 18-Hydroxydesoxycorticosterone[a]



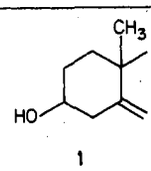
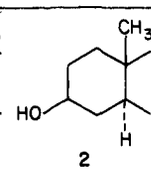
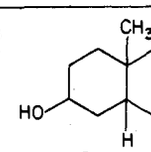
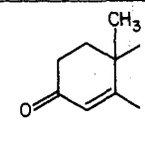
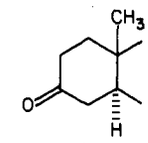
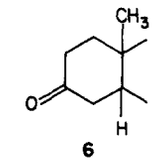
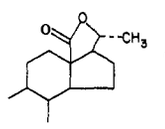
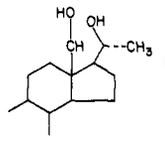
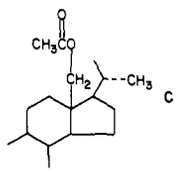
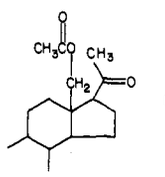
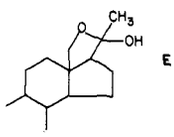
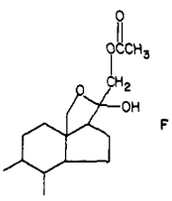
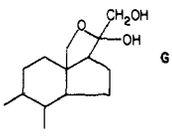

[a] Reagents: (a) ethylene glycol, *p*-toluenesulfonic acid, (b) sodium bis(methoxyethoxy)aluminum hydride, (c) aqueous acid, (d) 1.1 equiv of acetyl chloride, pyridine, −20 °C, (e) Jones reagent, (f) sodium hydroxide, (g) lead tetraacetate in acetic acid, (h) potassium hydroxide.

the 3-keto 4-ene lactone **4A** by using the modified Oppenauer oxidation.[23] Stereospecific reduction of **1A** and subsequent acetate hydrolysis furnished the 3β-hydroxy-5α series starting material **2A**.[24] Oxidation of **2A** then furnished the 3-keto 5α-lactone **5A**. The AB-cis (5β) series was entered by the catalytic hydrogenation of the enone **4A** in pyridine whereby the ketone **6A** was formed stereoselectively (**6A**/**5A** = 19/1).[25] Subsequent hydride reduction of the 3-ketone in **6A** yielded the 3α-hydroxy-AB-cis lactone **3A**.[26]

With the desired AB-rings oxidation patterns achieved in lactones **1A**–**6A**, introduction of the 18,21-dihydroxy 20-ketone functionality was accomplished as illustrated in Scheme II for enone **4A**. The 18,20β-diol **4B** was obtained by hydride reduction of the lactone ring in **4A** after ketal formation. The 18-hydroxyl group could be titrated in methylene chloride and pyridine with acetyl chloride at −20 to 0 °C to form the 18-acetoxy comp **4C** in very good yield.[27] The 20β-alcohol in **4C** was then readily oxidized

(17) Pappo, R. *J. Am. Chem. Soc.* **1959**, *81*, 1010.
(18) Meystre, Ch.; Heusler, K.; Kalvoda, J.; Wieland, P.; Anner, G.; Wettstein, A. *Helv. Chim. Acta* **1962**, *45*, 1317.
(19) Kalvoda, J.; Heusler, K. *Synthesis* **1971**, 501.
(20) (a) Kirk, D. N.; Rajagopalan, M. S. *J. Chem. Soc., Chem. Commun.* **1974**, 145. (b) *J. Chem. Soc., Perkin Trans 1* **1975**, 1860. (c) Biollaz, M.; Kalvoda, J.; Schmidlin, J. *Helv. Chim. Acta* **1975**, *58*, 1425.
(21) Kirk, D. N.; Rajagopalan, M. S. *J. Chem. Soc., Perkin Trans 1* **1976**, 1064.
(22) Hensler, K.; Wieland, P.; Meystre, Ch. "Organic Syntheses"; Wiley: New York, 1973; Collect. Vol. V, p 692.
(23) Raggio, M. L.; Watt, D. S. *J. Org. Chem.* **1976**, *41*, 1873.
(24) (a) Pierce, J. H.; Richards, H. C.; Shoppee, C. W.; Stephenson, R. J.; Summers, G. H. R. *J. Chem. Soc.* **1955**, 694. (b) Shoppee, C. W.; Agashe, B. D.; Summers, G. H. R. *Ibid.* **1957**, 3107.
(25) Tsuji, N.; Suzuke, J.; Shiota, M.; Takahashi, I.; Nishimura, S. *J. Org. Chem.* **1980**, *45*, 2729.
(26) Wheeler, D. M. S.; Wheeler, M. M. In "Organic Reactions in Steroid Chemistry"; Fried, J., Edwards, J. A., Eds.; Van Nostrand Reinhold: New York, **1972**; Vol. I, Chapter 2.

Table I. In Vivo and in Vitro Mineralocorticoid Activity of Metabolites of 18-OH-DOC and 18-OH-PROG

| compd | act. |  1 |  2 |  3 |  4 |  5 |  6 |
|---|---|---|---|---|---|---|---|
|  A | binding affinity[a] | 12.5 ± 10.5 | | | 65.0 ± 3.0[c] | 10.5 ± 9.5 | 0 ± 0 |
| | M/DOCA[b] | 0.72[d] | 0.61[d] | | 1.32[d] | 0.96 | 0.87 |
|  B | binding affinity | 5.0 ± 5.0 | 23.5 ± 22.5 | | 34.3 ± 7.9 ($N = 4$)[c] | 13.0 ± 9.0 | 6.5 ± 6.5 |
| | M/DOCA | 0.89 | 0.72[d] | | 1.33[d] | 1.03 | 0.43[d] |
|  C | binding affinity | | | | 13.0 ± 13.0 | 17.0 ± 7.0[c] | 10.5 ± 6.2 ($N = 4$)[c] |
| | M/DOCA | | | | 1.16 | 0.93 | 1.16 |
|  D | binding affinity | 0 ± 0 | 0 ± 0 | 14.25 ± 8.4[c] | 25.5 ± 2.5[c] | 0 ± 0 | 0 ± 0 |
| | M/DOCA | 1.95[d] | 0.99 | 1.13 | 1.19 | 1.10 | 1.08 |
|  E | binding affinity | 10.1 ± 10.1 | 30.0 ± 2[c] | | 34.5 ± 6.5[c] | 8.3 ± 0.2[c] | 0 ± 0 |
| | M/DOCA | 0.50[d] | 0.92 | 0.91 | 0.76[d] | 0.91 | 1.01 |
|  F | binding affinity | 7.0 ± 7.0 | 0 ± 0 | | 34 ± 3[c] | 20 ± 6[c] | 20 ± 7[c] |
| | M/DOCA | 0.55[d] | 1.10 | 0.83 | 0.71[d] | 0.80[d] | 0.96 |
|  G | binding affinity | 3.5 ± 2.5 | 0 ± 0 | 19.5 ± 4.5[c] | 29.5 ± 10.5[c] | 3.0 ± 3.0 | 32.0 ± 15.0[c] |
| | M/DOCA | 0.71[d] | 0.96 | 1.06 | 0.54[d] | 0.69[d] | 0.78[d] |

[a] Binding affinity expressed as the percent inhibition of $2 \times 10^{-9}$ M [$^3$H]aldosterone binding to the renal mineralocorticoid receptor by the presence of competing steroid ($8 \times 10^{-8}$ M). $N = 2$ unless otherwise indicated. [b] Mineralocorticoid activity in vivo expressed as a ratio (M/DOCA) of the urinary sodium/potassium excretion transformations (log Na × 10/K), after deoxycorticosterone acetate treatment, in the presence of competing steroid metabolite (M) and in the absence of that competing steroid (DOCA). [c] Significance is at $P < 0.05$ by Student's $t$ test. [d] Single determinations are compared against a mean control M/DOCA of 1.04 ± 0.19 (SD) ($N = 22$). Significance is concluded if an individual M/DOCA falls outside 1 SD. Ratios greater than 1.23 indicate mineralocorticoid antagonism and ratios less than 0.85 indicate mineralocorticoid-like (agonistic) activity.

to the 20-ketone **4D**, 18-acetoxyprogesterone, a compound which was accessible with difficulty by forced acetylation of 18-hydroxyprogesterone (**4E**).[18,21] Hydrolysis of the acetate in **4D** yielded 18-hydroxyprogesterone (**4E**), which was converted into 18-hydroxydeoxycorticosterone (**4G**) via the 21-acetate **4F**.[20a,b] In the 3-hydroxy series (**1A–3A**) the hydroxyl group was protected as its tetrahydropyranyl ether prior to reduction and carried through the reaction sequence from **1B–3B** to the 18-acetoxy 20-ketone compound **1D–3D** without isolation of intermediates. Buffered pyridinium chlorochromate was used to effect oxidation of the 20β-alcohol.[28] The triols **1B** and **2B** were prepared by direct hydride reduction of the acetates of **1A** and **2A**.

## Discussion

In the model series containing the 3β-hydroxy 5-ene moiety, mineralocorticoid agonistic activity was observed in vivo for the 18-hydroxy 20-ketone (**1E**) and 18,21-dihydroxy 20-ketone (**1G**) side chains (Table I). However,

(27) Graf, W.; Berner-Fenz, L.; Gossinger, E.; Imhof, R.; Wehrli, H. *Helv. Chim. Acta* **1970**, *53*, 2267.

(28) Corey, E. J.; Biggs, J. W. *Tetrahedron Lett.* **1975**, 2647.

since neither compound possessed significant in vitro receptor binding affinity, metabolic oxidation may have occurred. Biological conversion of the homoallylic alcohol in **1E** and **1G** to the conjugated enone would have formed the parent mineralocorticoids 18-hydroxyprogesterone (**4E**) and 18-hydroxydeoxycorticosterone (**4G**), respectively.

Reduction of the double bond in **4E** and **4G** formed the stereoisomeric potential dihydro metabolites, the 3-keto-AB-trans compounds **5E** and **5G** and the 3-keto-AB-cis compounds **6E** and **6G**. In the AB-trans series, the 18-hydroxyprogesterone derivative **5E** demonstrated significantly reduced receptor binding affinity in vitro and no mineralocorticoid activity in vivo. On the other hand, the 18-hydroxy DOC derivative **5G** did not possess in vitro receptor affinity but functioned as a mineralocorticoid by an unknown mechanism. The dihydro derivatives in the AB-cis series (**6E** and **6G**) demonstrated an interesting contrast. The dihydro-18-hydroxyprogesterone derivative **6E** had neither receptor affinity nor demonstrable mineralocorticoid activity. However, the 18-hydroxy DOC analogue **6G** was a potent mineralocorticoid with significant aldosterone receptor affinity.

Further reduction of the dihydro derivatives yielded the potential tetrahydro metabolites of 18-hydroxyprogesterone and 18-OH DOC in the 3$\beta$-hydroxy-5$\alpha$ series (**2E** and **2G**) and 3$\alpha$-hydroxy-5$\beta$ series (**3E** and **3G**). The 18-hydroxyprogesterone potential metabolite **2E** possessed high receptor affinity without observable mineralocorticoid activity, while the 18-hydroxy DOC derivative **2G** did not show any activity. In the epimeric series, the 18-hydroxyprogesterone analogue **3E** was not active while the 18-hydroxy DOC compound **3G** had moderate receptor affinity without in vivo mineralocorticoid activity.

Reduction of the 20-ketone generated a series of 18,20-diols in the di-, tetra-, and hexahydro series which show an interesting inversion in in vivo mineralocorticoid activity. The parent compound in this series, **4B**, a 3-keto 4-ene, showed strong receptor affinity and an aldosterone antagonistic profile in vivo. This latter activity may be intrinsic or the result of metabolic oxidation to the lactone **4A** which possesses the same biological profile as **4B**. However, reduction of the diol enone to the 3-keto-AB-cis derivative **6B** restored the mineralocorticoid activity while receptor affinity was lost. The diol 3-keto-AB-trans **5B** was inactive. Further reduction to the hexahydro derivative having the 3$\beta$-hydroxy-5$\alpha$ configuration **2B** generated a mineralocorticoid without receptor affinity.

## Conclusions

The synthesis and subsequent biological evaluation of 18-hydroxyprogesterone and 18-hydroxycorticosterone as well as their observed and potential reduced metabolites has allowed the following conclusions.

(1) 18-Hydroxyprogesterone and 18-hydroxydeoxycorticosterone are both mineralocorticoid like and have an affinity for the renal aldosterone receptor.

(2) Reduction of both 18-hydroxyprogesterone and 18-hydroxydeoxycorticosterone to their potential di-, tetra-, and hexahydro metabolites leads to either a reduction or a loss of mineralocorticoid effects. A causative role for these metabolites in hypertension is therefore unlikely.

(3) An exception to the above generalization is the 3-keto 4-ene-18,20-diol (**4B**) which not only has high receptor affinity but acts as an aldosterone antagonist.

## Experimental Section

**General Procedures.** Melting points were determined on a Thomas-Hoover Unimelt capillary apparatus and are uncorrected. IR spectra were run in KBr unless otherwise stated. Ultraviolet spectra were run in methanol and were not recorded if only n → $\pi^*$ absorption was observed. NMR spectra were recorded on Varian A-60, FT-80, and XL-100 spectrometers and were run in deuteriochloroform with tetramethylsilane as an internal standard. The NMR results are reported in chemical shifts ($\delta$), followed by the signal shape: s, singlet; d, doublet; t, triplet; m, multiplet. The multiplicity is followed by the coupling constant where applicable and the integrated signal intensity. ORD/CD curves were run on a Jasco ORD/UV-5 spectrometer and were run in methanol. Optical rotations were determined in chloroform on a Perkin-Elmer Model 141 polarimeter. GLC analyses were run on a Perkin-Elmer 900 gas chromatograph. Microanalyses were determined by the Searle Laboratories microanalytical Service under the supervision of E. Zielinski.

**3$\beta$,20(*R*)-Dihydroxy-5$\alpha$-pregnan-18-oic Acid $\gamma$-Lactone (2A).** A solution of lactone **1A** (10.0 g) in 150 mL of 2-propanol and 100 mL of tetrahydrofuran was hydrogenated for 4 h at 60 psi and 60 °C in the presence of 1.0 g of 10% Pd on carbon. After removal of the catalyst by filtration and evaporation of the tetrahydrofuran, the 3-acetate of 4,5$\alpha$-dihydro lactone **2A** (8.45 g) crystallizes, mp 202–204 °C (lit.[29] mp 190–193 °C and 212–213 °C, lit.[18] mp 197–202 °C, lit.[30] 207–209 °C). The 3-acetate was cleaved in 99% yield by adding 40 mL of 25% aqueous sodium hydroxide to a solution of 15.2 g of acetylated lactone in 500 mL of tetrahydrofuran. After 2 h at room temperature, the mixture was acidified with concentrated hydrochloric acid, diluted to 4 L with water and the precipitated **2A**, mp 215–216 °C (lit.[18] mp 213–215 °C, lit.[30] 217–218 °C), collected, and washed with water.

**20(*R*)-Hydroxy-3-oxo-5$\alpha$-pregnan-18-oic Acid $\gamma$-Lactone (5A).** A solution of 8.79 g (26.6 mmol) of alcohol **2A** in 0.5 L of acetone was oxidized at 0 °C with excess Jones reagent. After quenching of the excess oxidant with isopropyl alcohol, the ketone **5A** which had crystallized was collected and washed with water until colorless. The mother liquor was reduced in volume and diluted with water to obtain the remaining ketone **5A**, total yield 100%, mp 248–250 °C (lit.[29,31,32] mp 246–248 °C).

**20(*R*)-Hydroxy-3-oxopregn-5-en-18-oic Acid $\gamma$-Lactone (4A).** To a dry solution of 26.6 g (80.5 mmol) of alcohol **1A** in 1.5 L of toluene and 0.1 L of *N*-methyl-4-piperidone at room temperature was added 35 g of aluminum isopropoxide.[23] After refluxing for 18 h, the reaction mixture was cooled and transferred to a separatory funnel and extracted with aqueous hydrochloric acid until the washings were strongly acidic. The organic layer was separated and dried with sodium sulfate and the solvent was removed. The residue was crystallized from acetone and petroleum ether to yield 18.2 g of enone lactone **4A**. The mother liquor was flash chromatographed[33] with use of 15:85 ethyl acetate–methylene chloride to yield an additional 5.6 g (total yield 72.5 mmol, 90%) of enone **4A**, mp 200.5–204.5 °C (lit.[34] mp 206–208 °C).

**20(*R*)-Hydroxy-3-oxo-5$\beta$-pregnan-18-oic Acid $\gamma$-Lactone (6A).** A solution of 4.45 g (13.6 mmol) of enone lactone **4A** in 100 mL of pyridine was hydrogenated at 2 psi of hydrogen in the presence of 0.43 g of 5% palladium on carbon for 5 h. After filtration of the catalyst, the solvent was removed under reduced pressure and the residue crystallized from acetone–water to yield 4.3 g (13 mmol, 96%) of an epimeric mixture, determined to be 19:1 5-$\beta$:$\alpha$ by GLC. Recrystallization from acetone and hexane yielded pure **6A**: mp 215–218 °C; IR 1750, 1712 cm$^{-1}$; NMR $\delta$ 4.36 (q, $J \sim 7$ Hz, 1 H, C20), 1.37 (d, $J \sim 7$ Hz, C21), 1.12 (s, 3 H, C19);[39] ORD $[\phi]_{306}$ −151°, $[\phi]_{299}$ 0°, $[\phi]_{270}$ +1120°; CD $[\theta]_{289}$ −856°. Anal. ($C_{21}H_{30}O_3$) C, H.

**3$\alpha$,20-Dihydroxy-5$\beta$-pregnan-18-oic Acid $\gamma$-Lactone (3A).** To a solution of 7.00 g (21.2 mmol) of 5$\beta$-keto lactone **6A** in 250 mL of dry THF was added 2 equiv (10.8 g) of lithium aluminum

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tri-*tert*-butoxyhydride. After 5 h of stirring at room temperature, the mixture was carefully poured into dilute HCl and extracted with chloroform. The chloroform extracts were diluted with an equal volume of toluene and then dried with sodium sulfate. After evaporation of the solvent, the residue was crystallized from ether–hexane to give 6.75 g (20.3 mmol, 96%) of the hydroxy lactone **3A**; mp 198–202 °C; IR 3450 1755 $cm^{-1}$; NMR $\delta$ 4.33 (q, $J \sim 6$ Hz, 1 H, C20), 3.60 (m, 1 H, C3$\beta$), 1.01 (s, 3 H), 0.86 (d, $J \sim 6$ Hz, 3 H, C21). Anal. ($C_{21}H_{32}O_3$) C, H.

**18,20(*R*)-Dihydroxypregn-4-en-3-one (4B).** A suspension of 17.55 g (52.8 mmol) of enone lactone **4A** in 750 mL of ethylene glycol, containing 0.2 g of *p*-toluenesulfonic acid, is vacuum distilled at 55 °C (ca. 1 mmHg) until 400 mL has been distilled. The mixture is cooled to room temperature, 5 mL of pyridine added, and the mixture poured into 1.5 L of water. The precipitate is filtered and washed with water containing 1% pyridine and the 3-ketal subsequently dried. The crude dry ketal is dissolved in 1 L of benzene and placed under nitrogen and 30 mL of a 70% solution of sodium bis(methoxyethoxy)aluminum hydride in toluene carefully added. After addition of hydride, the resultant solution is refluxed 1 h, cooled, and decomposed with saturated Rochelle salt solution. After separation of the aqueous phase, the organics were filtered through Filter-Aid, dried with sodium sulfate, and evaporated. The residue was taken up in 1 L of acetone and 1.5 g of *p*-toluenesulfonic acid added. The solution was refluxed for 1 h, cooled, and quenched with 2 mL of pyridine and the solvent evaporated. The residue was taken up in 200 mL of hot acetone and 800 mL of water added slowly. After filtration and drying, the yield of the 18,20(*R*)-diol **4B** was 15.75 g (47.4 mmol, 90%), mp 203–205 °C (lit.[35] mp 206–209 °C).

Similarly prepared was 18,20(*R*)-dihydroxy-5$\alpha$-pregnan-3-one (**5B**), mp 207–211 °C, from keto lactone **5A**, in 77% overall yield. Anal. ($C_{21}H_{34}O_3$) C, H. Also the 5$\beta$-epimer, 18,20(*R*)-dihydroxy-5$\beta$-pregnan-3-one (**6B**) was prepared from keto lactone **6A** in 53% yield, mp 160–161.5 °C. Anal. ($C_{21}H_{34}O_3$) C, H.

**Pregn-5-ene-3$\beta$,18,20(*R*)-triol (1B).** To a solution of 1.5 g (3.98 mmol) of the 3-acetate of lactone **1A** in 40 mL of THF was added 4 molar equiv of lithium aluminum hydride (13.1 mL of a 1.22 M solution in THF). After the mixture was stirred for 18 h, the excess hydride was quenched with excess ethyl acetate and the mixture poured into dilute hydrochloric acid. The precipitate was filtered, washed with water, and dried. After recrystallization from methanol–toluene, 1.0 g (3.0 mmol, 75%) of the triol **1B**, mp 240–245 °C (lit.[36] mp 241–243 °C) was obtained.

Similarly prepared was 5$\alpha$-pregnane-3$\beta$,18,20(*R*)-triol (**2B**), mp 233–234 °C (lit.[37] mp 232–234 °C), in 95% yield.

**18-(Acetyloxy)-20(*R*)-hydroxypregn-5-en-3-one (4C).** The diol **4B** (22.62 g, 68.1 mmol) was dissolved in 500 mL of methylene chloride, which had been passed through an alumina column, and 50 mL of dry pyridine. After being placed under argon, the solution was cooled to −20 °C and 53.5 mL (75 mMol) of a 1.40 M of acetyl chloride solution in methylene chloride added. There was an immediate white precipitate which slowly dissolved. TLC (ethyl acetate on silica) indicated residual diol and an additional 8 mL of acetyl chloride solution was added at −20 °C. After a total of 2 h, the solution was washed with water, twice with dilute HCl, and finally water. After being dried with sodium sulfate, the solvent was removed under reduced pressure and the residue taken up in a little ether and then diluted with petroleum ether. Scratching induced immediate crystallization, and after further dilution with petroleum ether, 18.25 g (48.7 mmol, 72%) of the 18-acetate **4C**, mp 135–140.5 °C, was obtained.[38]

Similarly prepared was 18-(acetyloxy)-20(*R*)-hydroxy-5$\alpha$-pregnan-3-one (**5C**) in 72% yield: mp 125.5–128 °C; IR 3520, 1740, 1610, 1250 $cm^{-1}$; NMR $\delta$ 4.33, 3.85 (AB, $J \sim 12.5$ Hz, 2 H, C18), 2.13 (s, 3 H, $CH_3CO$), 1.14 (d, $J \sim 6.3$ Hz, 3 H, C21), 1.03 (s, 3 H, C19); $[\alpha]^{25}_{589}$ +32°, $[\alpha]^{25}_{365}$ + 165° (*c* 0.117). Anal. ($C_{23}H_{36}O_4$) C, H.

18-(Acetyloxy)-20(*R*)-hydroxy-5$\beta$-pregnan-3-one (**6C**) was prepared in 79% yield: mp 138.5–139.5 °C; IR 3520, 1745, 1713 $cm^{-1}$; NMR $\delta$ 4.33, 3.85 (AB, $J$ = 12 Hz, 2 H, C18), 3.63 (br s, 1 H, C20), 1.13 (d, $J$ = 6 Hz, 3 H, C21), 1.02 (s, 3 H, C19); $[\alpha]^{25}_{589}$ +16.7°, $[\alpha]^{25}_{365}$ +27.1° (*c* 0.096). Anal. ($C_{23}H_{36}O_4$) C, H.

**18-(Acetyloxy)-pregn-4-ene-3,20-dione (4D).** A solution of 18.25 g (48.7 mmol) of the 20-alcohol **4C** in 1 L of acetone was cooled in an ice bath and oxidized with the calculated amount of Jones reagent.[39] TLC on silica, using 1:1 ethyl acetate–toluene, indicated incomplete oxidation, and small amounts of oxidant were added until TLC indicated that **4D** had been consumed. The small amount of excess oxidant was quenched with isopropyl alcohol and the reaction mixture then diluted with water. after evaporation of the majority of the acetone, the aqueous residue was extracted three times with chloroform. The combined organics were dried with sodium sulfate and the solvent evaporated. The residual oil was taken up in ether and scratched to yield 16.51 g (44.3 mmol, 91%) of 18-acetoxyprogesterone (**4D**), mp 135–137 °C [lit.[18] mp 136.5–137.5 °C).

In a similar manner, 18-(acetyloxy)-5$\alpha$-pregnane-3,20-dione (**5D**), mp 100–104 °C, was prepared in 91% yield: IR 1750, 1720, 1715 $cm^{-1}$; NMR $\delta$ 4.12, 3.79 (AB, $J \sim 11$ Hz, 2 H, C18), 2.18 (s, 3 H, $CH_3CO_2$), 1.97 (s, 3 H, C21), 1.00 (s, 3 H, C19); $[\alpha]^{25}_{589}$ +627°, $[\alpha]^{25}_{365}$ +106° (*c* 0.100). Anal. ($C_{23}H_{34}O_4$) C, H. Also 18-(acetyloxy)-5$\beta$-pregnane-3,20-dione (**6D**) was synthesized in 64% yield; mp 102–106 °C; IR 1735, 1712 $cm^{-1}$; NMR $\delta$ 4.12, 3.80 (AB, $J \sim$ 11 Hz, 2 H, C18), 2.19 (s, 3 H, $CH_3CO_2$), 1.97 (s, 3 H, C21), 1.02 (s, 3 H, C19); $[\alpha]^{25}_{589}$ +82.8°, $[\alpha]^{25}_{365}$ +428° (*c* = 0.108). Anal. ($C_{23}H_{34}O_4$) C, H.

**18-(Acetyloxy)-3$\alpha$-hydroxy-5$\beta$-pregnan-20-one (3D).** The 3$\alpha$-hydroxy 5$\beta$-lactone **3A** (3.75 g, 11.3 mmol) was dissolved in 50 mL of methylene chloride and 1.55 mL (16.9 mmol) of dihydropyran. To this solution was added 300 mg (1.13 mmol) of pyridinium tosylate. After 1.25 h at room temperature, the solution was washed twice with water and dried with sodium sulfate and the solvent evaporated. The resultant 3$\alpha$-tetrahydropyranyl ether was dissolved in 200 mL of benzene, placed under argon, and the solution dried by refluxing, using a Dean–Stark trap. After cooling, 16 mL of a 70% solution of sodium bis(methoxyethoxy)aluminum hydride in toluene was added. After the mixture was stirred at room temperature for 18 h, the excess hydride was quenched by the addition of 26.4 mL of 20% sodium hydroxide solution. The organic layer was separated, dried with sodium sulfate, and stripped to a gel. The gel was crystallized from acetone and water to yield 4.4 g of the 3$\alpha$-tetrahydropyranyl ether 18,20-diol. This protected diol was dissolved in 100 mL of alumina-dried methylene chloride and 10 mL of dry pyridine in a 250-mL flame-dried flask and placed under argon. The solution was cooled to −20 °C and 1.1 equiv (8.05 mL) of a stock solution of 10% (v/v) of acetyl chloride in methylene chloride was added. After allowing to warm to room temperature overnight, the solution was washed three times with water and dried with sodium sulfate. The solvent was evaporated and the residue taken up in 200 mL of methylene chloride and oxidized with 22 mmol (4.75 g) of pyridinium chlorochromate, buffered with 1.8 g of sodium acetate. After 6 h, the reaction mixture was diluted with 0.25 L of ether and decanted through Celite. The residue was washed by slurrying with 0.5 L of a 1:1 mixture of ether and methylene chloride and then twice with 0.125 L of ether. The combined organics were allowed to stand overnight and then decanted again through Celite, and the solvent was evaporated. The residue was dissolved in 200 mL of methanol containing 1 g of *p*-toluenesulfonic acid and the solution stirred overnight at room temperature to remove the blocking group. The methanol solution was diluted with 200 mL of water and the methanol was then evaporated under reduced pressure. The resultant oil was extracted with 0.5 L of methylene chloride in portions, dried with sodium sulfate, and evaporated. The residue was flash chromatographed with use of 2:3 ethyl acetate–methylene chloride to yield 2.0 g (5.04 mmol, 45%) of 18-(acetyloxy)-3$\alpha$-hydroxy-5$\beta$-pregnan-20-one (**3D**): mp 171.5–173.5 °C (acetone–water); IR 3555, 1745, 1705, 1245 $cm^{-1}$; NMR $\delta$ 4.10 (AB, $J$ = 12 Hz, 1 H,

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C18), 3.78 (AB, 1 H, C18), 3.63 (m, 1 H, C3β), 2.19 (s, 3 H), 1.97 (s, 3 H), 0.93 (s, 3 H, C19); $[\alpha]^{25}_{589}$ +74°, $[\alpha]^{25}_{365}$ +498° (c = 0.108%). Anal. ($C_{23}H_{36}O_4$) C, H.

Similarly prepared was the 3β-hydroxy 5-ene derivative **1D**: mp 150–151.5°C; IR 3540, 1745, 1705 $cm^{-1}$; NMR δ 5.43 (m, 1 H, C6), 4.13 (AB, J ~ 11 Hz, 1 H, C18), 3.20 (AB, 1 H, C18), 2.20 (s, 3 H), 1.95 (s, 3 H), 1.00 (s, 3 H, C19); $[\alpha]^{25}_{589}$ +21°, $[\alpha]^{25}_{365}$ +260° (c 0.103%). Anal. ($C_{23}H_{34}O_5 \cdot 0.5H_2O$) C, H. The lactone **2A** was also transformed into the 3β-hydroxy 5α-derivative **2D**: mp 175.5–176.5 °C (acetone–water); IR 3510, 1735, 1722, 1708 $cm^{-1}$; NMR δ 4.16 (AB, J ~ 12 Hz, 1 H, C18), 3.94 (AB, 1 H, C18), 2.21 (s, 3 H), 1.98 (s, 3 H), 0.82 (s, 3 H, C19); $[\alpha]^{25}_{589}$ +82°, $[\alpha]^{25}_{365}$ +439° (c 0.119%). Anal. ($C_{23}H_{36}O_4$) C, H.

**18,20-Epoxy-20-hydroxypregn-4-en-3-one (18-Hydroxyprogesterone, 4E).** To a magnetically stirred solution of 13.0 g of 18-acetoxyprogesterone (**4D**) (34.9 mmol) in 1 L of methanol, under argon, was added 250 mL of a 2 N sodium hydroxide solution. After refluxing for 1 h, the solution was cooled and 20 g of citric acid in 75 mL of distilled water added to partially neutralize the excess base. The final pH was 12.5. The methanol was stripped, whereupon the product **4E** crystallized. It was filtered and washed well with water to yield 9.92 g (30.0 mmol, 86%): mp 175–178 °C, (lit.[17] mp 173–182 °C).

Similarly prepared was 18,20-epoxy-20-hydroxy-5α-pregnan-3-one (**5E**), mp 141–146 °C (lit.[40] mp 143–145 °C) in 97% yield. The previously unknown 5β-epimer **6E** [mp 171.5–176.5 °C; $[\alpha]^{25}_{589}$ +64°, $[\alpha]^{25}_{365}$ +184° (c 0.100)] was prepared in 73% yield from the 18-acetoxy compound **6D**. Anal. ($C_{21}H_{32}O_3$) C, H. 18,20-Epoxy-5β-pregnane-3α,20-diol (**3E**) [mp 185 °C dec; $[\alpha]^{25}_{589}$ +71°, $[\alpha]^{25}_{365}$ +226° (c 0.105] was synthesized from the 18-acetate in 99% yield. Anal. ($C_{21}H_{34}O_3$) C, H. The 5α-pregnan-3β-ol (**2E**), mp 158 °C (lit.[40] mp 159–161 °C), was a known compound, as was the 5-ene compound **1E**, mp 146–149 °C.[20]

**18,20-Epoxy-20,21-dihydroxypregn-4-en-3-one (18-Hydroxydeoxycorticosterone Acetate, 4F).** A solution of 9.3 g of 18-hydroxyprogesterone (**4E**) in 200 mL of glacial acetic acid was cooled in an ice bath and 12 g of lead tetraacetate added. The mixture was stirred magnetically for 45 min and then quenched by the addition of 30 mL of glycerine. After 15 min further stirring, the mixture was poured into water and stirred. The product was slow to crystallize. After 1 h of stirring at room temperature, the product was filtered and washed with water and dried to give 7.7 g (19.0 mmol, 71%) of 18-hydroxydeoxycorticosterone acetate (**4f**): mp 157–159 °C (lit.[17] mp 158–159 °C).

In a similar fashion, the known pregn-5-en-3β-ol 21-acetate (**1F**), mp 141–151 °C (lit.[20] mp 172–176 °C) (77% yield), was prepared, as well as the following derivatives: 21-(acetyloxy)-18,20-epoxy-20-hydroxy-5α-pregnan-3-one (**5F**), mp 146–150 °C (lit.[15b] mp 158–161 °C) (42% yield); 21-(acetyloxy)-18,20-epoxy-20-hydroxy-5β-pregnan-3-one (**6F**), mp 155.5–157 °C (lit.[15b] mp 158–162.5 °C) (66% yield); 21-(acetyloxy)-18,20-epoxy-5α-pregnane-3β,20-diol (**2F**), mp 174.5–178.5 °C (lit.[15b] mp 192–193 °C), (83% yield); 21-(acetyloxy)-18,20-epoxy-20-hydroxy-5β-pregnan-3α-ol (**3F**), mp 92–101 °C; [lit.[15b] mp 128–134 °C) (42% yield).

**18,20-Epoxy-20,21-dihydroxypregn-4-en-3-one (18-Hydroxydeoxycorticosterone, 4G).** A solution of 5.8 g of 18-hydroxydeoxycorticosterone 21-acetate (**4F**) (14.95 mmol) in 350 mL of 9:1 methanol–water containing 2 g of potassium hydroxide was refluxed under argon for 0.5 h. The solution was rapidly filtered through filter aid and the majority of the methanol evaporated. The solution was diluted with distilled water and filtered. The product was washed with water and dried. The residue was dissolved in chloroform and treated with decolorizing carbon. After filtering, the colorless chloroform solution was diluted with petroleum ether and the resultant **4G** collected; 3.35 g, 9.7 mmol (65%), mp 189–193 °C (lit.[17] mp 191–195 °C).

The 3β-hydroxy 5-ene analogue **1G**,[20] mp 153–156 °C, and the 3α-hydroxy-5β-pregnane derivative **3G**, mp 179–182 °C (lit.[15a] mp 180–182 °C) are known compounds, as are the following derivatives: 18,20-epoxy-20,21-dihydroxy-5β-pregnan-3-one (**5G**), mp 166–169 °C (lit.[15b] mp 178–180 °C); 18,20-epoxy-20,21-dihydroxy-5α-pregnan-3-one (**6G**), mp 177.5–181.5 °C (lit.[15b] mp 127–131 °C); 18,20-epoxy-5α-pregnane-3β,20,21-triol (**2G**), mp 150 °C dec (lit.[15b] mp 183–184 °C).

**Biological Methods. Mineralocorticoid Activity.** The biological assay procedure for in vivo characterization of these compounds was derived from Kagawa.[41] Briefly, male Charles River rats (180–220 g) were adrenalectomized 24 h prior to testing and maintained overnight on tap water and sucrose. On the day of testing, each animal received a 2.5-mL saline load and 12 μg of deoxycorticosterone acetate (DOCA) subcutaneously in oil. Compounds were administered by subcutaneous injection in corn oil at a dose of 2.4 mg/animal. Animals were than placed in metabolism cages, and urine was collected for 4 h. At the end of the collection period, the animals were forced to void by suprapubic pressure. Urinary sodium and potassium concentrations were determined by flame photometry, and the urinary excretion was expressed as the logarithmic transformation of the $Na^+ \times 10/K^+$ urinary excretion ratio (log Na/K).[42] Log Na/K in the saline-loaded, adrenalectomized rat is depressed with exogenous mineralocorticoid treatment with DOCA. A ratio was calculated between the log Na/K values resulting from each metabolite studied relative to that of a DOCA-treated control group (M/DOCA). Values of M/DOCA greater than 1.0 indicated mineralocorticoid antagonism whereas M/DOCA values less than 1.0 demonstrated an enhancement of the mineralocorticoid effect of DCA.

**Renal Aldosterone Receptor Binding.** Renal aldosterone receptor binding was evaluated by the method of Funder et al.[43] Washed tissue slices of adrenalectomized rat kidney were incubated with $2 \times 10^{-9}$ M [$^3$H]aldosterone in the presence of $8 \times 10^{-8}$ M 18-hydroxy steroids or unlabeled aldosterone and $2 \times 10^{-8}$ M dexamethasone in a buffered medium (135 mmol of NaCl, 5 mmol of $KH_2PO_4$, 5 mmol of Tris buffer, 0.5 mmol of $MgCl_2$, 5 mmol of dextrose, and 1 mmol of $CaCl_2$ at pH 7.2). After tissue homogenization and centrifugation at 20 000 rpm (30000g) for 30 min, the high-speed supernatant was transferred in 1.0-mL aliquots to tubes containing 0.1 mL of a 5:0.5% charcoal–dextran solution. The tubes were mixed by vortex and centrifuged at 2400 rpm (1200g) for 15 min to separate bound [$^3$H]aldosterone from the free ligand. Radioactivity of the samples was calculated by scintillation counting of the supernatant, as cpm/mg of protein corrected for nonspecific binding attained at $2 \times 10^{-7}$ M unlabeled aldosterone. Relative binding affinity of the metabolites was expressed as the percent inhibition of [$^3$H]aldosterone binding observed in the presence of each of the competing steroids at $8 \times 10^{-8}$ M.[44]

Control animals treated with only DOCA exhibited log Na/K ratios of 0.70 ± 0.19 (mean ± SD). The ratio of the individual log Na/K values and the overall mean Na/K ratio (M/DOCA control) was 1.04 ± 0.19. The M/DOCA ratios of the individual metabolites were calculated and are shown in Table I. A calculated M/DOCA value was judged significant if the ratio fell outside the standard deviation of the mean control ratios obtained with DOCA alone.

A significant deviation below 1.0 was indicative of an additive mineralocorticoid effect due to the administered potential metabolite and the resultant decrease in sodium excretion. Conversely, a metabolite such as **4A**, which demonstrated an elevation above 1.0, was indicative of an antimineralocorticoid effect since the effect of DOCA was antagonized and manifested as an increased sodium excretion. Theoretically metabolites which were mineralocorticoid or antimineralocorticoid in nature would be expected to demonstrate significant receptor binding as well. Those which did not show binding characteristics may have, in turn, been further metabolized to an active structure capable of effective receptor interaction. Inversely, structures demonstrating in vitro binding but no effects on urinary electrolyte excretion may have been converted to inactive metabolites in vivo.

In studies of in vitro aldosterone receptor affinity, inhibition of [$^3$H]aldosterone binding was the index of receptor affinity of

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18-OH-DOC (4G), 18-OH-PROG (4E), and their metabolites. Statistical significance of this index was evaluated by using Student's $t$ test against a hypothetical binding affinity of 0%. Both compounds were mineralocorticoid-like in their in vivo activity with M/DOCA ratios of 0.76 and 0.71, respectively. Binding of [$^3$H]aldosterone to the renal aldosterone receptor was identically inhibited with 18-OH-PROg (4E) and 18-OH-DOC (4G) by 34%.

**Registry No.** 1A, 60325-73-7; 1A acetate, 3020-10-8; 1B, 55388-46-0; 1D, 93716-45-1; 1E, 53620-26-1; 1F, 53803-17-1; 1G, 53512-61-1; 2A, 1238-51-3; 2A acetate, 2878-66-2; 2B, 3599-24-4; 2D, 93716-46-2; 2E, 38863-68-2; 2F, 93781-10-3; 2G, 81940-97-8; 3A, 93781-07-8; 3D, 93716-43-9; 3E, 1900-47-6; 3F, 86698-70-6; 3G, 31935-07-6; 4A, 3246-19-3; 4A ketal, 93716-38-2; 4B, 4813-43-8; 4C, 93716-39-3; 4D, 58210-92-7; 4E, 22618-71-9; 4F, 53512-59-7; 4G, 10385-97-4; 5A, 2858-27-7; 5A ketal, 26302-63-6; 5B, 26302-65-8; 5C, 93755-93-2; 5D, 93716-41-7; 5E, 14425-53-7; 5F, 86698-72-8; 5G, 86698-67-1; 6A, 93781-08-9; 6A ketal, 93859-83-7; 6B, 93781-09-0; 6C, 93716-40-6; 6D, 93716-42-8; 6E, 93716-47-3; 6F, 86698-68-2; 6G, 86698-69-3; ethylene glycol, 107-21-1; 18,20-dihydroxy-3$\alpha$-tetrahydropyranyloxy-5$\beta$-pregnane, 93716-44-0.

# *Notes*

## Bile Acids with Cyclopropane-Containing Side Chain. 2.[1] Synthesis and Properties of 3$\alpha$,7$\beta$-Dihydroxy-22,23-methylene-5$\beta$-cholan-24-oic Acid (2-Sulfoethyl)amide

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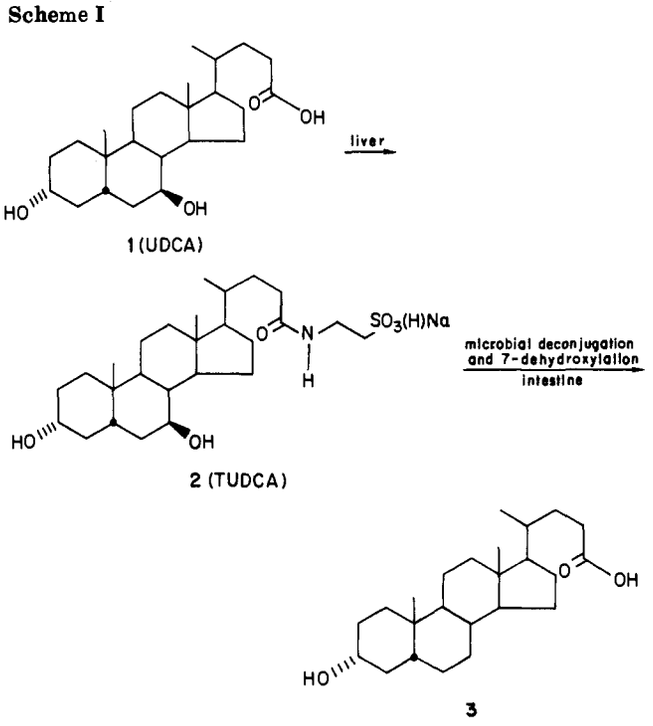
The preparation of 3$\alpha$,7$\beta$-dihydroxy-22,23-methylene-5$\beta$-cholan-24-oic acid (2-sulfoethyl)amide (5) by the one-step EEDQ-induced conjugation between ursodeoxycholic acid "cyclopropylog" (4) and taurine is described. The presence of a cyclopropyl ring adjacent to the amide bond is shown to make it resistant to degradation by intestinal bacteria. This new cyclopropylog is neither deconjugated at the C-24 amide bond nor 7-dehydroxylated when incubated with human stools in anaerobic conditions.

We have recently started a program aimed at the design of structurally modified bile acids (BA) in an attempt to increase the biological activity as well as to limit undesirable side effects exhibited by members of this class of compounds, such as ursodeoxycholic acid (UDCA),[2a] which is widely employed in several countries as cholesterol gallstone dissolving agent.[2a,b] Recent studies have established the conditions for successful therapy, such as the cholesterol nature of the gallstone crystalline structure and the high dose and length of treatment. The latter requirements, in particular, are motivated by the biotransformations which UDCA and the other natural BA undergo during enterohepatic cycling.[4] The fate of UDCA can be summarized as follows: after ingestion, it enters the small intestine and is passively absorbed throughout the intestine by the portal route. It has been estimated that the first-pass hepatic uptake for UDCA is 50%, with a resulting spillover into the systemic circulation of an analogous amount. Once in the hepatocyte, it is conjugated with glycine or taurine to form the corresponding $N$-acyl conjugate. The preferential conjugation with glycine can be explained by a reduced availability of taurine in the hepatocyte and has been considered negative since the taurine conjugate of UDCA is currently thought to have superior cholesterol-solubilizing properties. In order to overcome this problem, taurine, taurine jointly with UDCA, tauroursodeoxycholic acid (TUDCA 2), and TUDCA supplied with taurine have been administered to patients, and preliminary results seem to indicate a shift toward taurine in the UDCA conjugation pattern as a consequence of these approaches.[5,6]

**Scheme I**




A second problem arises once UDCA, as a conjugate, is secreted from the hepatocyte into the bile and with the

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(1) See ref 11.
(2) (a) Danzinger, R. G.; Hofmann, A. F.; Schoenfield, L. J.; Thistle, J. L. *N. Engl. J. Med.* **1972**, 288. (b) Makino, I.; Shinozaki, K. J.; Yoshino, K.; Nakagawa, S. *Nippon Shokakibyo Gakkai Zasshi* **1975**, *72*, 690.

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