phosphonate (2.2 g, 10 mmol) was added dropwise to a stirred suspension of NaH (240 mg, 10 mmol) in dry dimethoxyethane (15 mL). In a few minutes the entire reaction mixture solidified. After 0.5 h **9** (2.8 g, 10 mmol) in dimethoxyethane (5 mL) was added in one portion. The reaction mixture became stirrable although a precipitate was present. After 20 h the reaction mixture was poured into water (150 mL), acidified, and extracted with ethyl acetate (2 × 75 mL). The organic phase was washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. The resulting oil was dissolved in EtOH (15 mL) and cooled in an ice bath and sodium borohydride (380 mg, excess) was added in small portions. After 1.5 h the reaction mixture was poured into water (150 mL), carefully acidified (dilute HCl), and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification was effected by chromatography on silica gel using ethyl acetate–hexane (3:2) to elute. There was obtained 1.6 g (41%) of **10** as a clear oil: NMR δ 0.90 (3 H, m, CH_3CH_2–), 2.85 (3 H, s, CH_3SO_2–), 5.8 (2 H, m, trans –HC=CH–). Anal. ($C_{19}H_{33}NO_5S$) H, N; C: calcd, 58.88; found, 59.49.

(e) **7-[*N*-(4-Hydroxy-2-*trans*-nonenyl)methanesulfonamido]-5-*cis*-heptenoic Acid Hydrate (11).** Compound **10** (1.5 g, 3.8 mmol) was saponified by the method described for **2a**. The acid thus obtained was hydrogenated by the procedure described for **6d**. Thus there was obtained 1.2 g (85%) of **11** as a pale yellow oil: NMR δ 0.90 (3 H, m, CH_3CH_2–), 2.85 (3 H, s, CH_3SO_2–), 5.5 (2 H, m, cis –HC=CH–), 5.8 (2 H, m, trans –HC=CH–). Anal. ($C_{17}H_{31}NO_5S \cdot H_2O$) C, H, N.

Mouse Ovary Prostaglandin Assay.[4] Virgin female mice over 70 days old (Charles River CD-1) were killed and the ovaries dissected and denuded of adhering fatty tissue. Three ovaries were weighed (15–25 mg) and placed in 2 mL of aerated Krebs–Ringer phosphate buffer, pH 7.2, containing 1 μCi of adenine-8-^{14}C. The tissues were incubated 1 h at 37 °C with moderate shaking to cause a pool of intracellular ATP-^{14}C to accumulate.

The following additions were then made: 0.2 mL of 0.05 M theophylline in 0.15 M NaCl and the test compound in 0.1 mL of Me_2SO. The ovaries were again incubated at 37 °C for 30 min. The reactions were terminated by the addition of 0.4 mL of 10% trichloroacetic acid, and 50 μL of a nucleotide mixture solution[10] was added to facilitate recovery of the labeled nucleotides. The incubation mixture was transferred to a glass homogenizer and the ovarian tissue was homogenized into the acidified incubation solution. The homogenate was centrifuged 1000g for 5 min and the cAMP-^{14}C was isolated from the supernatant fluid as described by Humes and co-workers[10] including the final paper chromatography step.

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Synthesis and Antimineralocorticoid Activities of Some 6-Substituted 7α-Carboalkoxy Steroidal Spirolactones

Richard M. Weier* and Lorenz M. Hofmann

Departments of Chemical and Biological Research, Searle Laboratories, Division of G. D. Searle & Co., Chicago, Illinois 60680.
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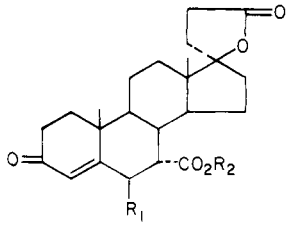
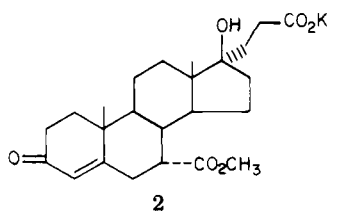
Several analogues of the previously reported steroidal spirolactone **1a** were synthesized. These analogues bear C-6 substituents and include the 6β-deuterio (**1c**), the 6β-bromo (**1d**), the 6β-methyl (**1e**), and the 6α-methyl (**7**) compounds. The 6β-hydroxy compound **1b**, a human and animal metabolite of **1a**, was also synthesized. On subcutaneous administration to adrenalectomized rats, all these compounds exhibited the ability to block the effects of administered deoxycorticosterone acetate (DCA) (MED $\leq$ 0.58 mg). Only **7** failed to show anti-DCA effects at the standard test level on oral administration. None was significantly superior in potency to the parent compound **1a**.

An earlier report[1] described the synthesis and antimineralocorticoid potency of **1a**, a steroidal spirolactone substituted in the 7α position with a carbomethoxy function. During the course of supplemental biological studies on **1a**, administered intragastrically as its potassium salt **2**, metabolism studies revealed that a principal biotransformation product in both animals and man was the 6β-hydroxy compound **1b**.[2] This communication describes the chemical synthesis and antimineralocorticoid potencies of both metabolite **1b** and of other compounds designed to determine the effects on potency of substitution in both 6α and 6β positions. These compounds include the 6β-deuterio (**1c**), the 6β-bromo (**1d**), the 6β-methyl (**1e**), and the 6α-methyl (**7**) derivatives. One other hydroxylated compound was also synthesized, namely **1g**, the 6β-hydroxy derivative of the corresponding C-7 isopropyl ester **1f**.

Synthesis. All target compounds were prepared through the intermediacy of either the enol ethers **3a** and **3c** or the enol acetate **3b**. These compounds reacted as typical 3,5-dien-3-ol systems and underwent substitution at C-6 when treated with appropriate electrophilic reagents.[3] Both **3a** and **3c** were prepared according to conventional procedures[4] by treatment of **1a** and **1f** with triethyl orthoformate in EtOH in the presence of *p*-TsOH at room temperature. Compound **3b** was prepared by



1a, R_1 = H; R_2 = CH_3
b, R_1 = OH; R_2 = CH_3
c, R_1 = D; R_2 = CH_3
d, R_1 = Br; R_2 = CH_3
e, R_1 = CH_3; R_2 = CH_3
f, R_1 = H; R_2 = i-C_3H_7
g, R_1 = OH; R_2 = i-C_3H_7



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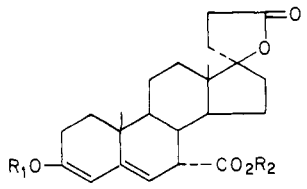


3a, R_1 = C_2H_5; R_2 = CH_3
b, R_1 = $COCH_3$; R_2 = CH_3
c, R_1 = C_2H_5; R_2 = i-C_3H_7

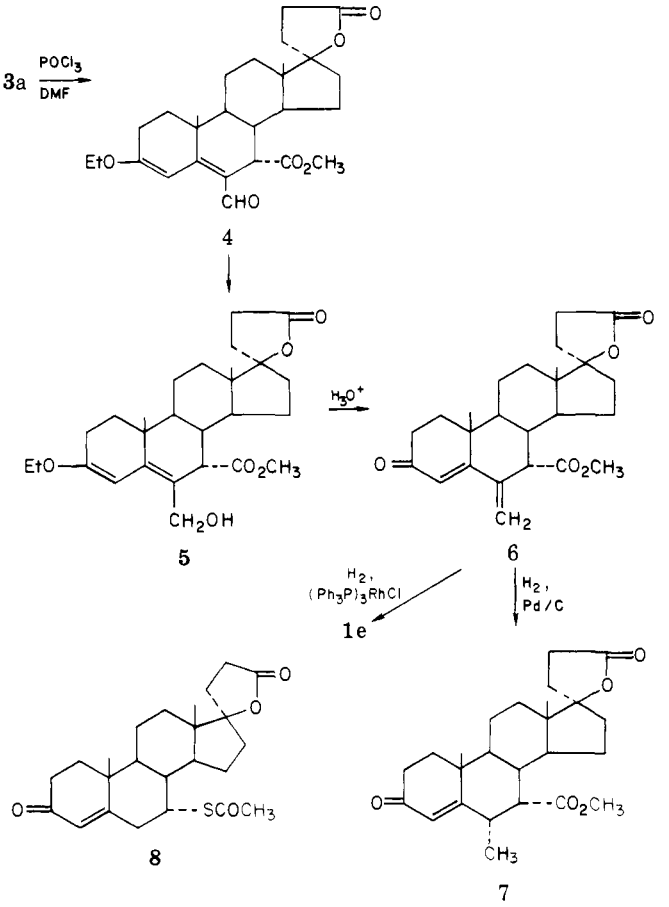
treatment of **1a** with *p*-TsOH and isopropenyl acetate at room temperature with periodic concentration on the rotary evaporator to remove by-product acetone. Although enol acetates are customarily prepared using this reagent by slow distillation of the excess isopropenyl acetate,[5] these higher temperatures were avoided in this case in order to preserve the acid-sensitive spirolactone ring.

Attention was first directed to the synthesis of the metabolite **1b** in order to prepare sufficient quantities of it for biological testing. To this end, the enol ether **3a** was oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) according to the procedure of Kirk and Wiles.[6] An aqueous dioxane solution of **3a** was treated with *m*-CPBA that had been buffered by half-neutralization with NaOH. Chromatography of the crude product and recrystallization gave a low yield (23%) of analytically pure **1b**. The spectral properties and chromatographic behavior of **1b** were identical with that of samples of isolated metabolite. Of particular importance was the appearance of the signal for the C-4 proton in the NMR spectrum. This proton, in the absence of allylic coupling with a 6β hydrogen, appeared as a very narrow singlet (half-bandwidth < 2 Hz) at 5.79 ppm. This observation is consistent with theoretical considerations[7] and was used as a proof of stereochemistry in the cases of other 6β-substituted steroids which were synthesized in this series.

Using the same procedure, the isopropyl ester **1f** was converted to the 6β-hydroxy derivative **1g** through the intermediacy of enol ether **3c**.

The 6α- and β-methyl compounds were synthesized from enol ether **3a** according to procedures previously utilized in other steroid series.[8] Thus, **3a** was converted to the 6-formyl derivative **4** by treatment with the Vilsmeier reagent generated from $POCl_3$ and DMF. Compound **4** was reduced with lithium tri-*tert*-butoxyaluminum hydride (Bu_3LiAlH) at 0 °C to give the 6-hydroxymethyl compound **5**, which was converted without characterization to the 6-methylene compound **6** by hydrolysis in aqueous acetone at pH 1.5–2.0.

Because the in vivo metabolic process yielded the 6β-hydroxy derivative, it appeared that a 6β-substituent should be the more effective blocker of enzymatic hydroxylation. For this reason, the 6β-methyl compound **1e** was the more desirable of the two possible epimers. Initial hydrogenation experiments on **6** were carried out using 5% Pd/C as catalyst. The product, isolated in 55% yield by direct crystallization from the crude reaction mixture, was identified as the 6α-methyl compound **7** by its elemental analysis and its UV and NMR spectra. The UV spectrum showed λ_{max} 244 nm, proving that the 4-en-3-one system



remained, while the NMR spectrum showed a *C*-methyl doublet at 1.08 ppm (J = 7 Hz). The methyl group was shown to have α stereochemistry by the signal for the C-4 proton. Because of allylic coupling with the 6β-hydrogen, the C-4 proton appears as a doublet at 5.77 ppm (J = 2 Hz). Examination of the mother liquors from this reaction by VPC showed that they consisted largely of **7** and that only 18% of the total reaction mixture consisted of a new, unidentified product, presumably the 6β-methyl compound.

In selecting an alternate approach to the problem of reduction to the 6β-methyl compound **1e**, consideration was given to the presence of the carbomethoxy group at C-7. This substituent is in an axial position on the α face of the steroid. Coordination of this group with a suitable catalyst and subsequent transfer of hydrogen from the catalyst to the C-6 *exo*-methylene group of **6** would, of necessity, yield the 6β-methyl compound **1e**. To this end, **6** was hydrogenated using the Wilkinson catalyst, chlorotris(triphenylphosphine)rhodium.[9] After chromatography on silica to remove the catalyst and recrystallization of the combined steroid fractions from MeOH, there was obtained a low (26%) yield of a new reduction product, different from the 6α-methyl compound **7**, whose spectral data were clearly consistent with that expected for the 6β-methyl steroid **1e**. The UV spectrum (λ_{max} 243–244 nm) showed that the 4-en-3-one system remained. The NMR spectrum showed a new *C*-methyl doublet at 1.33 ppm (J = 7.5 Hz), slightly downfield from the *C*-methyl doublet of **7** (1.08 ppm). The stereochemistry of the methyl group as β was shown both by this downfield shift[10] and by the narrow half-bandwidth (<2 Hz) of the C-4 proton at 5.76 ppm.

Although the isolated yields of **1e** from this reaction were always low due to the elaborate purification procedure necessary to remove the Wilkinson hydrogenation catalyst,

Table I. DCA Blocking Potencies

Compd	MED[a] sc[b]	MED[a] ig[c]
8[d]	0.33	0.48
1a[e]	0.33	0.71
1b	0.42	2.12
1c	0.30	1.0
1d	0.58	1.05
1e	0.20	0.58
1g	0.28	1.81
6	>2.4	>2.4
7	0.47	>2.4

[a] Median effective dose (mg/rat) necessary for 50% inhibition of urinary electrolyte induced by subcutaneously administered DCA. Rats weighed 150–200 g. See text. [b] Subcutaneous administration. [c] Intragastric administration. [d] Potency values for 8 (spironolactone) are taken from R. C. Tweit and C. M. Kagawa, *J. Med. Chem.*, 7, 524 (1964), and ref 8. [e] See ref 1.

NMR spectra of the crude product before chromatography showed much more of **1e** to be present. Comparison of the *C*-methyl doublets, the methoxyl signals, and the shape of the signal for the C-4 protons indicated that perhaps as much as 60% of the steroid product was the 6β-methyl compound **1e**.

In order to be sure that the reduction of **6** with the Wilkinson catalyst did indeed pursue a different course from that using 5% Pd/C as catalyst, it was necessary to establish that **1e** had not initially formed and then rearranged to **7** under these latter reduction conditions. Treatment of **1e** with 5% Pd/C at room temperature under a nitrogen atmosphere for 4 h resulted in no epimerization of the 6β-methyl group of **1e** as shown by NMR.

The 6β-deuterio compound **1c** was designed after consideration of the possible mechanism of enzymatic hydroxylation. During the course of this reaction, it is obvious that a C–H bond must be broken. Should this bond-breaking process be the rate-determining step in the mechanism, then replacement of the 6β-hydrogen atom with deuterium should slow the biotransformation reaction because of deuterium's primary isotope effect.[11] The synthesis of **1c** was effected by hydrolysis of **3a** using 0.5 N DCl–D_2O in diglyme.[12] The reaction mixture was warmed gently on the steam bath and, on cooling, crystals of analytically pure **1c** deposited. Mass spectral analysis showed this material to consist of 83.6% mono-, 4.2% di-, and 1.1% trideuterated material, as well as 11.1% of undeuterated compound.

Treatment of enol acetate **3b** with *N*-bromosuccinimide in buffered aqueous acetone[13] in the cold furnished the 6β-bromo compound **1d** in 50% yield.

That the stereochemistry of the C-6 substituent in both **1c** and **1d** was indeed β was shown by their NMR spectra, wherein the signals for the C-4 protons of both clearly showed the lack of any allylic coupling.

Biological Data. The compounds were assayed in a 4-h test in groups of four adrenalectomized male rats, each animal being treated subcutaneously with 12 μg of deoxycorticosterone acetate (DCA) and 2.5 mL of isotonic saline prior to administration of the test compound.[14] The median effective dose (MED) for anti-DCA activity was established by determining the dosage (mg/rat) necessary for 50% inhibition of urinary electrolyte effects (i.e., increase in Na:K ratio) of administered DCA. Test results are shown in Table I.

The most important single feature of Table I is that the biotransformation product **1b** has good anti-DCA potency upon sc administration. Although the potency decreases on ig administration, the possibility that **1b** may have activity when biosynthesized from **1a** in man cannot be dismissed. The other 6β-hydroxy compound **1g** followed a similar trend. While showing good potency on sc administration, the potency decreased by some sixfold on ig administration.

With the exception of compound **6** which bears an *exo*-methylene function at C-6, the MED values of the remaining compounds, determined on sc administration, range from 0.20 to 0.58 mg and thus are not much different from the parent structure **1a**. Although it is obvious that substitution at C-6 of the parent structure **1a** does not diminish potency, neither does it bring about marked improvement.

As for the ig test data, the 6β-deuterio (**1c**), the 6β-bromo (**1d**), and the 6β-methyl (**1e**) compounds exhibited reasonable potency, but none offered any distinct advantage over the parent compound **1a**. The MED of the 6β-deuterio compound **1c** was similar in magnitude to that previously determined for **1a**[1] and, therefore, no primary deuterium isotope effect was observed. Such a result could be rationalized by one or both of two possible situations. First, breaking of the C_6–H_β bond may not be rate determining and, thus, no enhancement of potency could be expected by substitution with deuterium. Second, deuterium could have been lost either by an enzyme-catalyzed exchange mechanism[15] or by chemical keto–enol equilibration subsequent to administration to the rat and prior to biotransformation. A third possibility, namely that metabolic hydroxylation involves the breaking of the C_6–H_α bond with substitution occurring at the 6β position, also exists. Such a mechanism, which calls for inversion of configuration at this carbon, would lead to metabolite containing deuterium in the 6α position and this seems incompatible with earlier data. Toft[15] found that in vitro metabolism of 6β-deuteriotestosterone yields 6β-hydroxytestosterone which no longer contains deuterium.

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were taken in $CDCl_3$ on a Varian A-60A spectrometer using tetramethylsilane as an internal standard. Ultraviolet spectra were obtained in MeOH on a Beckman DK-2A. Infrared spectra were obtained in $CHCl_3$ on a Beckman IR-12. Optical rotations are specific rotations taken in $CHCl_3$ on a Perkin-Elmer Model 141 polarimeter. Elemental analyses were also obtained on a Perkin-Elmer instrument, Model 240. Thin-layer chromatographies were carried out on plates coated with Woelm silica gel GF.

3-Ethoxy-17-hydroxy-17α-pregna-3,5-diene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone (3a). To a stirred slurry of **1a** (1.43 g, 3.57 mmol) in triethyl orthoformate (1.5 mL) and EtOH (3 mL) was added solid *p*-TsOH·H_2O (0.1 g). After 15 min at room temperature, the reaction mixture had become homogeneous. After a total reaction time of 25 min, excess anhydrous NaOAc (0.4 g) and pyridine (0.2 mL) were added and the reaction was concentrated in vacuo. The oily residue was treated with EtOAc, the insoluble precipitate filtered, and the filtrate concentrated in vacuo to give a viscous yellow oil. This was further dried on the vacuum pump (2 h) and treated with a minimum amount of MeOH containing a trace of pyridine. This yielded a crystalline precipitate, which, after being kept in the refrigerator overnight, was filtered and dried to give 0.89 g (58%) of pure **3a**: mp 90–93 °C; $[\alpha]^{25}_D$ −312° (*c* 0.998); λ_{max} 253 nm (ε 16 700); ν 1781, 1748, 1669, 1645 cm^{-1}; NMR 5.13 (C-4), 5.17 (d, J = 4 Hz, C-5), 3.18 (multiplet, C-7), 3.68 (–OCH_3), 3.81 (q, J = 7 Hz, –OCH_2CH_3), 1.02 (C-19), 0.96 ppm (C-18). Anal. ($C_{28}H_{36}O_5$) C, H.

6β,17-Dihydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone (1b). The enol ether **3a** was hydroxylated according to the procedure of Kirk and Wiles.[6] A solution of 81% *m*-chloroperbenzoic acid (0.74 g) in 10% aqueous

dioxane (5 mL) was half-neutralized with 0.985 N NaOH solution (2.01 mL). This was cooled to 0 °C with an ice bath and added in portions over a 2-h period to a stirred solution of enol ether **3a** (1.29 g, 3.0 mmol) in 10% aqueous dioxane (20 mL). This solution was stirred at room temperature overnight. The reaction mixture was poured into ice water and extracted four times with CH_2Cl_2. The organic layer was washed with water and then dried (Na_2SO_4). Concentration of the dried solution in vacuo yielded 0.99 g of a semicrystalline material whose TLC (50% EtOAc–C_6H_6) showed it to be largely a new product more polar than methyl ester **1a**.

This material was subjected to low-pressure liquid chromatography on a 1-in. column of Merck silica H using 80% EtOAc–20% CH_2Cl_2 as the eluent. At a column pressure of 60 psi and a flow rate of 10 mL min^{-1}, 401 mg of purified **1b** was obtained from fractions 41–68. This material was recrystallized from EtOAc–Skellysolve B to give 291 mg of analytically pure **1b**: mp 200–202 °C; $[\alpha]_D$ −11° (*c* 0.105); $[\alpha]_{365}$ −56° (*c* 0.105); λ_{max} 237 nm (ε 14 900); ν 1775, 1740, 1682, 3620, 3460 cm^{-1}; NMR (after D_2O exchange) 4.39 (d, *J* = 2 Hz, C-6), 3.66 (–OCH_3), 5.79 (sharp singlet, C-4), 2.95 (d of d, *J* = 2 and 4 Hz, C-7), 1.39 (C-19), 1.02 ppm (C-18). Anal. ($C_{24}H_{32}O_6$) C, H.

6β,17-Dihydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic Acid 7-Isopropyl Ester γ-Lactone (1g). A stirred slurry of ester **1f** (4.85 g, 11.3 mmol) in EtOH (10 mL) and ethyl orthoformate (5 mL) was treated with *p*-TsOH·H_2O (200 mg) at room temperature. After 1 min, the reaction became homogeneous. After 20 min, the reaction was stopped by the addition of pyridine (1 drop) and anhydrous sodium acetate (5 g). Solvent was removed in vacuo and the mushy residue stirred with EtOAc and filtered. The filtrate was concentrated in vacuo to give the enol ether **3c** as a light yellow oil (5 g) which, after drying on the oil pump, was suitable for use in the next step.

The enol ether **3c** was hydroxylated according to the procedure of Kirk and Wiles.[6] To a cold (+5 °C) solution of 81% *m*-chloroperbenzoic acid (*m*-CPBA) (2.5 g) in dioxane (20 mL) was added 0.985 N NaOH solution (7.1 mL, 6.99 mmol). This half-neutralized peracid solution was added in portions over a 2-h period to a stirred solution of enol ether **3c** (5 g, 11.0 mmol) in dioxane (50 mL) at room temperature. The reaction was stirred for 5 h and kept in the refrigerator overnight. One final treatment with 81% *m*-CPBA (0.5 g) half-neutralized with 0.985 N NaOH (1.2 mL, 1.18 mmol) was carried out. The reaction was concentrated in vacuo and the residue dissolved in CH_2Cl_2. The organic layer was washed twice with H_2O and dried (Na_2SO_4).

The crude product thus isolated (5.8 g) was subjected to chromatography on a 2-in. column of Mallinckrodt SilicAR CC-7 (580 g). Elution with 30% EtOAc–C_6H_6 gave 1.89 g of the desired hydroxy compound. Recrystallization of this material from EtOAc–Skellysolve B gave 1.20 g of analytically pure **1g**: mp 188–189 °C (Fisher-Johns); $[\alpha]_D$ −21° (*c* 0.108); λ_{max} 237 nm (ε 14 900); ν 1770, 1722, 1680, 3610, 3450 cm^{-1}; NMR (after D_2O exchange) 5.78 (C-4), 4.98 [septet, *J* = 6 Hz, –$OCH(CH_3)_2$], 4.38 (d, *J* = 2 Hz, C-6), 2.88 (C-7), 1.30 [d, *J* = 6 Hz, –$CH(CH_3)_2$], 1.15 (C-19), 1.00 ppm (C-18). Anal. ($C_{26}H_{36}O_6$) C, H.

17-Hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone-6β-*d* (1c). A solution of **3a** (4.57 g, 11.4 mmol) in diglyme (25 mL) was treated with 10 mL of 0.5 N DCl in D_2O. An oily precipitate formed but this disappeared on brief (0.5 min), gentle warming on the steam bath. The warm solution was allowed to cool to room temperature and after 1 h, product had crystallized out as white needles. This material was filtered and air-dried to give 3.20 g of analytically pure **1c**: mp 191–193 °C; λ_{max} 241 nm (ε 16 860); $[\alpha]_D$ +27° (*c* 1.020); $[\alpha]_{365}$ −10° (*c* 1.020); ν 1778, 1740, 1675, 1625 cm^{-1}; NMR 5.72 (s, half-bandwidth < 2 Hz, C-4), 2.84 (C-7), 3.65 (OCH_3), 1.22 (C-19), 0.98 ppm (C-18); M^+ 403 (1.1%), 402 (4.2%), 401 (83.6%), 400 (11.1%). Anal. ($C_{24}H_{32}O_5$) C, H.

3-Ethoxy-6-formyl-17-hydroxy-17α-pregna-3,5-diene-7α,-21-dicarboxylic Acid 7-Methyl Ester γ-Lactone Hemihydrate (4). The Vilsmeier reagent was prepared in DMF (20 mL) at 0 °C using $POCl_3$ (3.13 g, 20.4 mmol). After 5 min, a solution of **3a** (2.91 g, 6.79 mmol) in DMF (4 mL) was added. Immediately, the originally colorless solution of Vilsmeier reagent took on a deep red color. The reaction was stirred at 0 °C for 2 h and at room temperature overnight. The deep red reaction mixture was poured onto excess aqueous NaOAc solution and stirred for 2 h. The resulting yellow precipitate was filtered and air-dried to give 1.58 g of crude **4**. One further recrystallization from MeOH yielded 0.92 g of analytically pure **4** as the hemihydrate: mp 226–228 °C; λ_{max} 222, 251, 322 nm (ε 8200, 7000, 10 100); $[\alpha]_D$ −281° (*c* 0.103); $[\alpha]_{436}$ −730° (*c* 0.103); ν 1762, 1723, 1710, 1650, 3680 cm^{-1} (weak); NMR 10.2 (d, *J* = 1.5 Hz, CHO), 6.36 (C-4), 3.68 (OCH_3), 1.18 and 0.99 ppm (C-18,19). Anal. ($C_{27}H_{36}O_6 \cdot 0.5H_2O$) C, H.

17-Hydroxy-6-methylene-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone (6). To a stirred, cold (0 °C) solution of lithium tri-*tert*-butoxyaluminum hydride (2.17 g, 8.5 mmol) in THF (40 mL) was added the solid aldehyde **4** (2.18 g, 4.8 mmol). The steroid dissolved readily and the reaction was allowed to come to room temperature and stirred for 4.5 h. The reaction was worked up by adding H_2O and then a few drops of HOAc, care being taken to keep the reaction slightly basic. The resulting mixture was stripped in vacuo, slurried in EtOAc, and filtered through Celite. The filter cake was washed thoroughly with EtOAc and the combined filtrate dried (Na_2SO_4). This solution yielded 2.09 g of a white foam whose TLC (50% EtOAc–C_6H_6) showed a major new spot and two trace impurities.

Hydrolysis of this material was carried out by dissolving it in 133 mL of $(CH_3)_2CO$–H_2O (3:1) that had been previously adjusted to pH 1.5–2.0 with 0.1 N HCl. After stirring for 45 min at room temperature, the reaction solution was concentrated in vacuo to give a gummy precipitate which solidified on standing. The solid was filtered, washed with H_2O, and dried to give 1.52 g of crude product. This material was chromatographed on Mallinckrodt SilicAR CC-7 (152 g), eluting with mixtures of EtOAc–Skellysolve B. The product was obtained on elution with 40% EtOAc–Skellysolve B. This material was washed with Et_2O, filtered, and air-dried to give 0.43 g of analytically pure **6**: mp 165–166 °C; $[\alpha]_D$ +216° (*c* 0.111); $[\alpha]_{365}$ +1538° (*c* 0.111); λ_{max} 261–262 nm (ε 12 400); ν 1770, 1730, 1668, 1605 cm^{-1}; NMR 5.17 (d of d, *J* = 1 and 9.5 Hz, =CH_2), 5.89 (C-4), 3.63 (–OCH_3), 3.42 (C-7), 1.11 (C-19), 0.97 ppm (C-18). Anal. ($C_{25}H_{32}O_5$) C, H.

17-Hydroxy-6α-methyl-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone (7). Dienone **6** (500 mg, 1.21 mmol) was hydrogenated in THF (140 mL) at atmospheric pressure using prereduced 5% Pd/C (35 mg) as catalyst. After 2.5 h, reduction was complete. The catalyst was filtered and the filtrate stripped in vacuo to give a white foam that crystallized under Et_2O. The material thus isolated was recrystallized from MeOH to give 274 mg of analytically pure **7**: mp 191–194.5 °C; $[\alpha]^{25}_D$ +11° (*c* 0.094); $[\alpha]^{25}_{365}$ −47° (*c* 0.094); λ_{max} 244 nm (ε 15 900); ν 1770, 1734, 1665, 1615 cm^{-1}; NMR 5.77 (d, *J* = 2 Hz, C-4), 3.64 (–OCH_3), 2.81 (C-7), 1.08 (d, *J* = 7 Hz, C_6-CH_3), 1.23 (C-19), 0.99 ppm (C-18). Anal. ($C_{25}H_{34}O_5$) C, H.

17-Hydroxy-6β-methyl-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone (1e). Compound **6** (1.03 g, 2.49 mmol) was hydrogenated at atmospheric pressure in 180 mL of C_6H_6–EtOH (4:1) using chlorotris(triphenylphosphine)rhodium (1.03 g) as catalyst. The reaction was complete after 6.5 h. The reaction mixture was stripped in vacuo to give 2.2 g of a red gum which was chromatographed on Mallinckrodt SilicAR CC-7 (220 g). Elution was carried out with CH_2Cl_2 and 5, 10, and 15% EtOAc–CH_2Cl_2. Product was contained in the early cuts of this last elution mixture. The crude product thus isolated was recrystallized from MeOH to give 263 mg of analytically pure **1e**: mp 222–224 °C; $[\alpha]_D$ +4° (*c* 0.100); $[\alpha]_{365}$ +440° (*c* 0.100); λ_{max} 243–244 nm (ε 16 700); ν 1772, 1734, 1668, 1611 cm^{-1}; NMR 5.76 (C-4), 3.67 (–OCH_3), 1.33 (d, *J* = 7.5 Hz, C_6-CH_3), 1.31 (C-19), 1.03 ppm (C-18). Anal. ($C_{25}H_{34}O_5$) C, H.

3-Acetoxy-17-hydroxy-17α-pregna-3,5-diene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone (3b). A solution of **1a** (900 mg, 2.24 mmol) and *p*-TsOH·H_2O (200 mg) in isopropenyl acetate (50 mL) was stirred at room temperature for 24 h. During this time, the by-product acetone was periodically removed (three times) by concentrating the reaction solution on the rotary evaporator at low temperature ($t \leq 30$ °C). The isopropenyl acetate thus removed was replaced and the reaction allowed to proceed. After 24 h, the reaction mixture was poured onto a vigorously stirred mixture of toluene and saturated aqueous $NaHCO_3$ solution. The layers were separated and the aqueous layer was washed once more with toluene. The combined organic extracts were washed four times with 5% $KHCO_3$ solution and

once with saturated NaCl solution and dried (Na_2SO_4). The solvent was stripped in vacuo to give a crude brown oil that was further dried on the oil pump. This material crystallized under MeOH and was recrystallized from MeOH to give 409 mg of analytically pure **3b**: mp 226–231 °C; λ_{max} 242 nm (18500); ν 1770, 1741, 1679, 1645 cm^{-1}; NMR 5.71 (C-4), 5.32 (d, J = 5.0 Hz, C-6), 3.19 (C-7), 2.13 ($-COCH_3$), 1.05 (C-18), 0.96 ppm (C-19). Anal. ($C_{26}H_{34}O_6$) C, H.

6β-Bromo-17-hydroxy-3-oxo-17α-pregn-4-ene-7α,21-dicarboxylic Acid 7-Methyl Ester γ-Lactone (1d). Dienol acetate (440 mg, 1 mmol) was added to a cold (0 °C) stirred solution of sodium acetate (300 mg) and HOAc (1 mL) in 30% aqueous acetone (22 mL). Next, *N*-bromosuccinimide (202 mg, 1.13 mmol) was added. Even though the reaction was initially heterogeneous, a new crystalline material could be observed after 10 min. The reaction was stirred 1 h at 0 °C and H_2O (30 mL) was added. The resulting crystalline precipitate was filtered and air-dried to give 0.50 g of a white powder. Recrystallization of this material from CH_2Cl_2–Et_2O afforded 0.24 g (50%) of analytically pure **1d**: mp 168–170 °C dec; $[\alpha]_D$ −11° (c 1.063); λ_{max} 249 nm (ϵ 11700); ν (KBr) 1780, 1730, 1682 cm^{-1}; NMR 5.90 (C-4), 4.97 (d, J = 2 Hz, C-6), 3.13 (d of d, J = 2, 4.5 Hz, C-7), 3.67 ($-OCH_3$), 1.54 (C-19), 1.05 ppm (C-18). Anal. ($C_{24}H_{31}BrO_5$) C, H, Br.

Stability of 1e. A slurry of 5% Pd/C (21 mg) in a solution of the 6β-methyl compound **1e** (64 mg) in anhydrous THF (11 mL) was stirred at room temperature under a nitrogen atmosphere for 4 h. The reaction was filtered through filter aid and the filtrate stripped and dried on the vacuum pump to give 0.7 g of a clear colorless oil that crystallized on standing. Thin-layer chromatography (50% EtOAc–C_6H_6) of this material showed the appearance of no new materials. The NMR spectrum of this product is exactly the same as that of pure untreated **1e**: 5.76 (s, half-bandwidth < 2 Hz, C-4), 3.64 ($-OCH_3$), 1.32 ppm (d, J = 7 Hz, C_6-CH_3). There were no new signals present for new methoxyl protons or for a 6α-methyl doublet.

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Synthesis of Spin-Labeled Nitroxyl Esters of Steroids

Walter R. Benson,* Millard Maienthal,

Division of Drug Chemistry

George C. Yang,

Division of Chemistry and Physics

Eric B. Sheinin,

Division of Drug Chemistry

and C. W. Chung

Division of Toxicology, Food and Drug Administration, Washington, D.C. 20204. *Received November 8, 1976*

The preparations of spin-labeled nitroxyl esters of prednisone, prednisolone, cortisone, deoxycorticosterone, and cholesterol are described. The ESR spectra indicate that the esters of the three steroids with an oxygen atom at the 11 position have narrower band widths than those with only protons at that position. Mass spectral, chemical, and ESR studies confirmed the structures and purity of the compounds prepared. Reaction of four esters with prednisone antibodies showed reversible binding and large crossover binding.

Rapid and specific techniques involving a minimum of sample manipulation are needed to aid in the analysis of biological fluids for organic molecules. Rapid techniques are also desired for the estimation of the specificity, quality, and yield of antibodies after their preparation and isolation. Radioimmunoassay (RIA) and spin immunoassay (SIA)[1-4] have both been utilized for these purposes. However, RIA requires a time-consuming separation of