Asymmetric Reduction of 20-Steroidal Ketone: Synthesis of Corticosteroid Derivatives Containing the 20α-ol 21-al Side Chain

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Recent in vivo and in vitro studies on corticosteroid metabolism in humans[1,2] and hamsters[1,3] have led to the conclusion that steroids with a 20-hydroxy 21-aldehyde (aldol) side chain are important intermediates in the metabolism of cortisol and other corticosteroids. Therefore, it was important to synthesize this class of metabolites for further biological studies.

A procedure for the synthesis of the 20β epimer of the aldol side chain through a 20-keto 21-oxime intermediate was developed by Oh and Monder.[4] By use of sodium borohydride as the reductant, the 20β steroids were produced preferentially. Generally, side chains of the pregnane and 21-hydroxypregnane series are readily reduced by hydride reagents to the 20β configuration.[5] Reduction to the 20α form is more difficult. Chemical synthesis of these enantiomeric forms have been attempted, and several procedures appear in the literature. Fukushima et al.[6] applied solvolysis to invert 17,20β,21-triols via the 17α-acetate 20β-tosylate. Other approaches to the synthesis of the 20α-hydroxy steroid utilized the reduction by metal hydrides of 17α,20β-epoxy-20-acetoxy steroids,[7] Δ^{16} 20-ones,[8] and 16α,17α-oxido 20-ketone.[9] The reduction by sodium borohydride at C-20 of 17,21-cyclic acetals and alkyl ortho esters to 17,20α,21-triols proceeded efficiently with 11-keto-substituted steroids and slowly with 11-hydroxy or deoxy steroids.[10] Other procedures utilized sodium in 1-propanol,[11] hydrogenation with Raney nickel,[12] and alkaline hydrolysis of 20β-tosylates.[13] Biological reductions of corticosteroids to 20α carbinols have also been reported.[14,15]

The limitations of the available methods made it necessary to consider new approaches to the preparation of 20α-hydroxy steroids. Our goal was to develop broadly applicable methods for the synthesis of steroids with the 20α-hydroxy 21-aldehyde side chain. We now describe a novel method to obtain the 20α-hydroxy epimers of steroid aldols which utilizes many of the techniques we developed for the synthesis of the 20β isomers.

The approach is outlined in Scheme I. Glyoxal **2** derived from ketol **1** by cupric acetate catalyzed oxidation is converted to oxime **3**. Reduction of oxime in the cold in the presence of alkaline earth cations and under two-phase conditions (ethyl acetate/water for 17-hydroxy steroids; chloroform/water for 17-deoxy steroids) yields a mixture of 20β-hydroxy oxime **4** and 20α-hydroxy oxime **5**. The protecting group is removed to afford the aldols **6** and **7**. In early experiments, using a single-phase system, reduction of 10.6 μmol of **3a** with 5.3 or 79 μmol of sodium borohydride in *N,N*-dimethylformamide (DMF)–methanol (2/1 v/v) at 0 °C resulted in 20α/20β ratios of 0.17 and 0.42, respectively. The larger amount of sodium borohydride increased the proportion of the 20α isomer. Under these conditions, the production of the 20α isomer exceeded that obtained by known procedures. at −27 °C, the value with 5.3 μmol of sodium borohydride was 0.32. The temperature decrease doubled the proportion of 20α isomer. In DMF–methanol, significant reduction of the 4-en-3-one occurred. This was substantially diminished in the two-phase system (ethyl acetate–water). Since temperature had a significant effect on the stereochemistry of reduction in the homogeneous DMF–methanol system, we examined the two-phase ethyl acetate–water system to see how a change of solvent conditions influenced the temperature effect. Calcium chloride was added in order to keep the water phase from freezing.

Scheme I

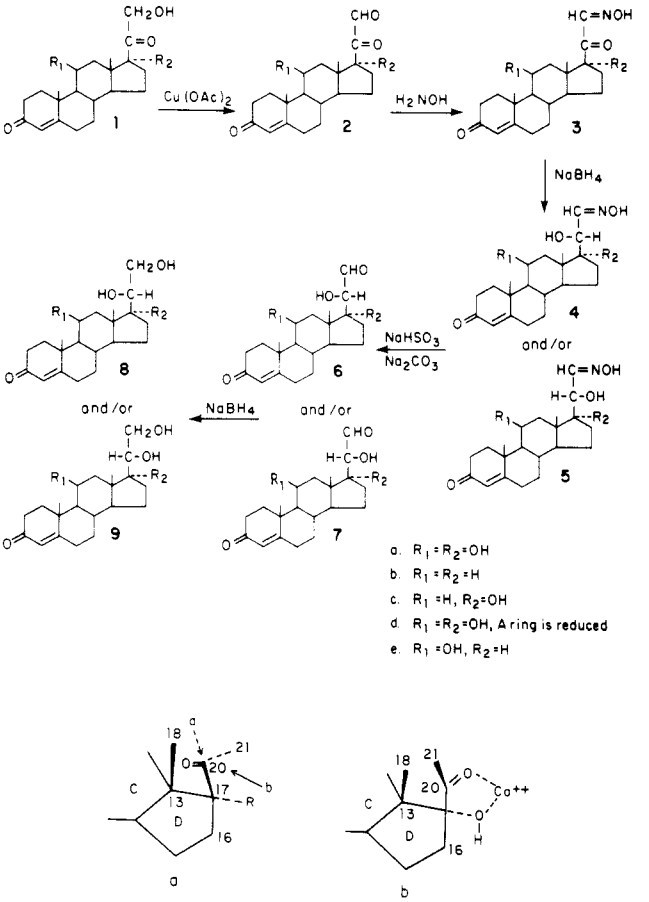

Figure 1. (a) Stereochemistry of attack of borohydride anion at C-20. An "a"-side attack results in 20α isomer; "b"-side attack results in 20β isomer. (b) Structure proposed for complex of calcium ion with the steroid side chain.

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Table I. Partition Coefficients[a] of Steroidal Keto Oxime in Ethyl Acetate-Water at −27 °C

compd	5.5 g of $CaCl_2$/ 10 mL of H_2O	7.0 g of $CaCl_2$/ 10 mL of H_2O	7.5 g of NaBr/ 10 mL of H_2O
3a	1.09 ± 0.02	14.5 ± 0.5	0.026 ± 0.001
3b	0.045 ± 0.005	0.11 ± 0.02	0.01 ± 0.003
3c	0.28 ± 0.01	6.0 ± 0.2	0.01 ± 0.0
3e	0.13 ± 0.01	0.85 ± 0.07	0.01 ± 0.0

[a] The compound (1 mg) was dissolved in 1 mL of ethyl acetate and was stirred with 2 mL of aqueous salt at −27 °C for 1 h. The mixture was kept at −27 °C during separation of phases. To 0.5 mL of aqueous phase was added 1 mL of saturated sodium sulfate solution. It was then extracted three times with 2 mL of ethyl acetate. The ethyl acetate solution was dried over sodium sulfate and evaporated under nitrogen. a 20-μL sample of organic phase was taken out and blown dry. Absorbances of aqueous and organic phases were measured in methanol at 230–240 nm (maximal absorbance). The partition coefficients are expressed as the ratio of the total absorbance of the aqueous phase to the total absorbance of the organic phase. Each value was an average of two independent experiments.

In the presence of $CaCl_2$ (4.2 g/10 mL of water), reduction favoring 20α orientation increased 9-fold as the temperature was lowered from 0 to −27 °C. At every temperature, the calcium salt greatly increased the formation of 20α isomer. When sodium chloride at saturation replaced calcium chloride, it had little effect on the stereochemistry of reduction. With sodium bromide (7.5 g/10 mL of water) as the polar phase, the ratio of **5a** to **4a** was unchanged at 0.4 from 0 to −20 °C. An examination of steroidal structures[16-18] shows that the steroidal 20-carbonyl group is, in general, pointing toward the D ring (Figure 1a). Therefore, attack by borohydride from the "b" side (Figure 1a) will result in a 20β isomer, and attack from "a" side (i.e., from the side of the 18-methyl group) results in a 20α isomer. The effect of calcium is undoubtedly due to its ability to bind with a variety of functional groups, including the carbonyl and hydroxyl groups. It may be postulated that the calcium ions are complexing with the side chain to stabilize a rotational isomer with the conformational characteristics shown in Figure 1b. This figure suggests that the calcium has altered the conformation of the side chain as a result of binding to the 17α-hydroxy and 20-carbonyl groups. Consequently, the sterically unhindered side is now the "a" side.

In Table I, we show that the partition coefficients of 17-hydroxylated steroid oximes undergo major increases favoring water with increasing concentrations of calcium ion, while the 17-deoxy oximes show much smaller changes. The spectacular increases caused by calcium ions in the aqueous solubilities of the 17α-hydroxy steroids, and the much smaller increases in the solubilities of the 17-deoxy steroids supports the model proposed. The formation of a bidentate chelate between calcium ions and the 17α-hydroxy steroids increases the solvent partition to favor the aqueous phase. The directing effect is considerably less with 17-deoxy steroids and is reflected in the unfavorable ratio of 20α/20β in this series. We investigated the effects of solvents on the stereochemistry of reduction of 17-deoxy steroids. Maximum proportionate yield of the 20α epimer occurred in a chloroform–water system (**4b**/**5b** ratio = 1.6) while ethyl acetate–water gave the lowest yield of 20α epimer (**4b**/**5b** ratio = 4.3). Ratios (**4b**/**5b**) with other systems were as follows: carbon tetrachloride–water, 2.8; benzene–water, 2.5; ether–water, 4.1. In the 17α-hydroxy series, calcium ions increased the rate of steroid reduction by borohydride because the steroid was now in more intimate contact with the reducing agent, and the polarizing effect of calcium increased the rate constant of hydride transfer to the carbonyl. The effect was not restricted to calcium but was also seen with strontium and barium ions. Magnesium, unlike other alkaline earth salts, did not increase the formation of the 20α isomers.

Table II. Effects of Metallic Salts on the Reduction of Compounds 3a[a] and 3b[b] with Sodium Borohydride at 0 °C

salt	ionic strength	% 20α[c] a	% 20α[c] b
$MgCl_2$	8.4	27.5	21.3
$CaCl_2$	8.4	66[e]	34.6[f]
$SrCl_2$	8.4	68.8	31
$BaCl_2$	5.4[d]	59	27.5
NaCl	5.1[d]	36.7	20
none		26	16.7

[a] Compound 3a (4 mg) in 1 mL of ethyl acetate, 2 mL of salt solution, and 3 mg of sodium borohydride was mixed in a flask and stirred for 1 h. [b] Compound 3b (5 mg) in 1 mL of $CHCl_3$, 2 mL of salt solution, and 15 mg of sodium borohydride was mixed in a flask and stirred for 3 h. [c] (20α/20α + 20β) × 100. [d] Because of the limited solubilities, the ionic strength of $BaCl_2$ and NaCl could not be used as high as that for others. [e] Since the reaction rate was much faster, the reaction was stopped after 10 min. [f] Since the reaction rate was much faster, the reaction was stopped after 30 min.

Table III. Effect of Varying Calcium Ion Level on Reductions of Steroidal Keto Oximes with Sodium Borohydride in an Ethyl Acetate-Aqueous System[a]

compd	% 20α[b] 4.2 g[d]	5.5 g[d]	7.0 g[d]
3a	90	96.5	100
3b	16	14.5	20
3c	53.7	93	96.4
3d		100	
3e[c]	31.3	35.7	31.3

[a] Reductions were carried out as described in Table II. [b] (20α/20α + 20β) × 100. [c] Because of the limited solubility, 2 mg of compound 3e was used instead of 4 mg. [d] Amount of $CaCl_2$ per 10 mL of H_2O.

The order of effectiveness favoring the 20α-hydroxy orientation for the 17-hydroxy series was $Sr^{2+} > Ca^{2+} > Ba^{2+} > Mg^{2+} = Na^+$ (Table II). For the reduction of the 17-deoxy steroid **3b** the order of efficiency was $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+} = Na^+$ (Table II). The effects of Mg^{2+} and Na^+, though less than those of the other ions, were greater than those obtained with no additions. This suggests that part of the effect of metal ions on the stereoselective reduction of **3b** with sodium borohydride may be nonspecific and associated with ionic strength effects rather than metal specific effects. In a two-phase system, the bulky hydrophobic ring skeleton of the steroid dissolves in the organic solvent, while the hydrophilic side chain dissolves in the aqueous phase. This phase partition is accentuated by the aqueous cationic environment. This accounts for our observation that under these conditions the side chain is reduced by sodium borohydride and the A ring is not. The conformation of the 17β side chain may change with different two-phase solvent systems. With compounds **3b** and **3e**, the chloroform–water system is more effective than other systems in orienting the 20-carbonyl group in a

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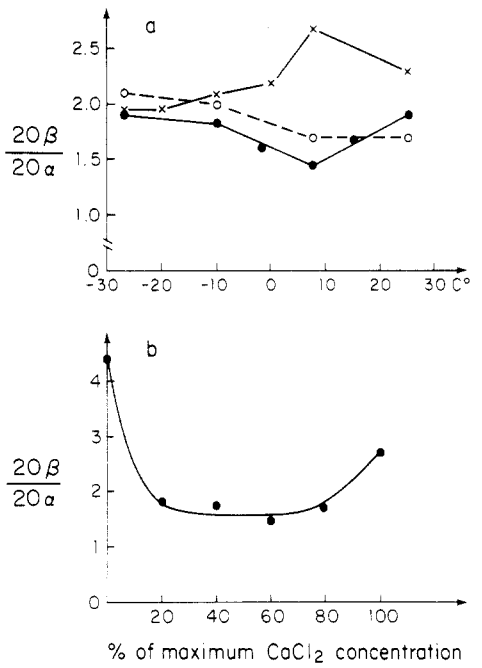

Figure 2. Reduction of 3,20-dioxopregn-4-ene 21-oxime by borohydride anion. The steroid (5 mg) in 1.0 mL of chloroform and 2.0 mL of calcium chloride was mixed with 15 mg of sodium borohydride and stirred for 3 h. The ordinate indicates ratio of 20β epimer to 20α epimer. (a) Effect of temperature. The calcium chloride concentrations (g/10 mL of water) were as follows: ●, 4.2 g; ○, 5.5 g; ×, 7.0 g. (b) Effect of calcium ion (as $CaCl_2$) at 7.5 °C. The abscissa is expressed as percent of maximum $CaCl_2$ concentration (7.0 g/10 mL of water). Other conditions are as described in part a except that the reaction time was 90 min.

conformation favorable to the production of the 20β isomer. With these 17-deoxy derivatives, calcium cannot form chelates. Therefore, the 20β isomers are still the predominant reduction products. The calcium ion probably serves primarily as a "salting-in" reagent for the side chain. The salting-in effect does not vary linearly with temperature and salt concentration.

In support of this concept is the observation that the formation of 20α isomers of both the 17-hydroxy and 17-deoxy series is increased by polar modifications of the steroid ring system. Table III shows that stereoselectivity of reduction was affected not only by the structure of the side chain but also by other structural features as well. An 11β-hydroxy group increased the ratio of 20α to 20β steroid relative to an 11-deoxy group (compare **3a** vs. **3c** and **3b** vs. **3e**). When the A ring was in the reduced form (3α,11β,17-trihydroxy-20-oxo-5β-pregnane-21-oxime) reduction at C-20 to the 20α-hydroxy steroid was greater than 99% at an intermediate concentration of calcium. The effects of 11β- and 17α-hydroxy groups on the same molecule were greater than would have been expected from either one alone (compare **3a** with **3c** and **3e**). The results suggest that the presence of a 17α-hydroxy and the polarity of the molecule both played an important role in directing the stereochemistry of reduction. The polarity influences the partition of the molecule between the organic phase and the aqueous phase.

Figure 2 shows that the stereochemistry of reduction of the 17-deoxy steroid **3b** was not appreciably influenced by the temperature within the range −27 to +25 °C. The ratio of 20β to 20α epimers ranged from 1.4 to 2.7 at 7.5 °C at various concentrations of Ca^{2+}. At other temperatures the ion effect was much less. At any given Ca^{2+} level, the effect of temperature was much less than 2-fold over the range studied. The best conditions for reduction of 17-deoxy keto oxime **3b** to 20α-hydroxy oxime **5b** were a two-phase system of chloroform and water, a calcium chloride concentration of 4.2 g/10 mL of water, and a temperature of 7.5 °C. The amount of sodium borohydride in the range of 5–15 mg had no effect. For reduction of **3e**, the conditions were the same, except that the calcium chloride concentration was 7.0 g/10 mL of water and the temperature was 0 °C.

Experimental Section

Chemical and Reagents. Corticosteroids were bought from Steraloids, Pawling, NY. Hydroxylamine hydrochloride and other reagents were used as purchased without further purification. Prepacked chromatographic columns, containing silica gel 60 were purchased from E. Merck Laboratories, Elmsford, NY. Zorbax and Hibar II (5 μm) octadecylsilyl (ODS) columns were purchased from Du Pont and E. Merck Laboratories, respectively. Elemental analysis was carried out by Integral Microanalytical Laboratories, Inc., Raleigh, NC.

Melting Points. Melting points were determined with a Hoover capillary apparatus and are uncorrected.

Absorption Spectra. The ultraviolet spectra were determined in a Cary Model 15 recording spectrophotometer. Infrared spectra were obtained of KBr micropellets with a Perkin-Elmer Model 521 spectrophotometer.

Nuclear Magnetic Resonance. NMR spectra were recorded in the Fourier transform mode by using a Varian HR-220 spectrometer equipped with a Nicolet 1080 accessory. All samples were prepared by dissolving 1–2 mg of the steroid in 0.5 mL of the appropriate deuterated solvent. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane (δ scale).

Mass Spectrometry. A Du Pont Model 21-492 double focusing magnetic sector mass spectrometer coupled to a VG Datasystem 2040 was used. The source temperatures were in the range of 120–150 °C. Isobutane was used as the reactant for chemical ionization.

MBTH Reaction. About 0.3 mg of steroid was dissolved in 1 mL of ethanol. To 100 μL of the sample solution was added 0.5 mL of 0.1% aqueous 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) solution followed by 1.2 mL of 0.1 M glycine buffer (pH 4.0). after the total volume was adjusted to 2.5 mL by adding water, the mixture was heated in a boiling water bath for 7 min. When the solution had cooled, 2.5 mL of ethanol was added and the absorbancy was determined spectrophotometrically between 200 and 450 nm.

Chromatographic Analysis. The products resulting from sodium borohydride reduction of steroid keto oximes (compounds **3a–e**) were dissolved in methanol and were injected into an ODS column [Zorbax or Hibar II, 250 mm (length) × 4.6 mm (i.d.)] with methanol–water as the mobile phase. Methanol in water (45%, 50%, and 60% v/v) was used to resolve the 20α- and 20β-hydroxy 21-oximes of compounds **a** and **d**, **c** and **e**, and **b**, respectively. The elution profiles were monitored with an ultraviolet spectrophotometer (Du Pont Model 837; 208 nm was used for compound **d**, and 254 nm was used for the others). The 20α and 20β epimers were identified by their mass spectra and comparison with the mobilities of standard 20β epimers made by the method of Oh and Monder.[4]

11β,17α-Dihydroxy-3,20-dioxopregn-4-en-21-al (2a) from 11β,17α,21-Trihydroxypregn-4-ene-3,20-dione (1a). The method of Oh and Monder[4] was used to prepare this compound: mp 160–163 °C dec (lit. mp 160–162 °C dec); UV λ_{max} (alcohol) 241 nm (ϵ 15900; lit. 15700). MBTH derivative: UV λ_{max} 386 nm; IR 5.81 (m), 6.08 (s), 9.60 (s) μm.

3,20-Dioxopregn-4-en-21-al (2b) from 21-Hydroxypregn-4-ene-3,20-dione (1b). The method of Oh and Monder[4] was used to prepare this compound: mp 90–93 °C dec (lit. 90–92 °C dec); UV λ_{max} (alcohol) 241 nm (ϵ 15300; lit. 15400). MBTH derivative: UV λ_{max} 372 nm (lit. 374 nm); IR 5.83 (m), 6.05 (s), 8.15 (m), 9.34 (s) μm.

17α-Hydroxy-3,20-dioxopregn-4-en-21-al (2c) from 17α,21-Dihydroxypregn-4-ene-3,20-dione (1c). The method of Oh and Monder[4] was used to prepare this compound: mp 98–103 °C dec (lit. 96–100 °C dec); UV λ_{max} (alcohol) 241 nm (ϵ 16700; lit. 15400).

MBTH derivative: UV λ_{max} 387 nm; IR 5.83 (m), 6.06 (s) μm.

3α,11β,17α-Trihydroxy-20-oxo-5β-pregnan-21-al (2d) from 3α,11β,17α,21-Tetrahydroxy-20-oxo-5β-pregnane (1d). Conversion of **1d** to **2d** was performed as described for the synthesis of **2a** except that ratio of **1d** to cupric acetate (final concentration 1 mg/1 mL of methanol) was 2 and the oxidation proceeded for 2 h: yield 77%; mp 142 °C dec; MBTH derivative UV λ_{max} 385 nm; IR 2.90 (s), 3.42 (s), 3.50 (m), 5.82 (s), 6.04–6.19 (m, multiple bands), 6.90 (s), 7.22 (m), 7.34 (m), 7.98 (w), 8.21 (w), 9.66 (s), 9.95 (w) μm.

Anal. Calcd for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85. Found: C, 69.45; H, 8.66.

11β-Hydroxy-3,20-dioxopregn-4-en-21-al (2e) from 11β,21-Dihydroxypregn-4-ene-3,20-dione (1e). The method of Oh and Monder[4] was used to prepare this compound: mp 112–116 °C dec (lit. mp 113–116 °C dec); UV λ_{max} (alcohol) 241 nm (ε 14 900; lit. 15 300). MBTH derivative: UV λ_{max} 373 nm; IR 5.84 (m), 6.05 (s), 9.38 (m), 9.64 (m) μm.

3,20-Dioxo-11β,17α-dihydroxypregn-4-ene 21-Oxime (3a) from 2a. The method of Oh and Monder[4] was used to prepare this compound. The product was boiled with ethyl acetate and was recovered by filtration, resulting in pure product: mp 202–204 °C dec (lit. mp 202–203 °C dec); IR 2.90–3.10 (s, multiple bands), 3.42 (s), 5.87 (m), 5.95–6.25 (s, multiple bands), 6.80–7.10 (s, multiple bands), 7.22 (m), 7.37 (m), 7.48 (m), 7.62 (w), 7.82 (m), 8.12 (m), 8.25 (w), 8.42 (m), 8.62 (w), 8.90 (w), 9.03 (m), 9.30 (m), 9.50 (m), 9.70 (m), 9.80 (w) μm.

3,20-Dioxopregn-4-ene 21-Oxime (3b) from 2b. The method of Oh and Monder[4] was used to prepare this compound. The product was boiled with ethyl acetate and was recovered by filtration, resulting in pure product: mp 205–233 °C dec (lit. mp 208–210 °C dec); IR 2.94–3.20 (s, multiple bands), 3.29 (m), 3.43 (s), 3.52 (m), 6.0–6.20 (s, multiple bands), 6.8–7.0 (s, multiple bands), 7.23 (m), 7.35 (m), 7.52 (m), 7.82 (m), 7.90 (w), 8.02 (m), 8.12 (m), 8.26 (w), 8.42 (m), 8.60 (m), 9.02 (m), 9.92 (s) μm.

3,20-Dioxo-17α-hydroxypregn-4-ene 21-Oxime (3c) from 2c. The method of Oh and Monder[4] was used to prepare this compound. The product was boiled with ethyl acetate and was recovered by filtration, resulting in pure product: mp 196–198 °C dec (lit. mp 195–197 °C dec); IR 2.92 (s), 3.42 (s), 3.48 (w), 5.9–6.2 (s, multiple bands), 6.8–7.05 (m, multiple bands), 7.22 (w), 7.37 (w), 7.55 (w), 7.92 (w), 8.14 (m), 8.27 (m), 8.45 (m), 8.95 (m), 9.25 (m), 9.40 (m) μm.

20-Oxo-3α,11β,17α-trihydroxy-5β-pregnane 21-Oxime (3d) from 2d. The compound **3d** was prepared as described for the synthesis of **3a**. The oxime was purified on a silica gel column (chloroform/methanol, 94:6; yield 65%). The product was recrystallized from ethanol–water: mp 138–141 °C dec; IR 2.92 (s), 3.42 (s), 3.51 (m), 5.88 (w), 5.97 (w), 6.92 (m), 7.23 (w), 7.35 (w), 7.74 (w), 7.98 (w), 8.23 (w), 8.37 (w), 9.71 (m), 9.95 (m) μm.

Anal. Calcd for $C_{21}H_{33}O_5N{\cdot}0.5CH_2H_5OH$: C, 65.65; H, 9.01; N, 3.48. Found: C, 65.96; H, 8.62; N, 3.24.

3,20-Dioxo-11β-hydroxypregn-4-ene 21-Oxime (3e) from 2e. The method of Oh and Monder[4] was used to prepare this compound. The product was boiled with ethyl acetate and was recovered by filtration. Further purification was not attempted: mp 205–218 °C dec (lit. mp 184–188 °C dec); IR 2.95–3.20 (s, multiple bands), 3.31 (w), 3.42 (s), 3.49 (w), 5.95–6.20 (s, multiple bands), 6.80–7.10 (m, multiple bands), 7.22 (m), 7.29 (w), 7.35–7.48 (m, multiple bands), 7.64 (w), 7.82 (w), 7.92 (w), 8.13 (m), 8.49 (m), 8.71 (m), 9.00 (m), 9.25 (w), 9.40 (m) μm.

11β,17α,20α-Trihydroxy-3-oxopregn-4-ene 21-Oxime (5a) from 3a. Compound **3a** (200 mg) was dissolved in 25 mL of ethyl acetate and was stirred vigorously with 50 mL of calcium chloride solution (7 g/10 mL of H_2O) at −27 °C. To the mixture was added 25 mg of sodium borohydride in 1 mL of the above calcium chloride solution. The solution was stirred at −27 °C for 60 min. Water (15 mL) was added, and the two phases were separated. The aqueous phase was extracted three times with ethyl acetate. The combined solvent extract was washed with water (15 mL) twice and then dried over anhydrous sodium sulfate. HPLC analysis showed only a trace amount of 20β isomer. The product was recrystallized from ethanol–water: purified yield 132 mg (66%); mp 232–234 °C; IR 2.82 (w), 2.96 (s), 3.40–3.49 (s, multiple bands), 6.06–6.21 (s, multiple bands), 7.08 (m), 7.23 (w), 7.53 (m), 7.86 (m), 8.15 (m), 8.45 (m), 9.00 (m), 9.38 (m), 9.62 (w), 9.76 (w), 9.96 (w), 10.14 (w), 10.82 (s) μm.

Anal. Calcd for $C_{21}H_{31}O_5N$: C, 66.82; H, 8.28; N, 3.71. Found: C, 66.55; H, 8.24; N, 3.62.

20β-Hydroxy-3-oxopregn-4-ene 21-Oxime (4b) and 20α-Hydroxy-3-oxopregn-4-ene 21-Oxime (5b) from 3b. Compound **3b** (200 mg) was dissolved in 40 mL of chloroform and was stirred vigorously with 80 mL of calcium chloride solution (4.2 g/10 mL of H_2O) at 7.5 °C. To the mixture 600 mg of sodium borohydride was added. The solution was stirred at 7.5 °C for 80 min. The aqueous layer was separated from the chloroform layer and was extracted with ethyl acetate (50 mL) three times. The combined ethyl acetate extracts and the chloroform layer were each washed with 50 mL of water. The combined organic solution was shaken and dried with anhydrous sodium sulfate. The organic solvent was removed under vacuum. HPLC analysis showed a **4b** (20β)/**5b** (20α) ratio of 1.5:1. The combined steroid fraction **4b** and **5b** dissolved in 2.5 mL of chloroform–methanol (92/8 v/v) was purified on a silica gel column (E. Merck, size B) with chloroform as the eluent. **4b** came off the column before **5b**: yield of **4b**, 80 mg (40%); yield of **5b**; 40 mg (20%). The yield of **5b** was less than that shown in HPLC analysis because of overlap of **4b** and **5b** and the tailing of **5b** during elution. We have recently found that a reversed-phase HPLC column [Du Pont RP-18, 250 mm × 7.6 mm (i.d.)] gave improved separation. The sample was introduced into the column with methanol and eluted with methanol–water (55/45 v/v) as the mobile phase. If **5b** contaminates **4b**, it can be washed off (no heating; both **4b** and **5b** decompose on heating) with methanol or ethyl acetate. Both **4b** and **5b** were recrystallized from ethanol–water.

4b: mp 217.5–218.5 °C; IR 2.88–3.13 (s, multiple bands), 3.42 (s), 3.49 (w), 6.00–6.09 (s, multiple bands), 6.24 (m), 6.82–7.07 (m, multiple bands), 7.31 (w), 7.47 (w), 7.57 (w), 7.93 (m), 8.09 (w), 8.20 (m), 8.50 (m), 9.10 (m), 9.50 (w), 9.82 (w), 10.01 (w) μm.

Anal. Calcd for $C_{21}H_{31}O_3N$: C, 73.01; H, 9.05; N, 4.05. Found: C, 72.85; H, 8.72; N, 4.01.

5b: mp 192–193.5 °C; IR 2.97–3.09 (s, multiple bands), 3.43 (s), 3.50 (w), 6.05–6.10 (s, multiple bands), 6.21 (w), 6.82–7.08 (m, multiple bands), 7.32 (w), 7.38 (w), 7.57 (m), 7.88 (m), 7.93 (m), 8.10 (m), 8.18 (m), 8.49 (m), 8.60 (w), 9.10 (w), 9.47 (w), 9.60 (w) μm.

Anal. Calcd for $C_{21}H_{31}O_3N{\cdot}0.5H_2O$: C, 71.15; H, 9.10; N, 3.95. Found: C, 70.75; H, 8.94; N, 3.91.

17α,20α-Dihydroxy-3-oxopregn-4-ene 21-Oxime (5c) from 3c. The preparation of **5c** was performed as described for the synthesis of **5a** except that 13 instead of 25 mg of sodium borohydride was used, and the reduction proceeded for 80 min. HPLC analysis showed a **4c** (20β)/**5c** (20α) ratio of 1:15.4. The product was recrystallized from ethanol–water; yield 173 mg (86%). HPLC analysis showed only a trace amount of 20β isomer. Extremely pure products can be obtained by recrystallization from ethanol: mp 217–218 °C; IR 2.92 (s), 3.42 (s), 3.49 (w), 6.00–6.18 (s, multiple bands), 6.78–7.05 (m, multiple bands), 7.28 (w), 7.34 (m), 7.52 (w), 7.91 (m), 8.13 (m), 8.44 (m), 8.92 (m), 9.52 (m), 10.10 (m) μm.

Anal. Calcd for $C_{21}H_{31}O_4N$: C, 69.78; H, 8.64; N, 3.87. Found: C, 70.08; H, 8.60; N, 3.59.

3α,11β,17α,20α-Tetrahydroxy-5β-pregnane 21-Oxime (5d) from 3d. The preparation of **5d** was performed as described for the synthesis of **5a** except that calcium chloride solution was 5.5 g/10 mL of H_2O instead of 7 g/10 mL of H_2O; yield 150 mg (75%). HPLC analysis showed only a trace amount of 20β isomer. The product was recrystallized from ethanol–water: mp 192–193.5 °C; IR 2.92 (s), 3.43 (m), 3.51 (w), 6.02–6.24 (m, multiple bands), 6.82–7.05 (m, multiple bands), 9.02 (m), 9.40 (w), 9.67 (m).

Anal. Calcd for $C_{21}H_{35}O_5N{\cdot}2H_2O$: C, 60.41; H, 9.41; N, 3.35. Found: C, 60.20; H, 9.12; N, 3.06.

11β,20β-Dihydroxy-3-oxopregn-4-ene 21-Oxime (4e) and 11β,20α-Dihydroxy-3-oxopregn-4-ene 21-Oxime (5e) from 3e. Compound **3e** (50 mg) was dissolved in 50 mL of chloroform and was stirred vigorously with 100 mL of calcium chloride solution (7 g/10 mL of H_2O) at 0 °C. To the mixture was added 300 mg of sodium borohydride. The solution was stirred at 0 °C for 60 min. The aqueous layer was separated from the chloroform layer and was extracted with ethyl acetate (25 mL) three times. The combined ethyl acetate extracts and the chloroform layer were each washed with 50 mL of water. The combined organic solution was shaken and dried with anhydrous sodium sulfate. The organic

solvent was removed under vacuum. HPLC analysis showed a **4e** (20β)/**5e** (20α) ratio of 1.3:1. The steroid fraction was dissolved in methanol, and **4e** and **5e** were separated and purified on a reversed-phase HPLC column [Du Pont RP-18, 250 nm × 7.6 mm (i.d.); mobile phase methanol–water (45/55 v/v)]. **5e** came off before **4e**: yield of **4e**, 18 mg (36%); yield of **5e**, 12 mg (24%). **4e** and **5e** were recrystallized from ethanol–water.

4e: mp 112 °C dec; IR 2.93 (s), 3.42 (s), 3.49 (w), 6.03–6.18 (s, multiple bands), 6.8–7.06 (m, multiple bands), 7.47 (m), 7.59 (m), 7.89 (m), 8.07 (m), 8.16 (m), 8.43 (m), 8.68 (m), 9.47 (m), 9.76 (m) μm.

Anal. Calcd for $C_{21}H_{31}O_4N·1.5H_2O$: C, 64.92; H, 8.82; N, 3.61. Found: C, 64.82; H, 8.80; N, 3.49.

5e: mp 200 °C dec; IR 2.94 (s), 3.42 (s), 3.48 (w), 6.02–6.18 (s, multiple bands), 6.79–7.05 (m, multiple bands), 7.52 (m), 7.89 (m), 8.15 (m), 8.44 (m), 8.68 (m), 9.08 (m), 9.90 (m) μm.

Anal. Calcd for $C_{21}H_{31}O_4N$: C, 69.78; H, 8.64; N, 3.87. Found: C, 69.50; H, 8.92; N, 3.68.

11β,17α,20α-Trihydroxy-3-oxopregn-4-en-21-al (7a) from 5a. Compound **7a** was prepared by hydrolysis of the oxime **5a** as described for the synthesis of **6a** by Oh and Monder,[4] except that hydrolysis by sodium carbonate proceeded for 5 min instead of 10 min: yield 35 mg (44.6%); mp 151–153 °C; IR 2.82 (w), 2.92 (s), 3.44 (s), 3.51 (w), 5.83 (m), 6.06–6.23 (s, multiple bands), 6.88–7.12 (m, multiple bands), 7.50 (w), 7.60 (m), 7.90 (m), 8.18 (m), 8.52 (m), 8.98 (m), 9.73 (m) μm; UV (methanol) λ_{max} 241 nm (ε 15 300), MBTH derivative λ_{max} 312 nm (ε 22 000); NMR ($CDCl_3$) 1.25 (18-CH_3); 1.46 (19-cH_3), 4.38 (11-H), 4.46 (20-H, d, J = 2.9 Hz), 9.90 (21-CHO); mass spectrum, m/e (relative intensity) 363 ($M^+ + 1 - CHOCH_2OH$, 100), 285 ($M^+ + 1 - (CHOCH_2OH + H_2O)$, 12).

Anal. Calcd for $C_{21}H_{30}O_5$: C, 69.59; H, 8.34. Found: C, 69.59; H, 8.57.

20β-Hydroxy-3-oxopregn-4-en-21-al (6b) and 20α-Hydroxy-3-oxopregn-4-en-21-al (7b) from 5b. Compounds **6b** and **7b** were prepared by hydrolysis of the oximes **4b** and **5b**, respectively, as described for the synthesis of **6b** by Oh and Monder.[4]

6b: yield 35 mg (52%); mp 142–142.5 °C (lit. 140 °C dec); IR 2.92–2.98 (s, multiple bands), 3.42 (s), 3.50 (w), 5.82 (s), 6.03 (s), 6.21 (w), 6.94 (m), 7.02 (m), 7.32 (w), 7.44 (m), 7.56 (w), 7.90 (m), 8.13 (m), 8.19 (m), 8.49 (m), 9.08 (w), 9.44 (m), 9.82 (w), 9.95 (m) μm; UV (methanol) λ_{max} 241 nm (ε 16 600; lit. 16 400), MBTH derivative λ_{max} 312 nm (ε 26 900; lit. 26 900); NMR ($CDCl_3$) 0.89 (18-CH_3), 1.20 (19-CH_3), 4.17 (20-H, d, J = 10.5 Hz), 5.73 (4-H), 9.72 (21-CHO); mass spectrum, m/e (relative intensity) 331 ($M^+ + 1$, $C_{21}H_{31}O_3$, 100), 301 ($M^+ + 1 - CH_2O$, 16).

Anal. Calcd for $C_{21}H_{30}O_3$: C, 76.33; H, 9.15. Found: C, 75.94; H, 9.54.

7b: yield 40 mg (80%); mp 134–135 °C; IR 2.92–3.01 (s, multiple bands), 3.43 (s), 3.52 (m), 5.79 (s), 6.07 (s), 6.23 (m), 6.94 (m), 7.02 (m), 7.10 (w), 7.30 (w), 7.40 (m), 7.57 (m), 7.69 (w), 7.90 (m), 8.17 (m), 8.43 (m), 8.56 (w), 8.72 (w), 8.79 (m), 9.06 (m), 9.20 (m), 9.45 (w), 9.66 (m), 9.85 (m), 10.68 (m), 10.83 (m) μm; UV (methanol) λ_{max} 241 nm (ε 15 500), MBTH derivative λ_{max} 312 nm (ε 21 800); NMR ($CDCl_3$) 0.87 (18-CH_3), 1.21 (19-CH_3), 4.32 (20-H, d, J = 2.9 Hz), 5.75 (4-H), 9.67 (21-CHO); mass spectrum, m/e (relative intensity) 331 ($M^+ + 1$, $C_{21}H_{31}O_3$, 100), 329 ($M^+ + 1 - H_2$, 21), 301 ($M^+ + 1 - CH_2O$), 7).

Anal. Calcd for $C_{21}H_{30}O_3·0.5H_2O$: C, 74.4; H, 9.20. Found: C, 74.67; H, 8.91.

17α,20α-Dihydroxy-3-oxopregn-4-en-21-al (7c) from 5c. Compound **7c** was prepared by hydrolysis of the oxime **5c**, as described for the synthesis of **6c** in Oh and Monder.[4] The product was isolated from ethyl acetate–hexane: yield 38 mg (55%); mp 132 °C dec; IR 2.92 (s), 3.42 (s), 3.49 (w), 5.77–5.86 (m, multiple bands), 5.97–6.20 (s, multiple bands), 6.80–7.05 (m, multiple bands), 7.28–7.52 (m, multiple bands), 7.89 (m), 8.06 (w), 8.13 (m), 8.44 (m), 8.89 (m), 8.98 (w) μm; UV (methanol) λ_{max} 241 nm (ε 15 100), MBTH derivative λ_{max} 312 nm (ε 21 500); NMR ($CDCl_3$) 1.01 (18-CH_3), 1.21 (19-CH_3), 4.37 (20-H), 5.74 (4-H), 9.89 (21-H); mass spectrum, m/e (relative intensity) 347 ($M^+ + 1$, $C_{21}H_{31}O_4$, 34), 329 ($M^+ + 1 - H_2O$, 14), 317 ($M^+ + 1 - CH_2O$, 70), 301 ($M^+ + 1 - (H_2O + CO)$, 287 ($M^+ + 1 - CHOCH_2OH$, 100).

Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.57; H, 8.58.

3α,11β,17α,20α-Tetrahydroxy-5β-pregnan-21-al (7d) from 5d. Compound **7d** was prepared by hydrolysis of the oxime **5d** as described for the synthesis of **7a**. The product was isolated from ethyl acetate–hexane: yield 49 mg (70%); mp 111 °C dec; IR 2.92 (s), 3.42 (s), 3.49 (m), 5.76–5.88 (s, multiple bands), 6.04–6.18 (m, multiple bands), 6.78–6.95 (s, multiple bands), 7.20 (w), 7.33 (m), 7.97 (m), 8.90 (m), 9.40 (m), 9.63 (m), 9.90 (m) μm; UV (MBTH derivative) λ_{max} 312 nm (ε 19 200); NMR (acetone-d_6) 1.17 (18-CH_3), 1.21 (19-CH_3), 3.53 (3-H, m), 4.17 (20-H), 4.26 (11-H, m), 9.78 (21-H, d, J = 0.81 Hz); mass spectrum, m/e (relative intensity) 367 ($M^+ + 1$, $C_{21}H_{35}O_5$, 30), 349 ($M^+ + 1 - H_2O$, 20), 337 ($M^+ + 1 - CH_2O$, 16), 331 ($M^+ + 1 - 2H_2O$, 29), 307 ($M^+ + 1 - (CHO-CH_2OH)$, 57), 289 ($M^+ + 1 - (H_2O + CHOCH_2OH)$, 100), 271 ($M^+ + 1 - (2H_2O + CHOCH_2OH)$, 43).

Anal. Calcd for $C_{21}H_{34}O_5·0.5H_2O$: C, 67.17; H, 9.40. Found: C, 67.31; H, 9.59.

11β,20β-Dihydroxy-3-oxopregn-4-en-21-al (6e) and 11β,20α-Dihydroxy-3-oxopregn-4-en-21-al (7e) from 5e. Compounds **6e** and **7e** were prepared by hydrolysis of the oximes **4e** and **5e**, respectively, as described for the synthesis of **6e** by Oh and Monder.[4]

6e: isolated from ethyl acetate–hexane; yield 29 mg (44%); mp 136 °C dec (lit., 138 °C dec); IR 2.92 (s), 3.42 (s), 3.48 (w), 3.51 (w), 5.77–5.90 (m, multiple bands), 6.02–6.18 (s, multiple bands), 6.79–7.06 (m, multiple bands), 7.28–7.47 (m, mutliple bands), 7.89 (m), 8.14 (m), 8.43 (m), 8.71 (m), 11.58 (m) μm; UV (methanol) λ_{max} 241 nm (ε 13 000; lit. 15 100), MBTH derivative λ_{max} 312 nm (ε 22 600; lit. 17 500); NMR ($CDCl_3$) 1.13 (18-CH_3), 1.46 (19-CH_3), 4.16 (20-H, d, J = 10.63 Hz), 4.34 (11-H, m), 5.68 (4-H), 9.71 (21-H); mass spectrum, m/e (relative intensity) 347 ($M^+ + 1$, $C_{21}H_{31}O_4$, 100), 345 ($M^+ + 1 - H_2$, 29), 331 ($M^+ + 1 - O$, 20), 329 ($M^+ + 1 - H_2O$, 13.8), 317 ($M^+ + 1 - CH_2O$, 65), 299 ($M^+ + 1 - (CH_2O + H_2O)$, 12), 289 ($M^+ + 1 - (CHO-CHO)$, 17).

Anal. Calcd for $C_{21}H_{30}O_4·0.5H_2O$: C, 70.96; H, 8.79. Found: C, 70.92; H, 8.53.

7e: isolated from dichloromethane–hexane; yield 35 mg (52%); mp 144–146 °C; IR 2.92 (s), 3.42 (s), 3.48 (w), 3.51 (w), 5.76–5.88 (m, multiple bands), 5.93–6.21 (s, multiple bands), 6.77–7.04 (m, multiple bands), 7.33–7.57 (m, multiple bands), 7.86 (m), 8.13 (m), 8.43 (m), 8.70 (m), 9.00 (m) μm; UV (methanol) λ_{max} 241 nm (ε 12 700), MBTH derivative λ_{max} 312 nm (ε 17 900); NMR ($CDCl_3$) 1.10 (18-CH_3), 1.46 (19-CH_3), 4.30 (20-H, d, J = 3.59 Hz), 4.41 (11-H, m,), 5.68 (4-H), 9.69 (21-H); mass spectrum, m/e (relative intensity) 347 ($M^+ + 1$, $C_{21}H_{31}O_4$, 100), 345 ($M^+ + 1 - H_2$, 23), 331 ($M^+ + 1 - O$, 24), 329 ($M^+ + 1 - H_2O$, 19), 317 ($M^+ + 1 - CH_2O$, 77), 301 ($M^+ + 1 - (CO + H_2O)$, 21), 299 ($M^+ + 1 - (CH_2O + H_2O)$, 25), 289 ($M^+ + 1 - (CHO-CHO)$, 48).

Anal. Calcd for $C_{21}H_{30}O_4·0.5H_2O$: C, 70.96; H, 8.79. Found: C, 71.17; H, 8.82.

Determination of Stereochemistry at C-20. Compounds **6** and **7** (1 mg) were reduced to 20,21-glycols by addition of 0.04 mg of sodium borohydride in 0.08 mL of methanol in an ice bath. After the mixture was stirred 10 min, 0.2 mL of water was added, and methanol was blown off under a stream of nitrogen. The aqueous solution was extracted with ethyl acetate and chromatographed with authentic standard on a silica gel coated TLC plate which had been dipped into 0.1 M sodium borate solution (pH 9.0) and dried at 45 °C overnight. R_f values with chloroform–methanol (90:10) as the developing solvent were as follows (mobilities of authentic standards in parentheses): **9a**, 0.18 (20β, 0.25; 20α, 0.18); **8b**, 0.37; **9b**, 0.26 (20β, 0.37; 20α, 0.26); **9c**, 0.37 (20β, 0.47; 20α, 0.37). With chloroform–methanol (85:15): **9d**, 0.38 (20β, 0.43; 20α, 0.38). With ethyl acetate–methanol–formic acid (50:50:1): **8e**, 0.32; **9e**, 0.24 (20β, 0.32; 20α, 0.24).

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Registry No. 1a, 50-23-7; **1b**, 64-85-7; **1c**, 152-58-9; **1d**, 53-02-1; **1e**, 50-22-6; **2a**, 14760-49-7; **2b**, 853-27-0; **2c**, 20287-95-0; **2d**, 80753-74-8; **2e**, 20287-97-2; **3a**, 59005-51-5; **3b**, 59005-48-0; **3c**, 59005-49-1; **3d**, 80753-75-9; **3e**, 59005-50-4; **4b**, 59005-52-6; **4e**, 59005-54-8; **5a**, 80764-23-4; **5b**, 80753-76-0; **5c**, 80753-77-1; **5d**, 80753-78-2; **5e**, 80753-79-3; **6b**, 59005-56-0; **6e**, 59005-58-2; **7a**, 80753-80-6; **7b**, 75056-44-9; **7c**, 80753-81-7; **7d**, 80753-82-8; **7e**, 80753-83-9; **8b**, 298-35-1; **8e**, 1048-51-7; **9a**, 1719-79-5; **9b**, 26437-06-9; **9c**, 3946-10-9; **9d**, 516-38-1; **9e**, 977-22-0.