extracted with CH_2Cl_2 (3 × 10 mL) and then treated with activated charcoal. The resulting colorless solution was evaporated to dryness. The residue was slurried in H_2O and the solid collected on a filter and rinsed several times with cold H_2O. This material and a second crop obtained after concentration of the filtrate provided pure **4f** (64.0 mg, 0.30 mmol, 15%): mp 198–200 °C dec (lit.[18] mp 198–200 °C); TLC (3:1:1:1 EBAW) R_f 0.6; NMR (1 N DCl in D_2O) 4.59 (1 H, d, J = 10), 6.06 (1 H, dd, J = 16, 10), 6.72 (1 H, d, J = 10), 7.1–7.3 (5 H, m); MS(FAB), m/e 178 ($M^+ + 1$). Anal. Calcd for $C_{10}H_{11}NO_2 \cdot {}^1/_4H_2O$: C, 66.10; H, 6.38; N, 7.70. Found: C, 65.81; H, 6.24; N, 7.62.

Preparation of Amino Acid 4e from NH_4OAc. A mixture of (*E*)-2-methyl-2-butenal (0.168 g, 2.0 mmol), NH_4OAc (0.442 g, 6.0 mmol) and KCN (0.130 g, 2.0 mmol) in EtOH (4 mL) was stirred for 5 h under nitrogen. The reaction mixture was evaporated to dryness and the residue combined with 6 N HCl (30 mL). The solution was heated at reflux for 18 h and then treated as described above for **4c**, affording pure amino acid (69.4 mg, 0.538 mmol, 27%), identical with that prepared as described above.

2-(Benzylamino)-5,5-dimethylbutyrolactone Hydrochloride (6). A mixture of 3,3-dimethylacrolein (1.00 g, 11.9 mmol), benzylamine (1.28 g, 11.9 mmol), and 4-Å molecular sieves (2.0 g) in CH_2Cl_2 (25 mL) was stirred under nitrogen for 1 h. Then trimethylsilyl cyanide (1.75 mL, 13.1 mmol) was added and stirring was continued for 1 h. The mixture was filtered and evaporated to dryness. The residue was combined with concentrated HCl (80 mL) and the mixture heated at reflux for 18 h. The mixture was diluted with H_2O (100 mL) and treated, while still warm, with activated charcoal. Filtration and cooling of the solution gave a white solid, which was collected and rinsed with H_2O. This and a second crop obtained upon concentration of the filtrate provided 2.08 g (8.16 mmol, 68%) of lactone: mp 258–260 °C dec; TLC (50:1 CH_2Cl_2:CH_3OH) R_f 0.2; NMR (CD_3OD) 1.48 (3 H, s), 1.56 (3 H, s), 2.23 (1 H, t, J = 13), 2.74 (1 H, dd, J = 13, 8), 4.43 (2 H, AB, J_{AB} = 12, δ_{AB} = 52), 4.75 (1 H, dd, J = 13, 8), 7.5–7.7 (5 H, m); MS, m/e 176 ($M^+ + 1 - CO_2$). Anal. Calcd for $C_{13}H_{17}NO_2 \cdot HCl$: C, 61.05; H, 7.09; N, 5.48; Cl, 13.86. Found: C, 61.15; H, 7.16; N, 5.35; Cl, 13.81.

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Registry No. **3** (R = $PhCH_2$; R_1 = R_2 = Me; R_3 = H), 90461-23-7; (*E*)-**3** (R = $CH(C_6H_4$-*p*-$OMe)_2$; R_1 = Me; R_2 = R_3 = H), 90481-27-9; (*E*)-**4** (R = R_2 = R_3 = H; R_1 = Ph), 90461-24-8; (*E*)-**4a**, 90528-90-8; (*Z*)-**4a**, 90528-91-9; (*E*)-**4b**, 90461-20-4; (*Z*)-**4b**, 90461-21-5; (*E*)-**4c**, 90461-19-1; (*E*)-**4d**, 90461-22-6; (*E*)-**4e**, 80744-99-6; (*E*)-**4f**, 90528-92-0; **4g**, 56512-51-7; **5**, 19293-62-0; **6**, 77694-17-8; $NH_3 \cdot HOAc$, 631-61-8; $PhCH_2NH_2$, 100-46-9; $CH_3CH{=}CHCHO$, 4170-30-3; $(CH_3)_2CHNH_2$, 75-31-0; $CH_2{=}CHCHO$, 107-02-8; $(p\text{-}MeOC_6H_4)_2CHCl$, 7525-23-7; 4,4'-dimethoxydiphenylmethanol, 728-87-0; *trans*-2-hexenal, 6728-26-3; *trans*-cinnamaldehyde, 14371-10-9; (*E*)-2-methyl-2-butenal, 497-03-0; 3,3-dimethylacrolein, 107-86-8.

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Synthesis of 6β-(Bromoacetoxy)cortisol 21-Bromoacetate: A Novel Reagent for Labeling the Catalytic Site of Enzymes

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Earlier, we synthesized the first steroid with two alkylating functional groups, 2,4-bis(bromomethyl)-3-hydroxy1,3,5(10)-estradien-17-one, and found it to be a long-acting antiestrogen.[1] 2,4-Bis(bromomethyl)estradiol, prepared by reduction of this compound, was a long-acting estrogen and it also irreversibly inactivated the enzyme human placental 17β-estradiol dehydrogenase.[2] Several radioactive (bromoacetoxy)progesterone derivatives were synthesized and found to be useful for radiolabeling amino acids at the catalytic site of the enzyme 3α,20β-hydroxysteroid dehydrogenase (3α,20β-HSD).[3-6] 16α-(Bromoacetoxy)progesterone, 11α-(bromoacetoxy)progesterone, and 19-nortestosterone 17-bromoacetate terminated pregnancy in rats.[7,8] Medroxyprogesterone 17-bromoacetate maintained pregnancy in rats although it was equal to 16α-(bromoacetoxy)progesterone both in its chemical reactivity and also its capacity to inactivate 3α,20β-HSD by the mechanism of affinity alkylation.[9,10] The many intriguing and useful properties of these novel steroids inspired us to design an even more versatile bromoacetoxy steroid derivative for biochemical and reproductive biological experiments. This led to the present synthesis of 6β-(bromoacetoxy)cortisol 21-bromoacetate.

In general, the solubility in water of the various (bromoacetoxy)progesterones prepared by us[3-8] has been found to be about 0.1 mM, or approximately one-tenth that of progesterone. We assumed that the addition of two bromoacetoxy groups to progesterone would reduce its water solubility by as much as 2 orders of magnitude which would render a bis(bromoacetoxy)progesterone analogue unusable for experiments with enzymatic proteins. Enzymes are generally soluble and stable only in aqueous, buffer solutions. Therefore, to enhance its water solubility for use in enzyme experiments, a suitable bis(bromoacetoxy) steroid had to contain two free hydroxy groups. The polarity and hydrogen-bonding capability of the hydroxy groups were expected to compensate for the hydrophobicity of the two bromoacetoxy groups thus enhancing the water solubility of the steroid.

The main synthetic problem was the stepwise introduction of two bromoacetoxy groups at specific positions on a steroid molecule which contained four hydroxy groups (**4**, Scheme I). Furthermore, a progesterone analogue in which one bromoacetoxy group is at the C-6β position and the other group is at the C-21 position was required for extension of our previous work. These positional and configurational requirements were necessary for comparing the biochemical and biological properties of the new steroid with those of 6β-(bromoacetoxy)progesterone and 21-(bromoacetoxy)progesterone from our earlier enzymological[2,4,6] and reproductive biological studies.[7,8] This report describes the synthesis of **7** which had the desired structure and also enhanced water solubility.

Results and Discussion

Synthesis. Cortisol (**1**) was heated under reflux in a mixture of 2,2-dimethoxypropane, dimethylformamide, and a catalytic amount of *p*-toluenesulfonic acid and gave a 75% yield of the crude dienol methyl ether acetonide (**2**). The acetonide group in **2** blocked both the C-17 and C-21 hydroxy groups and the conjugated dienol function in the

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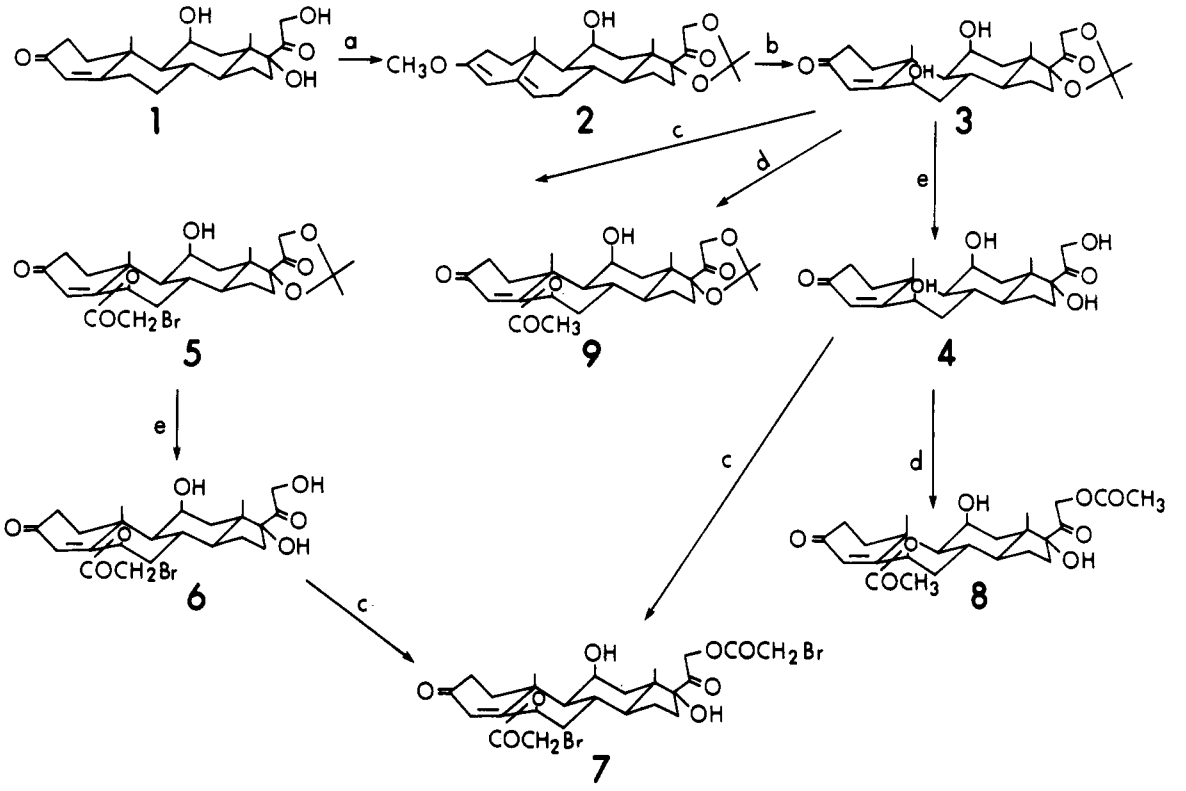
Scheme I[a]



[a] (a) $CH_3C(OCH_3)_2$/DMF/*p*-TsOH; (b) *hν* (UV); (c) $BrCH_2COOH$/DCC; (d) $(CH_3CO)_2O$/pyridine; (e) CH_3COOH/CH_3OH.

A/B ring system permitted introduction of the 6β-hydroxy group in the subsequent step. Photooxidation of **2** in ethanol for 2 weeks gave a 30% overall yield of **3**. Removal of the C-17/C-21 acetonide group with 1:1 acetic acid–methanol liberated the C-17 and C-21 hydroxy groups to give the tetrahydroxy steroid **4**. Reacting **4** with 2 equiv of bromoacetic acid and an excess of dicyclohexylcarbodiimide (method A) gave **7** (mp 155 °C) as the sole product in 75% yield. The structure assigned to **7** as represented in Scheme I was supported by elemental analysis and spectroscopic data. The stepwise introduction of the C-6β and C-21 bromoacetoxy groups (method B), via **6** which contained the C-6β bromoacetoxy group (Scheme I), produced a compound with identical physical and spectroscopic properties to that of **7** from method A. The stepwise and stereoselective introduction of the C-6β and C-21 bromoacetoxy groups suggests that the C-6β [^{3}H] and C-21 [^{14}C] radioisotopic derivatives of **7** can be produced.

The assignments of the chemical shifts for the NMR signals due to the methylene protons of the bromoacetoxy groups in **7** deserve some comments. As a point of reference, the signal from the methylene protons in the bromoacetoxy group of the known 6β-(bromoacetoxy)progesterone[4] (in $CDCl_3$) appears as a sharp singlet at δ 3.90. Similarly, the corresponding signals due to the C-6β bromoacetoxy group in **5** ($CDCl_3$) and **6** (CDCN) are δ 3.85 and 3.95, respectively. On the basis of these NMR data, the range of the chemical shifts for the methylene protons in a bromoacetoxy group at the C-6β position of a 4-pregnene steroid is from δ 3.85 to 3.95. The bis(bromoacetoxy) steroid **7** produces two sharp singlets at δ 3.90 and 3.75. Therefore, we assigned the δ 3.90 signal to the C-6β bromoacetoxy group, and the δ 3.75 signal (which falls out of the above range) was assigned to the C-21 bromoacetoxy group.

Reactions of 6 and 7 with 3α,20β-Hydroxysteroid Dehydrogenase (3α,20β-HSD). The bis(bromoacetoxy)cortisol derivative **7** exceeded the water solubility of the mono(bromoacetoxy)progesterone derivatives which were synthesized earlier.[2-8] Thus the plan for enhancing the water solubility of the steroid by including the C-11β and C-17 hydroxy groups succeeded and **7** was tested with the steroid-specific enzyme 3α,20β-HSD. Testing **7** with 3α,20β-HSD was carried out by measuring changes in the 3α and 20β enzyme activity. This experiment was designed to determine whether or not the steroid reacts with the enzyme at the catalytic site.

Allowing a solution of **7** and 3α,20β-HSD to react in 0.05 M phosphate buffer, pH 6.0, at 25 °C, caused *simultaneous* loss of 3α and 20β enzyme activity. The two enzyme catalytic activities disappeared at equal rates (panel A, Figure 1) by a first-order kinetic process ($t_{1/2}$ = 12 h). When the reaction between **7** and 3α,20β-HSD was carried out at pH 7.0 the loss of 3α and 20β enzyme activity was more rapid but it did not conform to simple first-order kinetics (panel B, Figure 1). A similar result from earlier experiments[6,9] led us to suspect that at pH 7.0 hydrolysis of **7** to **6** slowly occurred and was responsible for the complex kinetics of enzyme inactivation. Extraction of the pH 7.0 reaction mixture with ether followed by TLC analysis of the ethereal extract revealed that about half of **7** had been converted to **6** during the first 5 h. By contrast, hydrolysis of **6** to **4** at pH 7.0 and 25 °C was not observed during 24 h. Therefore, the kinetics of the reaction between **7** and 3α,20β-HSD (panel B, Figure 1) were complex at pH 7.0 because the hydrolytic conversion of **7** to **6** with a $t_{1/2}$ of 5 h competes against the reaction of **7** with 3α,20β-HSD (also about $t_{1/2}$ of 5 h).

The reaction of **6** with 30α,20β-HSD at pH 7.0 showed that the monobromoacetoxy compound inactivated 3α,20β-HSD by simple first-order kinetics with a $t_{1/2}$ of 12 h (Figure 1). When a solution of **7** was buffered at pH 6.0 and allowed to stand at 25 °C, practically no hydrolysis of the C-21 bromoacetate group (estimated by TLC

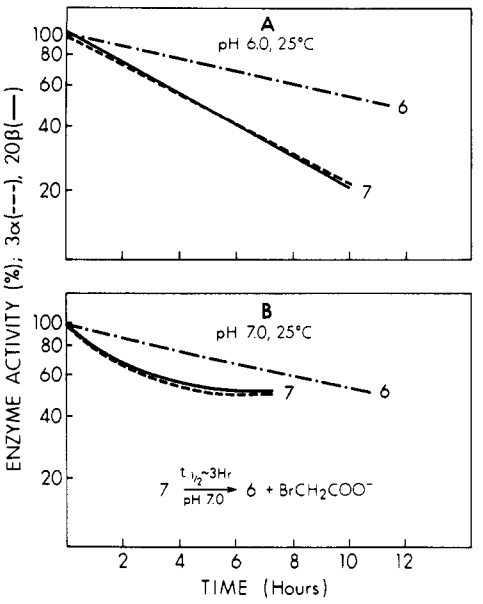


Figure 1. Reactions of **6** or **7** with 3α,20β-hydroxysteroid dehydrogenase. Both 3α (---) and 20β (—) enzyme activities were assayed. (A) Plots of the logarithms of the enzyme activities (as percents of the controls) vs. time when 3α,20β-HSD (0.5 mg, 5 nmol) in 5 mL of 0.05 M phosphate buffer at pH 6.0 (25 °C) was treated with 500 nmol of either **6** (-·-) or **7** (---, —). (B) Plots of the data obtained from experiments under conditions similar to those represented in A except at pH 7.0 (25 °C). At pH 7.0, **7** underwent hydrolysis of the C-21 bromoacetoxy group to form **6** (monitored by TLC analysis) which gave the nonlinear plot shown for **7** (---, —). The enzyme inactivation kinetics obtained from the reaction of **6** with 3α,20β-HSD were practically the same at pH 6.0 and at pH 7.0.

analysis) was observed during 24 h. These were the conditions under which **7** reacted with 3α,20β-HSD wherein 3α and 20β enzyme activities were lost by simple first-order kinetics (panel A, Figure 1).

The present results suggest that both C-6β and C-21 bromoacetate groups react with nucleophilic amino acid residues at the catalytic center of 3α,20β-HSD. However, **6** required 3 times as long as **7** to inactivate 3α,20β-HSD. The greater reactivity of **7** toward enzyme inactivation must be due to the additional bromoacetoxy group at the C-21 position. To prove that both the C-6β and C-21 bromoacetoxy groups of **7** alkylate two different amino acids at the catalytic site of 3α,20β-HSD will require the synthesis of **7** in which each of the bromoacetoxy groups contains a different radioactive isotope. The appropriate ^3H and ^{14}C derivatives of **7** are presently being synthesized for this purpose.

Experimental Section

Materials and General Methods. Melting points were determined with a Laboratory Devices Mel-Temp apparatus and are reported uncorrected. Ultraviolet spectra were obtained with methanol solutions on a Beckman Model 25 spectrophotometer. Infrared spectra with KBr pellets were recorded on a Beckman Acculab 4 spectrometer. Nuclear magnetic resonance spectra with solutions of $CDCl_3$ or CD_3CN (containing tetramethylsilane as an internal standard) were recorded with a Varian T-60 spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. The synthesis reactions were monitored by thin layer chromatography (TLC) with fluorescent silica gel G plates from Eastman. Silica gel G by E. Merck-AG-Darmstadt was used for column chromatography.[11] Mixtures of $CHCl_3$ (Fisher Scientific, C-574, containing 0.75% EtOH) and EtOAc or benzene (PhH) and EtOAc in a 1:1 (v/v) ratio were used to develop all chromatograms. Iodine and/or UV light were used for visualization of the analytical TLC plates. Evaporation of solvents was carried out under reduced pressure in a Buchler flash evaporator. Optical rotations were determined in $CHCl_3$ with 2% solutions in a 1-dm semimicro (2.5 mL) tube with a Dr. Steeg & Reuter Model SR-5 polarimeter at 25 °C. Cortisol (11β,17,21-trihydroxy-4-pregnene-3,20-dione) was purchased from Steraloids, Inc.

6β,11β,17,21-Tetrahydroxy-4-pregnene-3,20-dione 17,21-Acetonide (3). 3-Methoxy-11β,17,21-trihydroxy-$\Delta^{3,5}$-pregnadien-20-one 17,21-acetonide (**2**) was prepared by modification of a known procedure.[12] A solution of cortisol (**1**, 15 g, 0.0414 mol) in 90 mL of DMF and 225 mL of 2,2-dimethoxypropane, containing *p*-toluenesulfonic acid (375 mg), was heated under reflux for 6 h. TLC analysis of the reaction mixture ($CHCl_3$–EtOAc) indicated conversion of **1** (R_f 0.1) to **2** (R_f 0.8). The reaction mixture was cooled to room temperature, stirred for 10 min with $NaHCO_3$ (1.6 g), and then filtered. The filtrate was concentrated to a residue of crude enol ether **2**, which was mixed with 300 mL of absolute EtOH and then photooxidized in sunlight for 3 weeks.[13] TLC analysis showed **3** to be contaminated with byproducts. Isolation of **3** by the method of Fukushima et al.[14] was unsuccessful in our hands. However, chromatography of the crude reaction mixture (7 g) on a column of SiO_2 (160 g) which was eluted with $CHCl_3$–EtOAc under pressure[11] gave 2.5 g of pure product after pooling the fractions of **3** (R_f 0.32). Recrystallization of **3** from benzene provided colorless needles with mp 204–206 °C (lit.[14] mp 200–204 °C). Optical rotation, IR, TLC, and NMR data were in agreement with the literature values.[14]

6β,11β,17,21-Tetrahydroxy-4-pregnene-3,20-dione (4). The treatment of **3** (84 mg, 0.2 mmol) with a mixture of 1.5 mL of MeOH and 2 mL of 1:1 HOAc–H_2O at 52 °C for 3 h showed by TLC ($CHCl_3$–EtOAc) progressive disappearance of **3** (R_f 0.32) and formation of **4** (R_f 0.05). The reaction mixture was concentrated to an oily residue which was taken up in 30 mL of EtOAc, washed with water, dried ($MgSO_4$), and filtered. The filtrate was concentrated to a solid residue. The solid was crystallized from acetone to give 56 mg of **4** with mp 222–225 °C (lit.[14] mp 220–225.5 °C) with characteristics (TLC, UV, and IR spectra, mmp) identical with that of an authentic sample (from Steraloids, Inc.).

6β-(Bromoacetoxy)cortisol 21-Bromoacetate (6β,21-Bis(bromoacetoxy)-11β,21-dihydroxy-4-pregnene-3,20-dione, 7). Method A. To 38 mg (0.1 mmol) of **4** in 2 mL of anhydrous CH_2Cl_2 at 0 °C was added 27.8 mg (0.2 mmol) of bromoacetic acid dissolved in 1 mL of dry CH_2Cl_2. Then 45.4 mg (0.22 mmol) of dicyclohexylcarbodiimide (DCC) in 1 mL of anhydrous CH_2Cl_2 was added to the reaction mixture at 0 °C. After 5 min, 5 μL of anhydrous pyridine was added and the mixture was stirred for 3 h at room temperature. The reaction was monitored by TLC with $CHCl_3$–EtOAc. The spot at R_f 0.05 due to **4** was gradually replaced by a new UV-absorbing spot at R_f 0.47 due to **7**. Isolation of **7** was carried out by a procedure developed earlier for the synthesis of various (bromoacetoxy)progesterone analogues.[3,4] This gave 26 mg of **7** from hexane as colorless crystals: mp 155 °C; UV 238 nm (ε 13 500); IR (KBr) 1735 (ester), 1705 (C-20, C=O), 1670 cm^{-1} (C-3, C=O, Δ^4); NMR ($CDCl_3$) δ 5.85 (s, 1, H-4), 5.35 (m, 1 H-6α, 5.00 (d, 2, J = 3 Hz, H-21), 4.40 (m, 1, H-11α), 3.90 (s, 2, C-6β, $BrCH_2COO$), 3.75 (s, 2, C-21, $BrCH_2COO$), 1.50 (s, 3, H-19); 0.95 (s, 3, H-18). Anal. Calcd for $C_{25}H_{32}O_8Br$: C, 55.56; H, 5.97; Br, 14.79. Found: C, 55.45; H, 5.92; Br, 14.65.

Method B. Although method A is convenient for a one-step synthesis of **7**, the projected synthesis of **7** in which the two bromoacetoxy groups each contains a different radioactive isotope (^3H, ^{14}C) will involve the sequential introduction of the groups. The sequential introducing of a C-6β and a C-21 bromoacetoxy group is provided by the following synthesis.

6β-(Bromoacetoxy)-11β,17,21-trihydroxy-4-pregnene-3,20-dione 17,21-Acetonide (5). To a stirred solution of **3** (418 mg, 1.0 mmol) in 25 mL of anhydrous CH_2Cl_2 at 0 °C was added bromoacetic acid (336 mg, 2.4 mmol) in 10 mL of CH_2Cl_2 and then

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DCC (487 mg, 2.36 mmol) in 10 mL of CH_2Cl_2. After the reaction mixture was stirred for 5 min, 50 μL of anhydrous pyridine in 1 mL of CH_2Cl_2 was added, stirring was continued at 0 °C for 1 h and then at room temperature for 1 h. TLC analysis (PhH–EtOAc) showed complete conversion of **3** (R_f 0.3) to **5** (R_f 0.53) during this period. The crude product was worked up by our general procedure.[3,4] Crystallization from acetone–petroleum ether gave 340 mg (80% yield) of **5**: mp 178–180 °C; UV 235 nm (ε 15400); IR 3525 (hydroxy), 1753 (ester), 1712 (C-20, C=O), 1670 (C-3, C=O), 1622 cm^{-1} (Δ^4); NMR ($CDCl_3$) δ 5.89 (s, 1, H-4), 5.14 (m, 1, H-6α), 4.50 (m, 1, H-11α), 4.17 (d, 2, J = 5 Hz, H-21), 3.85 (s, 2, C-6β, $BrCH_2COO$), 1.57 (s, 3, H-19), 1.25 (d, 6, J = 3, C-17/C-21, $C(CH_3)_2$), 0.95 (s, 3, H-18). Anal. Calcd for $C_{26}H_{35}O_7Br$: C, 57.89; H, 6.54; Br, 14.81. Found: C, 58.05; H, 6.74; Br, 14.62.

6β-(Bromoacetoxy)-11β,17,21-trihydroxy-4-pregnene-3,20-dione (6). A solution of **5** (350 mg, 0.65 mmol) in 4 mL of MeOH and 7 mL of 1:1 HOAc–H_2O was heated at 52 °C for 5 h. TLC (PhH–EtOAc) showed progressive disappearance of **5** (R_f 0.55) with formation of **6** (R_f 0.08). After the reaction was complete, the solvent was evaporated and the residue was extracted with EtOAc. The EtOAc extract was washed with water, dried ($MgSO_4$), and filtered. Evaporation of the solvent from the filtrate left a solid residue which was crystallized from acetone–petroleum ether to give 160 mg of colorless crystals of **6**: mp 183–185 °C; UV 241 nm; IR 3440 (hydroxy), 1735 (ester), 1703 (C-20, C=O), 1677 (C-3, C=O), 1622 cm^{-1} (Δ^4); NMR (CD_3CN) δ 5.80 (s, 1, H-4), 5.40 (m, 1, H-6α), 4.35 (q, 2, J = 5 Hz, H-21), [4.35 (m, 1, H-11α)], 3.95 (s, 2, C-6β, $BrCH_2COO$), 1.50 (s, 3, H-19), 0.85 (s, 3, H-18). Anal. Calcd for $C_{23}H_{31}O_7Br$: C, 55.32; H, 6.26; Br, 16.00. Found: C, 55.21; H, 6.44; Br, 16.16.

6β,21-Bis(bromoacetoxy)-11β,17-dihydroxy-4-pregnene-3,20-dione (7). A procedure for the synthesis of **7** from **6** was used which is similar to that described above for the synthesis of **5**. Thus, 250 mg (0.5 mmol) of **6** was converted to 225 mg of **7** (colorless needles from cyclohexane, mp 156–158 °C) which was found to be identical in all respects (TLC, UV, IR, NMR, and mmp) with **7** which had been obtained by direct bromoacetylation of **4**.

6β-Acetoxy-11β,17,21-trihydroxy-4-pregnene-3,20-dione 17,21-Acetonide (9) from 3. Compound **3** (106 mg, 0.25 mmol) was acetylated in a mixture of 0.4 mL of Ac_2O in 2 mL of dry pyridine at room temperature. TLC (PhH–EtOAc) indicated complete conversion of **3** (R_f 0.33) to **9** (R_f 0.58) during 4 h. Recrystallization of the product from EtOAc–cyclohexane gave 60 mg of **9** with mp 195–199 °C (lit.[14] mp 207–211 °C). NMR and TLC agreed with the literature values.

6β,21-Diacetoxy-11β,17-dihydroxy-4-pregnene-3,20-dione (8). Starting with 77 mg (0.2 mmol) of **4**, a procedure similar to that used for the synthesis of **9** gave 50 mg of colorless needles from EtOAc–petroleum ether: mp 134–145 °C; UV 239 nm (ε 16100); IR 3260 (hydroxy), 1747 (ester), 1727 (C-20, C=O), 1660 (C-3, C=O), 1637 cm^{-1} (Δ^4). Anal. Calcd for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 65.01; H, 7.48.

Reaction of 7 with 3α,20β-Hydroxysteroid Dehydrogenase. To a solution of 0.5 μg (5 nmol) of 3α,20β-HSD in 5 mL of 0.05 M phosphate buffer at pH 6.0 and 25 °C was added 0.27 mg (500 nmol) of **7** in 200 μL of ethanol. At 30- to 60-min intervals, 3α or 20β enzyme activity was measured[9] in the 100-μL aliquots that were removed from the reaction mixture. Similar measurements were made on aliquots taken from an enzyme-control mixture which contained a quantity of **8** equivalent to **7** in the reaction mixture. The change in enzyme activity (calculated as a percent of enzyme activity in the control mixture) was plotted on a logarithmic scale as a function of time (Figure 1). The reactions of **6** or **7** with 3α,20β-HSD when carried out at pH 6.0 caused a time-dependent loss in enzyme catalytic activity which followed first-order kinetics (panel A, Figure 1). The reaction between **7** and 3α,20β-HSD at pH 7.0 was complicated by hydrolysis of **7** to **6**. This is reflected by the nonlinear logarithmic plot shown in panel B, Figure 1.

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Reductive Cleavage of Aromatic Disulfides Using a Polymer-Supported Phosphine Reagent

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The use of polymeric reagents in organic synthesis has been the subject of substantial interest in recent years.[1] Among the several advantages offered by such reagents,[2] the one most frequently utilized is the ease of workup, often consisting of a simple filtration. This feature is particularly noteworthy when the reaction products are noxious or toxic and when they are unstable during lengthy workups. The reductive cleavage of disulfides to afford the corresponding thiols is certainly a reaction that could benefit substantially by application of these polymer-supported reagents due to the well-recognized noxious properties of the sulfur products. In addition, many of the thiol products are subject to air oxidation to regenerate the disulfides, a process inhibited by simplifying the workup procedure. With this goal in mind, we have developed a mild and high yield method for the reductive cleavage of a variety of disulfides to their corresponding thiols using a polymer-supported reagent.

The reductive cleavage of disulfides has been accomplished in a number of ways including reductions with $NaBH_4$,[3] $LiAlH_4$,[4] and Zn/acetic acid.[5] One method that appeared to be particularly attractive because of its mild conditions and high yields was the use of triphenylphosphine in aqueous organic solvents.[6] The yields of this reaction were sufficiently high to allow it to be used for quantitation of disulfides.[7] First described by Schönberg in the 1930s,[8] the reaction has been extensively studied and the mechanism carefully detailed.[9-11] As indicated in Scheme I, the first step involves a thiophilic attack by phosphorus to generate the aryl thiolate anion and the (arylthio)phosphonium cation, the latter undergoing hydrolysis in the second step to afford the second equivalent of thiol. The reaction is catalyzed by either acid or base.

Scheme I

$$Ar\text{–}S\text{–}S\text{–}Ar + Ph_3P \rightleftarrows Ar\text{–}S\text{–}P^+Ph_3 + ArS^-$$

$$Ar\text{–}S\text{–}P^+Ph_3 + H_2O \rightarrow ArS^- + 2H^+ + Ph_3PO$$

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