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SYNTHESIS OF MULTI CARBON-13 LABELED DEXAMETHASONE

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Abstract—A synthesis of dexamethasone multiply labeled with ^{13}C about the A ring is described. 16-α-Methyl-17α,20-dihydroxypregn-1,4,9(11)-trien-3,20-dione (**1**) was blocked as the bismethylenedioxy derivative and degraded to the ring C,D-fragment, Des-A,B-9-keto-16α-methyl-17α, 20;20,21-bismethylenedioxypregn-8α-propionic acid (**9**). Conversion to the enol lactone and condensation with 1-diethylphosphono-4-pentanone-^{13}C ethylene ketal followed by methylation and A-ring closure afforded the BMD derivative of 16α-methyl-pregn-4,9(11)-diene-3-one-^{13}C (**14**). The steroidal intermediate was further transformed to dexamethasone-^{13}C.

The United States Food and Drug Administration (FDA) has listed twelve glucocorticoid drugs as having a bioequivalence/bioavailability problem potential. The apparent need for an *in vitro/in vivo* correlation is obvious. However, due to the low dosages of these glucocorticoids administered, new and innovative approaches for the analysis of picogram quantities in biological samples becomes of paramount importance. The desired deployment of mass spectrometry combined with the use of stable isotopes to establish relative and absolute bioavailabilities for these drug products was the analytical choice for method of analysis.[1,2] In particular the synthesis of (M + 3), (M + 6) and (M + 9) entities of dexamethasone using ^{13}C in steroid rings A and B would allow such a mass spectrometric approach to be adopted. The use of ^{13}C in rings A and B would permit these stable isotopes to be used either orally or intravenously in the simultaneous administration of a solid dose form without the subsequent worry of exchange phenomena associated with deuterium labeling. Additionally, the use of an (M + 9) as external standard for quantitation was required. With this protocol, the intrasubject variability is reduced in a cost-effective manner.[3] Simultaneous determination of relative and absolute bioavailabilities would become possible in a single controlled experiment. Furthermore, the judicious choice of labeling in ring A was made on the grounds of predictive metabolism occurring primarily at C-17. The intact ring A would then permit additional qualitative and quantitative monitoring of metabolites as well as the parent moiety in biological studies. This paper describes the synthesis of three labeled dexamethanones as the precursor to implementing the analytical approach of stable isotope dilution mass spectrometry.

Of the various methods available for total synthesis of cortical steroids the route developed by the Roussel-UCLAF laboratories[4] seemed to offer the most convenient opportunity to introduce multiple ^{13}C-labels. The route basically entails condensation of a ring C–D synthon with the Grignard reagent derived from 1-chloro-4-pentanone ethyleneketal, methylation and closure of ring-A. A modification of this process whereby a phosphonate reagent was used in place of the above Grignard reagents was described by Henrick *et al.*[5] It offered additional advantage for purposes of labeling ring A and forms the basis for our synthesis of dexamethasone-2,3,4-$^{13}C_3$, -1,2,3,4,10,19-$^{13}C_6$ and 1,2,3,4,10,19-$^{13}C_6$-19,19,19-$^{2}H_3$ as described herein.

Our fundamental strategy (as shown in Scheme 1) for synthesis of the A-ring labeled dexamethasone was to immobilize the side chain functionality, degrade rings A and B to the keto acid (**9**) and condense the relay intermediate with ^{13}C-labeled diethyl-1-phosphono-4-pentanone-ethylene ketal[6] to reconstruct dexamethasone with retention of chirality. Starting with the available 16α-methylcorticoid intermediate (**1**)[7] the cortical side chain was blocked as the bismethylenedioxy (BMD) group[8] by treatment of **1** with formaldehyde-hydrochloric acid to give a 74% yield of **2**. The BMD derivative **2** was aromatized with zinc in DMF[9] at 145° to afford the 3-hydroxyestratriene BMD **3** in quantitative yield. Methylation with dimethyl sulfate in tetrahydrofuran gave the 3-methylether (**4**) in an overall yield of 35% from **1**.

The aromatic A ring derivative **4** was then subjected to Li-ammonia reduction at −78° to give the 9α-3-OMe compound[10] **5** in nearly quantitative yield. The 9α-3-methoxydihydro derivative (**5**) was subsequently subjected to Birch reduction at −33° with ethanol as the proton source to give the desired diene-3-methylether (**6**) and recovered starting material **5** (9:1). The tetrahydro derivative **6** was crystallized from ethyl acetate to give a purified yield of 75%. Attempts to obtain **6** by direct Birch reduction

of **4** in the presence of ethanol were not successful since products with both 9α- and 9β-stereochemistry resulted.

Hydrolysis of the 3-enol ether **6** with oxalic acid[11] in methanolic-THF gave a quantitative conversion to the 3-keto-$\Delta^{5(10)}$-steroid (**7**). When **7** was treated with pyridinium bromide perbromide in pyridine at 85° a bromination-dehydrobromination process was effected[12] to afford the $\Delta^{4,9}$-diene-3-one (**8**) in 50% yields after purification by direct crystallization.

Degradation of the A–B ring system of the dienone **8** was accomplished with ozone in methanol-methylenechloride-acetic acid at −78° followed by hydrogen peroxide work up to give a crude yield of 70–80% of the bicyclic keto acid **9**. Ozonation of **8** at higher temperatures and in different solvent systems gave poorer yields of the desired product **9**. Pure bicyclic keto acid **9** was obtained in 50% yield by preparative HPLC.

The keto acid **9** could be dehydrated to the enol lactone **10** by brief treatment with acetic anhydride-perchloric acid[13] or heating for several hr at 100° in Ac_2O–NaOAc. Although the latter process was slower it was more reproducible and tended to give a product with less unclosed acetyl mixed anhydride as detected by a prominent signal at 2.2 ppm in the PMR. When **10** was condensed with the anion of the ketal phosphonate, as generated by n-BuLi,[5] the tricyclic enone ketal **11** was isolated as a crystalline solid in low yield (20–25%). After extensive investigation we were unable to improve the yield in this step, but were able to recover nearly all of the unreacted phosphonate reagent and over 50% of the keto acid **9**. It would appear that enolization of the lactone CO is the dominant process followed by opening to keto acid during work up. The enol lactone could exist as a mixture of pro 9-11 and pro 8-9 olefin isomers. The integrated PMR indicated at least 90% of the 9-11 olefin as determined by ratio of the signal cluster at 5.2 ppm (=CH, $-OCH_2O-$) vs the C-21 methylene singlet at 4.0 ppm.

Introduction of the angular Me group at the eventual C-10 carbon was carried out by the process reported by the Roussel workers.[14] When the enone **11** was treated with an equivalent of sodium t-amyloxide in refluxing toluene followed by quenching of the anion with methyl iodide the methyl ketone **12** was obtained as a mixture of the 10α and 10β isomers. These isomers could be separated by preparative TLC and were obtained in a 1.73:1 ratio of 10β/10α. Velluz *et al.*[15] had reported a high yield of pure 10β-product for a similar case. However, Stork *et al.*[16,17] have consistently found ratios of about 2:1 for 10β/10α. They have also repeated the work of the Velluz group and found that the claim of 100% 10β was in error and was in reality a 2:1 mixture of 10β/10α.

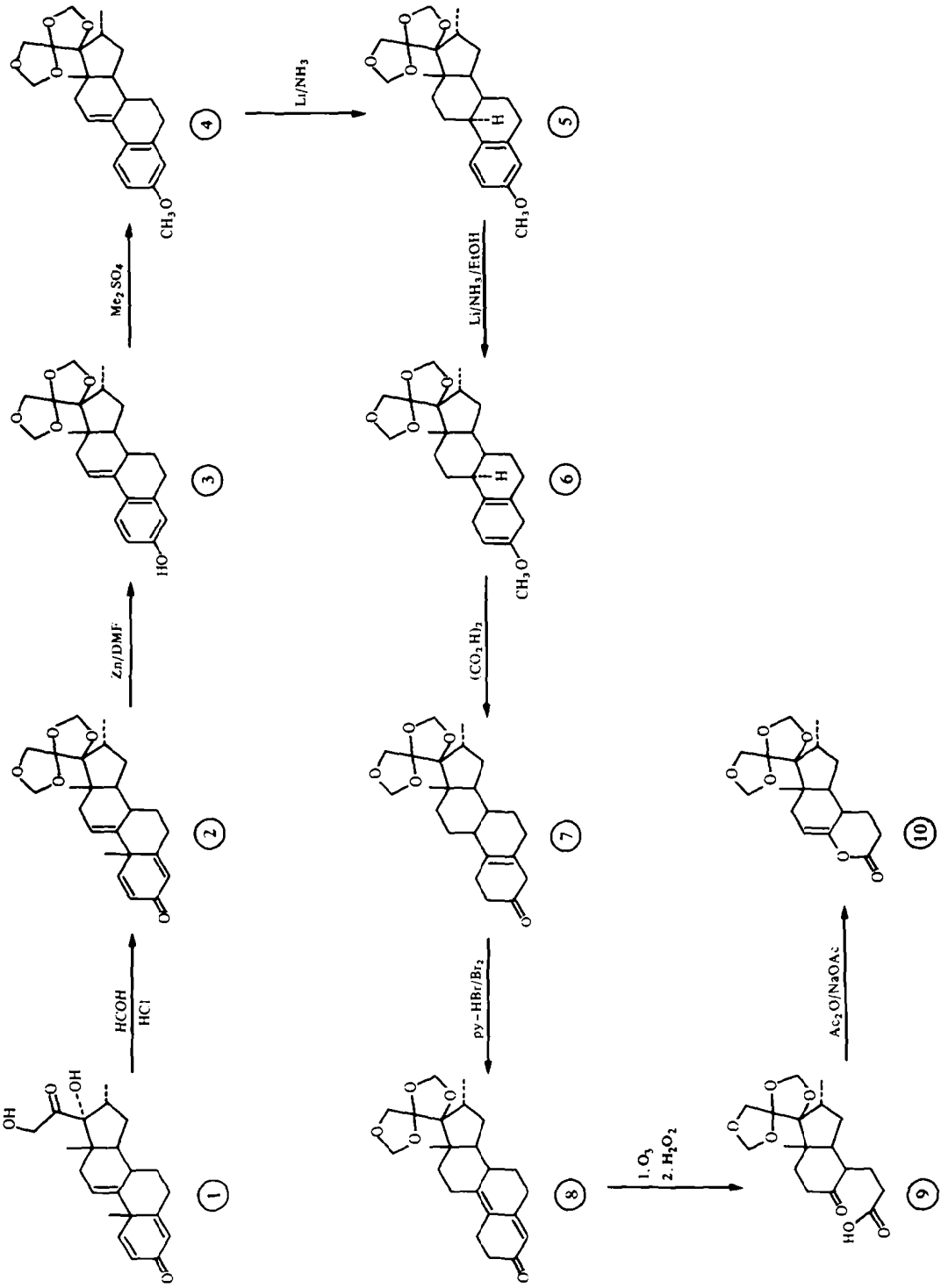
Our tentative assignment of 10β stereochemistry to the major isomer **12a** was based on the precedents cited above. Conclusive evidence was obtained by hydrolysis of the ketal group of **12a, b** in aqueous acetic acid to the dike-tone **13a, b**. Ring closure with t-BuOK in t-BuOH at room temperature afforded the isomeric $\Delta^{4,9(11)}$-diene-3-one (**14a, b**). ^{13}C-NMR spectra for **14a** and **14b** were compared with authentic dienone prepared by hydrogenation of compound **2** over Wilkinson's catalyst.[18] The authentic dienone had a C-4 peak at 123.89. M + 3 labeled **14a** (10β) had 2 sets of doublets centered at 123.76 and M + 3 labeled **14b** (10α) had 2 sets of doublets centered at 125.25. PMR spectra for **14a** (10β) showed a C-19 methyl signal at 1.32 ppm while **14b** (10α) gave a signal at 1.38 ppm. The authentic dienone showed the C-19 signal at 1.32 ppm.

To complete the synthesis compound **14a** was oxidized to the ring-A dienone **2** with dicyano-dichlorobenzoquinone (DDQ) in refluxing dioxane.[19] Unfortunately it was found that this procedure consistently promoted oxidation at C_6-C_7 and gave 15–20% of the $\Delta^{1,4,6,9}$-tetraene as a by-product. We were unable to prevent the by-product formation by variation in the reaction conditions. Attempts to remove the contaminant by crystallization or chromatography were tedious and unrewarding. To circumvent the problems caused by the DDQ oxidation we used a phenyl selenation-oxidation process.[20] Treatment of **14a** with 1.5 quivs of lithium diisopropylamide at 0° followed by 1.6 equivs of phenylselenylchloride afforded the 2-phenylselenide. Oxidation of the crude material with sodium periodate in THF-aqueous methanol yielded the desired triene-one **2** free of contamination by the tetraene. Reaction of **2** with N-bromoacetamide in dioxane at room temperature gave the bromohydrin **15** in 95% yield.[21] Treatment of **15** with potassium acetate in dioxane/absolute ethanol for 17 hr at reflux afforded the β-9,11-epoxide **16** in 87% yield.[21] When **16** was stirred with 48% hydrofluoric acid at 0° epoxide opening and concomittant cleavage of the BMD blocking group took place to afford dexamethasone (**17**) in 73% yield.

When 5-(diethylphosphono)-2-pentanone-1,2,3-^{13}C ethylene ketal was condensed with 10 and carried through the entire sequence we obtained dexamethasone-2,3,4-$^{13}C_3$ (**17**). Use of 5-(diethylphosphono)-2-pentanone-1,2,3,4,5-$^{13}C_5$[3] gave the intermediate **11**-$^{13}C_5$ labeled at carbons 1, 2, 3, 4 and 10. Methylation of **11**-$^{13}C_5$ with $^{13}CH_3I$ gave dexamethasone-1,2,3,4,10,19-$^{13}C_6$ while methylation of **11**-$^{13}C_5$ with $^{13}Cd_3I$ afforded dexamethasone-1,2,3,4,10,19-$^{13}C_6$-19,19,19-d_3.

EXPERIMENTAL

16α - Methyl - 17α,20;20,21 - *bismethylenedioxypregn*-1,4,9(11) - *triene*-3-*one* (**2**). To a stirred suspension of **1** (220 g; 0.63 mol) in 2.2 l. CH_2Cl_2 that was cooled to 0° under N_2, 370 ml cone HCl was added over 10 min while maintaining the mixture at 0°. To the resulting soln, 370 ml of a 37% formaldehyde soln was added in three equal portions at 4 hr intervals and then the mixture was stirred for an additional 18 hr at room temp. The aqueous phase was separated and washed with 800 ml CH_2Cl_2. The CH_2Cl_2 was combined and washed four times with 1 l. portions of water and then dried over Na_2SO_4. The CH_2Cl_2 was removed at reduced pressure and an oily residue remained. Crystallization from CH_2Cl_2-hexane-EtOH gave 182 g (74%) of **2**. An analytical sample was prepared by recrystallization from CH_2Cl_2–MeOH: m.p. 198–200°. IR (Nujol) 1665 cm^{-1} (Ring A-dienone); NMR ($CDCl_3$) δ 0.86 (s, C-18, 3H), 0.92 (d, C-16 CH_3, 3H), 1.4 (s, C-19 CH_3, 3H), 3.97 (s, C-21 CH_2, 2H), 4.95–5.18 (m, OCH_2O– 4H), 5.5 (d, C-11 H, 1H), 6.03 (s, C-4 H, 1H), 6.12 (d, C-2 H, 1H), 7.17 (d, C-1 H, 1H). (Found: C, 72.42; H, 7.38. Calc. for $C_{24}H_{30}O_5$: C, 72.34; H, 7.59%).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HCl
Zn/DMF
Me2SO4
Li/NH3
Li/NH3/EtOH
(CO2H)2
py-HBr/Br2
1. O3
2. H2O2
Ac2O/NaOAc
1
2
3
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7
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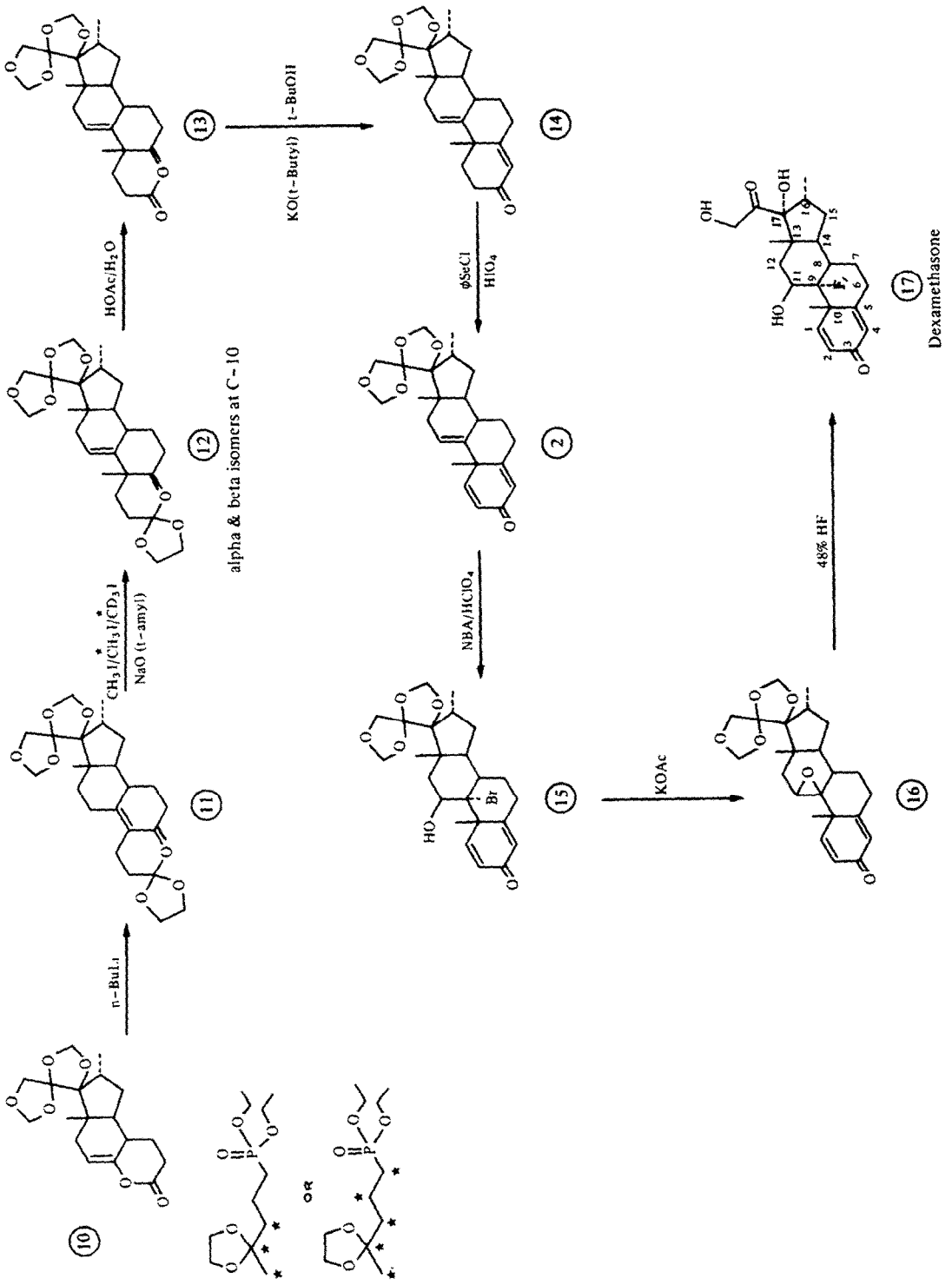
n-BuLi
CH3I/CH3I/CD3I
NaO (t-amyl)
HOAc/H2O
KO(t-Butyl) t-BuOH
φSeCl
HIO4
NBA/HClO4
KOAc
48% HF
alpha & beta isomers at C-10
OR
Dexamethasone

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxy* - 19 - *norpregn* - 1,3,5(10),9(11) - *tetraen* - 3 - *ol* (**3**). To a soln of 160 g (0.4 mol) of **2** in 200 ml DMF containing 16 ml water, under N_2, 640 g activated Zn was added. The stirred slurry was heated at 145–147° for 30 min then cooled to room temp and stirred for 2 hr. The reaction was filtered through a Celite pad and the cake was washed with four 200-ml portions of DMF. The light yellow filtrate was evaporated at reduced pressure and the residue was triturated with 1 l. water. The resulting white solid was filtered then air dried to afford 154 g (100%) of **3**. An analytical sample was prepared by crystallization from CH_2Cl_2–MeOH: m.p. 239–242°; IR (Nujol) 3300 (C-3 OH), 1080, 1730, 1770 (ring A aromatic and 9(11)-double bond); NMR ($CDCl_3$, 10% DMSO-d_6) δ 0.87 (s, C-18 CH_3, 3H), 0.95 (d, C-16 CH_3, 3H), 3.97 (s, C-21 CH_2, 2H), 5.0-5.15 (m, $-OCH_2O-$, 4H), 6.0 (s, C-11 H, 1H), 6.58 (q, C-2 H, 1H), 7.4 (d, C-1 H, 1H). (Found: C, 71.51; H, 7.25. Calc. for $C_{23}H_{28}O_5$: C, 71.55; H, 7.34%).

16α - *Methyl* - 17α,20;20,21-*bismethylenedioxy* - 19 - *norpregn* - 1,3,5(10),9(11) - *tetraen* - 3 - *ol* 3 - *methyl ether* (**4**). To a stirred soln of **3** (134 g; 0.35 mol) in 2680 ml THF and 1045 ml 0.5N NaOH that contained 1.18 g (3.48 mol) Bu_4NHSO_4, was cooled to 5° under N_2 then 87.9 g (0.697 mol) Me_2SO_4 was added over 5 min. The mixture was stirred at 5–10° for 0.5 hr then warmed to room temp and stirred for 16 hr. The reaction was cooled to 10° and 2N NH_4OH was added followed by warming to room temp and stirring for 2 hr. Most of the THF was removed at reduced pressure and 2 l. water was added to precipitate the product. The product was collected by filtration and air dried. Crystallization from $CHCl_3$–EtOH gave 66 g (48%) of **4**. An analytical sample was prepared by recrystallization from CH_2Cl_2–MeOH: m.p. 208–210°; IR (Nujol) 1640, 1720, 1740 (ring A aromatic and 9(11)-double bond). NMR ($CDCl_3$) δ 0.82 (s, C-18 CH_3, 3H), 0.94 (d, C-16 CH_3, 3H), 3.75 (s, OCH_3, 3H), 3.95 (s, C-21 CH_2, 2H), 5.0–5.17 (m, $-OCH_2O-$, 4H), 6.04 (s, C-11 H, 1H), 6.53 (d, C-4H, 1H), 6.62 (q, C-2H, 1H), 7.48 (d, C-1 H, 1H). (Found: C, 71.98; H, 7.37. Calc. for $C_{24}H_{30}O_5$: C, 72.34; H, 7.59%).

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxy* - 19 - *norpregn* - 1,3,5(10) - *trien* - 3 - *ol* 3-*methyl ether* (**5**). To 350 ml distilled ammonia that was mechanically stirred at −78° under N_2 was added a soln of **4** (9.2 g) in 200 ml warm dry THF, dropwise, over 10 min. The steroid **4** precipitated during the addition. To the suspension of the steroid in THF-ammonia at −78° was added 3 g of 2.3 mm Li wire cut into 1.5 cm pieces. The mixture turned blue and the color persisted during 2 hr of vigorous stirring at −78°. The mixture was warmed to −33° then 50 ml abs EtOH was added to discharge the blue color. After 15 min the ammonia was evaporated while maintaining a N_2 atmosphere. Then 200 ml water and 300 ml ether were added. The aqueous and organic phases were separated and the aqueous phase was extracted twice with 100 ml portions ether. The ether extracts were combined and washed twice with saline soln and once with water, then dried over Na_2SO_4. The solvent was removed at reduced pressure to afford 9 g of **5**. An analytical sample was prepared by crystallization from CH_2Cl_2–MeOH: m.p. 174–176°; IR (Nujol) 1640, 1720 cm^{-1} (ring A aromatic); NMR ($CDCl_3$) δ 0.83 (s, C-18 CH_3, 3H), 0.95 (s, C-16 CH_3, 3H), 3.72 (s, OCH_3, 3H), 3.97 (s, C-21 CH_2, 2H), 5.0-5.2 (s, $-OCH_2O-$, 4H), 6.2 (d, C-4 H, 1H), 6.63 (q, C-2 H, 1H), 7.18 (d, C-1 H, 1H): high resolution mass spectrum *m/e* 400.2229 (M^+) ($C_{24}H_{32}O_5$ requires 400.2250).

16α - *Methyl* - 17α,20;20,21-*bismethylenedioxy* - 19 - *norpregn* - 2,5(10) - *dien* - 3 - *ol* 3-*methyl ether* (**6**). To 350 ml distilled ammonia mechanically stirred at −78° under N_2 was added 50 ml abs EtOH followed by addition of 9 g of **5** in 200 ml dry THF. To the mixture, 3.0 g of 3.2 mm Li wire cut into 1.5 cm pieces was added over 10 min. During the Li addition the reaction turned blue and following the addition the dry ice–acetone bath was removed. After 15 min the blue color dissipated. An additional 1.5 g Li was added and after 20 min the blue color again dissipated. Then 1.5 g Li was added and after 20 min 25 ml abs EtOH was added to quench the reaction. The ammonia was evaporated under N_2 then 400 ml water and 300 ml ether were added. The phases were separated and the aqueous phase was washed with 200 ml ether. The ether extracts were combined and washed with a sat NaCl aq followed by a water wash then dried over Na_2SO_4. The solvent was removed at reduced pressure to afford 9.0 g of residue. NMR analysis showed that the residue contained 90% of **6** and 10% unreacted aromatic **5**. Crystallization from CH_2Cl_2–EtOAc gave 6.8 g of **6**. An analytical sample was prepared by recrystallization from CH_2Cl_2–EtOAc: m.p. 178–181°; NMR ($CDCl_3$) δ 0.83 (s, C-18 CH_3, 3H), 0.98 (d, C-16 CH_3, 3H), 3.56 (s, OCH_3, 3H), 4.5 (m, C-2 H, 1H), 5.0–5.26 (m, $-OCH_2O-$, 4H): high-resolution mass spectrum *m/e* 402.2424 (M^+) ($C_{24}H_{34}O_5$ requires: 402.2406).

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxy* - 19 - *norpregn* - 5(10) - *en* - 3 - *one* (**7**). To a stirred soln of **6** (9.8 g) in 200 ml THF under N_2 was added 400 ml MeOH followed by 77 ml 1M oxalic acid soln. When addition was complete the steroid crystallized out, but after stirring for 2.5 hr the mixture was homogeneous. The mixture was poured into 600 ml water and the product precipitated. The ppt was extracted into 500 ml ether and the aqueous phase was extracted with an additional 300 ml ether. The combined ether extract was washed with water, 5% $NaHCO_3$ aq and water, then dried over Na_2SO_4. The solvent was removed at reduced pressure to leave 9.5 g of residue. NMR analysis showed that the residue contained only **7**. An analytical sample was prepared by crystallization from CH_2Cl_2–EtOAc: m.p. 192–195°; IR (Nujol) 1720 cm^{-1} (3-ketone); NMR ($CDCl_3$) δ 0.88 (s, C-18 CH_3, 3H), 0.95 (d, C-16 CH_3, 3H), 4.0 (s, C-21 CH_2, 2H), 5.04–5.25 (m, $-OCH_2O-$, 4H). (Found: C, 70.71; H, 8.18. Calc. for $C_{23}H_{32}O_5$: C, 71.11; H, 8.30%).

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxy* - 19 - *norpregn* - 4,9(10) - *dien* - 3 - *one* (**8**). To a stirred soln of **7** (9.5 g; 24 mMol) in 240 ml dry pyridine under N_2, 7.7 g (24 mMol) pf pyridinium bromide perbromide was added. The mixture was stirred for 2 hr at room temp then heated at 85° for 1.0 hr. The mixture was cooled to room temp, then most of the pyridine was removed at reduced pressure. To the residue was added 500 ml 2N HCl and the product was extracted into 300 ml CH_2Cl_2. The phases were separated and the HCl phase was washed with 200 ml CH_2Cl_2. The CH_2Cl_2 extracts were combined, washed with 2N HCl, water, and a sat NaCl aq, then dried over Na_2SO_4. The CH_2Cl_2 was removed at reduced pressure to leave 9.0 g of residue. NMR analysis of the residue showed that it contained 90% of **8** and 10% of the unreacted starting material **7**. Crystallization from $CHCl_2$–EtOAc gave 4.5 g (50%) pure **8**. An analytical sample was prepared by crystallization from CH_2Cl_2–EtOAc: m.p. 249–251°; IR (Nujol) cm^{-1} 1670 (dienone), 1620 and 1580 (double bonds); UV (EtOH) 304 mμ (ε 20,400); NMR ($CDCl_3$) δ 0.93 (d, C-16 CH_3, 3H), 0.97 (s, C-18 CH_3, 3H), 4.0 (s, C-21 CH_2, 2H), 5.0–5.25 (m, $-OCH_2O-$, 4H), 5.65 (s, C-4 H, 1H). (Found: C, 71.28; H, 7.82. Calc. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82%).

Des - A,B - 9 - *keto* - 16α - *methyl* - 17α,20;20,21 - *bismethylenedioxypregn* - 8α - *propionic acid* (**9**). A stream of O_3† was passed through a soln of **8** (18 g) in 900 ml CH_2Cl_2, 450 ml MeOH and 180 ml of AcOH that was cooled to −72° under a N_2 at. The reaction was monitored by TLC (benzene–ether, SiGF) and after 3.5 hr most of the starting material had reacted and the mixture was blue indicating excess O_3. The O_3 flow was discontinued and the mixture was stirred at −72° for an additional 1.5 hr then N_2 was

†Welsbach model T408 ozone generator. Calibrated to deliver 1 mM/min of ozone at 6.0 psi oxygen pressure at 70 V for a delivery rate of 0.4 l./min.

bubbled through the soln for 0.5 hr to drive out excess O_3. A soln of 270 ml 10% H_2O_2 was added and the mixture was warmed to room temp. The two phase system was stirred vigorously for 18 hr then 200 ml water was added. The phases were separated and the aqueous phase was washed with 400 ml CH_2Cl_2. The combined CH_2Cl_2 extract was washed twice with water and once with a sat NaCl aq then dried over $NaSO_4$. The solvent was removed at reduced pressure and 16.5 g of residue remained. NMR analysis comparing the C-21 methylene signal and the C-18 Me signal showed that the crude mixture contained 70–80% of one major product. The crude acid was purified by HPLC (Si gel, methylene chloride:acetonitrile 9:1) to give 9.6 g (58% yield) of product. IR 5.9 μ (C=O); H NMR ($CDCl_3$) 5.1 (m, $-OCH_2O-$, 4H), 4.05 (s, C-21 CH_2, 2H), 1.1 (s, C-18 CH_3, 3H), and 1.0 (d, C-16 CH_3, 3H).

Enol lactone of keto acid, **9** (**10**). A mixture of **9** (5.90 g; 16.6 mMol) and 8.30 g (0.101 mole) of anhyd NaOAc in 90 ml Ac_2O was stirred at 100° for 2.5 hr. The excess Ac_2O was removed under high vacuum. The residual solid was partitioned between 20 ml ether and 200 ml sat $NaHCO_3$aq. The bicarbonate wash was extracted with 100 ml ether and the ether extracts were combined and dried over $MgSO_4$. The ether was removed *in vacuo* to leave a solid residue. Toluene was added and evaporated to remove the last traces of Ac_2O and the residue pumped under high vacuum overnight to leave 5.37 g (96%). The crude product was triturated with diethyl ether to afford 2.96 g (53%) of a crytalline solid. The mother liquor from the trituration was evaporated and the residual gum chromatographed on 30 g silica gel (Woelm, dry column chromatography, activity III) with EtOAc/hexane (1:2) as the solvent. Elution from an open column chromatography gave an additional 0.82 g of pure **10**. A sample was recrystallized from diethyl ether, m.p. 179–181°. IR (μ) 5.78 (C=O); 6.02 (C=C); H NMR ($CDCl_3$): 0.95 (s, C-18 CH_3, 3H), 1.00 (d, C-16 CH_3, 3H), 4.00 (s, C-21CH_2, 2H), 5.20 (m, =CH, $-OCH_2O-$, 5H). The integral ratio 5.20/4.00 averaged 2.45–2.50; TLC: silica-gel HF; EtOAc/hexane (1:1) R_f 0.80. (Found: C, 64.09; H, 7.08. Calc. for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19%).

16α - *Methyl* - 17α,20;20,21-*bismethylenedioxy* - 19 - *nor* - 3 - *ethylenedioxy* - 4,5 - *seco* - *pregna* - 9 - *ene* - 5 - *one* - 2,3,4 - $^{13}C_3$ (**11**). To a soln of 1 - (diethylphosphono)-4 - pentanone - 1,2,3 - $^{13}C_3$ ethylene ketal[6] (4.12 g; 15.5 mMol) in 180 ml freshly distilled THF chilled to −78° was added 10.3 ml (15.5 mMol) n-BuLi (1.05 M in hexane) via a syringe/septum. The clear mixture was stirred at −78° for 50 min under N_2. To the lithio ketal phosphonate reagent was added a soln of **10** (4.65 g; 13.8 mMol) in 50 ml dry THF, dropwise, over 20 min at −78°. The mixture was stirred an additional 4 hr at −78° and then at room temp for 15 hr. The mixture was concentrated to a gum. The crude product was extracted by triturating the gum twice with 125-ml portions of ether and collecting the ether insoluble solid. The ether extract was washed with 75 ml water and dried over $MgSO_4$. The ether was removed *in vacuo* to leave a syrup which crystallized on standing. The crystalline material was suspended in approximately 5 ml dry MeOH, triturated, and chilled to 0°. The pure product was filtered, collected, and dried *in vacuo* at 80° to leave 0.74 g (12%).

An additional crop of pure **11** was obtained from the mother liquor above by column chromatography using silica gel (Woelm dry column) and EtOAc/hexane (1:1) as the eluant. The slightly impure material from the column was further purified by preparative TLC with EtOAc/hexane (1:1) as the developing solvent. The product was detected by UV, extracted from the powdered silica gel with EtOAC, and the extract dried over $MgSO_4$. The solvent was removed to leave 30 mg of a crystalline solid; total yield 1.04 g (17%) recrystallized from MeOH, m.p. 157–158°. IR (μ): 6.0 (C=C–CO); TLC, silica gel HF; EtOAc/hexane (1:1); R_f 0.75. H NMR ($CDCl_3$): 0.92 (s, C-18 CH_3, 3H), 1.01 (d, C-16 CH_3, 3H), 3.95 (d, OCH_2CH_2O, 4H), 4.0 (s, C-21CH_2, 2H), 4.95–5.25 (m, $-OCH_2O-$, 4H); ^{13}C NMR ($CDCl_3$) 23.5 (C-4, CH_3), 38.0 (C-2, CH_2), 109.9 (C-3, –OC–O). Anal. calcd. for $^{12}C_{22}{}^{13}C_3H_{36}O_7$: C, 67.3; H, 8.00. Found: C, 66.9; H, 8.22. Compound **11**-$^{13}C_5$: ^{13}C NMR ($CDCl_3$). 19.9 (C-1), 23.4 (C-4), 38.0 (C-2), 109.9 (C-3), 133.9 (C-10).

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxy* - 4,5 - *seco* - *pregn* - 9(11) - *ene* - 3 - *ethylenedioxy* - 5 - *one* - 2,3,4 - $^{13}C_3$ (**12**). A mixture of dry t-amyl alcohol (distilled from Na) (0.3 ml; 2.7 mmol) and 130 mg (2.7 mmol) 50% NaH was heated at reflux in 10 ml dry toluene (distilled from Na) under an argon atmosphere for 1 hr. Argon was then bubbled through the hot reaction and a soln of **11** (1.023 g, 2.27 mmol) in 2.3 ml dry toluene that had been gassed with argon was added giving a deep red color. The reaction was refluxed for 10 min. The heat was removed and 8 ml dry toluene was placed in the addition funnel and gassed with argon. Freshly distilled MeI (1.42 ml, 22.8 mmol) was added to the toluene in the addition funnel and this soln was then added dropwise to the reaction over a 2-min period. The reaction was refluxed for 40 min.

The reaction was cooled and dry ice was added carefully to achieve neutrality. Water (20 ml) was added and the toluene layer was removed and the aqueous phase was extracted 3 times with $CHCl_3$ (20 ml each). The combined toluene and $CHCl_3$ extracts were dried over $MgSO_4$ and evaporated to dryness to give 0.927 g of crude product.

The product was chromatographed on 8 Analtech 2000 μm silica gel preparative plates with pentane:Et_2O, (1:1). The recovered **11** (34 mg) was detected by UV and the methylated isomers by spraying one side of each of the plates with H_2SO_4 and charring with a heat gun. The compounds were eluted from the silica gel with hot EtOAc. This separation gave 183 mg of the 10β-isomer at R_f 0.6, 146 mg of the 10α-isomer at R_f 0.5, and 121 mg of a mixture of 10α and 10β at R_f 0.55 for a total yield of both isomers of 450 mg (43%). The mixture of 10α and 10β was rechromatographed to give an additional 100 mg of 10β and 18 mg of 10α. The ratio of 10β:10α was 1.73:1; IR, 5.85 μ (C=O), was the same for both isomers. H NMR ($CDCl_3$) β-isomer **12a** 5.50 (m, C-11 H, 1H), 5.10 (m, O–CH_2–O, 4H), 4.00 (s, C-21 CH_2, 2H), 3.90 (d, ketal, 4H), 1.20 (s, C-19 CH_3, 3H), 1.00 (d, 16 CH_3, 3H), and 0.9 (s, C-18 CH_3, 3H). α-Isomer **12b** was the same as **12a** except that the C-19 CH_3 for **12b** was at 1.15 ppm.

Alkylation of **11**-$^{13}C_5$ with $^{13}CH_3I$ afforded **12a**-$^{13}C_6$ in 25% yield; ^{13}C NMR. 23.6 (C-4), 28.0–36.0 (C-1, 2, 19), 53.9 (C-10), 110.1 (C-3).

Alkylation of **11**-$^{13}C_5$ with $^{13}Cd_3I$ gave **12a**-$^{13}C_6$-d_3 in 15% yield; ^{13}C NMR. 23.6 (C-4), 28.0–36.0 (C-1, 2, 19), 53.7 (C-10), 110.0 (C-3).

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxy* - 4,5 - *seco* - *pregn* - 9(11) - *ene* - 3,5 - *dione* - 2,3,4 - $^{13}C_3$ (**13**). A soln of the 10β-isomer (**12a**-$^{13}C_3$) (315 mg; 0.676 mmol) in 3 ml glacial AcOH and 1 ml water was heated at 65° for 1.25 hr. The reaction was evaporated to dryness. The residue was dissolved in 20 ml ether and washed with 20 ml sat $NaHCO_3$aq, followed by 20 ml water. The ether extract was dried over $MgSO_4$ and evaporated to dryness to give 214 mg (75%) of a foam.

The 10α-isomer (291 mg, 0.625 mm) was deketalized in the same manner in a 71% yield (186 mg). IR 5.85 and 6.0 μ (C=O) was the same for both isomers. H NMR showed no ketal doublet at 3.9 ppm, otherwise they were the same as **12a** and **12b**.

Compounds **12a**-$^{13}C_6$ and **12a**-$^{13}C_6$-d_3 were similarly deketalized to yield **13a**-$^{13}C_6$ and **13a**-$^{13}C_6$-d_3.

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxy* - *pregn* - 4,9(11) - *diene* - 3 - *one* - 2,3,4 - $^{13}C_3$ (**14**). To a soln of 10β-isomer (**13a**) (214 mg, 0.508 mmol) in 14 ml dry t-BuOH (distilled from Na) under argon was added 1.27 ml (0.534 mmol) freshly prepared 0.42 M t-BuOK in t-BuOH. The reaction was stirred at room temp under argon for 17 hr.

The t-BuOH was evaporated and the residue was partitioned between ether (10 ml) and water (10 ml). The aqueous phase was re-extracted twice with ether (10 ml). The combined ether extracts were dried over $MgSO_4$ and evaporated to give 167 mg (82%) of a yellow solid, showing a single UV absorbing spot at R_f 0.6 on TLC (pentane: Et_2O, 1:1).

The 10α-isomer (**13b**) (185 mg, 0.439 mm) was ring closed in the same manner, but gave only a 57% yield (101 mg) of a much less pure product. It was purified on a silica gel preparative plate with pentane: Et_2O, 1:1 with elution of the UV absorbing band at R_f 0.50 with hot EtOAc. IR (μ) 6.15 (C=O) for both isomers; H NMR ($CDCl_3$) **14a** (10β-isomer), 0.85 (s, C-18 CH_3, 3H), 0.98 (d, C-16 CH_3, 3H), 1.32 (s, C-19 CH_3, 3H), 4.00 (s, C-21 CH_2, 2H), 5.10 (m, $-OCH_2O$, 4H), 5.50 (m, C-11H, 1H); **14b** (10α-isomer) 1.38 (s, C-19 CH_3, 3H); ^{13}C NMR ($CDCl_3$) **14a**, 34.20 (q, C-2), 123.76 (q, C-4), 198.38 (q, C-3); **14b** 34.54 (q, C-2), 125.25 (q, C-4), 199.03 (q, C-3).

Unlabeled authentic **14a** was prepared by hydrogenation of **2** in MeOH over Wilkinsons catalyst,[19] Tris (triphenylphosphine rhodium chloride). The reduction product was purified by column chromatography on silica gel (m.p. 200–210°) and was identical to **14a**-$^{13}C_3$ by TLC and HPLC analysis; H NMR ($CDCl_3$) 1.32 (s, C-19 CH_3, 3H); ^{13}C NMR ($CDCl_3$) 34.29 (C-2), 123.89 (C-4), 169.56 (C-5), 198.80 (C-3). (Found: C, 72.1; H, 8.08. Calc. for $C_{24}H_{32}O_5$: C, 72.0: H, 8.05%).

Ring closure of **13a**-$^{13}C_6$ afforded **14a**-1,2,3,4,10,19-$^{13}C_6$ in 76% yield; ^{13}C NMR. 26.4 (C-19), 32.0–36.0 (C-1,2), 40.9 (C-10), 124.0 (C-4), 199.0 (C-3).

Ring closure of **13a**-$^{13}C_6$-d_3 gave **14a**-1,2,3,4,10,19-$^{13}C_6$-19,19,19-d_3 in 78% yield; ^{13}C NMR. 26.4 (C-19), 32.0–37.0 (C-1, 2), 40.9 (C-10), 123.9 (C-4), 199.0 (C-3).

16α - *Methyl* - 17α,20;20,21 - *bismethylenedioxypregn* - 1,4,9(11) - *triene* - 3 - *one* (**2**). In a dry flask protected from moisture and over argon was placed 3.0 ml THF (freshly distilled) and 95 μl. (0.60 mmol) diisopropylamine (freshly distilled from KOH). The reaction soln was chilled to −78° and then 0.45 ml (0.60 mmol) n-BuLi (freshly titrated as 1.40 M in hexane) was added from a syringe. The mixture was stirred over argon at −78° for 20 min. To the LDA soln was added 2.0 ml soln of **14** (200 mg; 0.50 mmol) in dry THF. The mixture was stirred at −78° for 1 hr and then at 0° for 5 hr. The temp was reduced to −78° and a soln 134 mg (0.65 mmol) phenylselenenyl chloride in 3.0 ml THF added. The mixture was stirred at −78° for 3.5 hr and then poured into 15 ml cold 3N HCl (pH 1–2). The mixture was extracted with two 20 ml portions CH_2Cl_2. The extract was washed with 20 ml sat $NaHCO_3$ aq, and 20 ml water. The extract was dried over $MgSO_4$ and then, the solvent was removed *in vacuo* to leave 311 mg of the 2-phenylselenyl intermediate as a gum; TLC: silica-gel HF; EtOAc/hexane (1:2); R_f 0.70 two spots (α and β-phenylselenyl isomers at C-2); IR (μ): 5.98 (C=O); 6.18 (C=C); NMR (ppm) ($CDCl_3$); 0.85 d (H-16, 3H); 0.90 and 0.98 s (H-18, 3H); 1.25 and 1.30 s (H-19, 3H); 4.0 s (H-21, 2H); 5.0–5.3 m (BMD, 4H); 5.78 s (H-4, 1H); 7.2–7.7 m (PhSe, 5H).

To a soln of the phenylselenenyl corticoid in 30 ml THF/MeOH (1:5) was added with stirring a soln 246 mg (1.15 mmol) sodium metaperiodate in 5 ml water. The mixture was stirred at room temp for 2.5 hr. A white solid precipitated in 10 min. The mixture was poured into 25 ml sat $NaHCO_3$ aq. The product was extracted by two 25 ml portions CH_2Cl_2. The extract was washed with water and then dried over $MgSO_4$. The solvent was removed *in vacuo* to leave a residual foam. The crude product was purified on 5.0 g silica-gel (90–200 mesh) with EtOAc/hexane (1:2) as the eluting solvent cuts (approx. 5 ml) were collected and those containing pure product (by TLC) were collated. The solvent was stripped from the combined cuts to leave 125 mg 63%) of a solid; IR (μ): 5.95 (C=O); 6.15 (C=C); NMR ppm) ($CDCl_3$): 0.90 d (H-16, 3H); 1.00 s (H-18, 3H); 1.40 s (H-19, 3H); 4.05 (H-21, 2H); 5.0–5.2 q (BMD, 4H); 5.50 d (H-11, 1H); 6.05 d (H-4, 1H); 6.25 d.d. (H-2, 1H); 7.2 d (H-1, 1H). TLC: Silica gel HF; EtOAc/hexane (1:2); R_f 0.55.

Comparison of the pure product above with authentic **2** by HPLC, NMR and mass spectrum showed the two materials to be identical.

2-2,3,4-$^{13}C_3$: ^{13}C NMR 123.7 (q, C-4), 127.2 (q, C-2), 186.3 (t, C-3). Authentic **2** showed 123.7 (C-4), 127.2 (C-2), 186.2 (C-3).

2-1,2,3,4,9,10-$^{13}C_6$: ^{13}NMR 26.8 (C-19), 46.0 (C-10), 123.7 (C-4), 127.2 (C-2), 154.8 (C-1), 186.3 (C-3).

9α - *Bromo* - 11β - *hydroxy* - 16α - *methyl* - 17α,20;20,21 - *bis* - *methylenedioxy* - *pregna* - 1,4 - *diene* - 3 - *one* (**15**). To a soln of 16α - methyl - 17α,20;20,21 - bismethylenedioxy - pregna - 1,4,9(11) - triene - 3 - one (240 mg; 0.60 mMol) in 10 ml dioxane and 0.4 ml 0.4 N perchloric acid was added 270 mg (1.90 mMol) N-bromoacetamide. The reaction solution was stirred at room temp for 15 hr.

The mixture was chilled with ice chips and then 10 ml 10% Na_2SO_3 aq was added, until a negative starch iodide test was obtained. The mixture was extracted with three 25 ml portions CH_2Cl_2. After drying the extract over $MgSO_4$, the solvent was removed to leave 283 mg (95%) of a solid, m.p. 215° (dec). IR (μ) 3.0 (OH); 6.0 (C=C=O); TLC: silica-gel HF; EtOAc/hexane (1:2); R_f 0.25; H NMR ($CDCl_3$–MeOH-d_4) 0.98 (d, C-16 CH_3, 3H), 1.22 (s, C-18 CH_3, 3H), 1.75 (s, C-19 CH_3, 3H), 4.0 (s, C-21 CH_2, 2H), 4.70 (t, C-11H, 1H), 5.0-5.3 (q, OCH_2O, 4H), 6.08 (d, C-4H, 1H), 6.30 (q, C-2H, 1H), 7.40 (d, C-1H, 1H). (Found: C, 57.2; H, 6.29. Calc. for $C_{24}H_{31}BrO_6\cdot\frac{1}{2}H_2O$, C, 57.1; H, 6.38%). The M + 3, M + 6 and M + 9 labeled compounds of **15** were obtained in the same manner and were identical by TLc and HPLC to **15**.

16α - *Methyl* - 9β,11β - *oxido* - 17α,20;20,21 - *bismethylenedioxy* - *pregna* - 1,4 - *diene* - 3 - *one* (**16**). To a soln of anhyd KOAc (304 mg; 3.1 mMol) in 10 ml EtOH was added a soln of 280 mg (0.56 mMol) 9α - bromo - 11β - hydroxy - 16α - methyl - 17α,20;20,21 - bismethylenedioxy - pregna - 1,4 - diene - 3 - one-in 10 ml dry *p*-dioxane. The mixture was refluxed for 19 hr and concentrated to dryness. The residual solid was partitioned between 20 ml CH_2Cl_2 and 10 ml water. The aqueous fraction was extracted with an additional 10 ml CH_2Cl_2. The extracts were combined and dried over $MgSO_4$. The solvent was evaporated *in vacuo* to leave a white solid, 210 mg (87%), m.p. 165–175°. TLC: silica gel HF; EtOAc/hexane (1:2); R_f 0.45. H NMR ($CDCl_3$); 0.90 (d, C-16 CH_3, 3H), 1.05 (s, C-18 CH_3, 3H), 1.45 (s, C-19 CH_3, 3H), 3.15 (broad s, C-11 H, 1H), 3.95 (s, C-21 CH_2, 2H), 4.9–5.25 (m, OCH_2O, 4H); 6.12 (s, C-4H, 1H), 6.22 (d, C-2H, 1H), 6.60 (d, C-1H, 1H). (Found: C, 67.5; H, 7.50. Calc. for $C_{24}H_{30}O_6\cdot\frac{3}{4}H_2O$: C, 67.3; H, 7.42%). The M + 3, M + 6 and M + 9 forms of **16** were similarly obtained and were identical to unlabeled **16**.

9α - *Fluoro* - 16α - *methyl* - 11β,17α,21 - *trihydroxypregna* - 1,4 - *diene* - 3,20 - *dione* **17** (*Dexamethasone*-$^{13}C_3$). To 2.0 ml 48% hydrofluoric acid contained in a polyethylene test tube fitted with a stirring bar was added at 0°, dropwise with stirring, a soln of **16** (20 mg; 0.28 mMol) in 0.2 ml abs EtOH/THF mixture (1:1). The mixture was continually stirred vigorously at 0° for 20 hr.

The mixture was neutralized to approx. pH 7 by careful addition, dropwise, to 5 ml chilled sat K_2CO_3 aq; vigorous stirring is necessary to minimize foaming. The solid ppt was extracted with three 12-ml portions EtOAc. The extract was washed with 10 ml water, and then dried over $MgSO_4$. The solvent was evaporated *in vacuo* to leave 14 mg (73%) white solid. This material was identical to dexamethasone by TLC, HPLC, IR and NMR.

The labeled dexamethasones were similarly obtained from the appropriately labeled oxides **16**. They were chromatographically (TLC and HPLC) identical to authentic dexamethasone.

REFERENCES

[1]M. Eichelbaum, A. Somogyi, G. E. Von Unruh and H. J. Dengler, *Eur. J. Clin. Pharmacol.* **19**, 127 (1981).

[2]M. Eichelbaum, A. Somogyi, G. E. Von Unruh and H. J. Dengler, *Ibid.* **19**, 133 (1981).

[3]H. d'A Heck, S. E. Butrill, N. W. Flynn, R. L. Dyer, M. Anbar, T. Cairns, S. Dighe and B. E. Cabana, *J. Pharmacol. Biopharm.* **7**, 233 (1979).

[4]Roussel-UCLAF, *Neth. Appl.* 6,414,701, 18 June 1964.

[5]C. A. Henrick, E. Böhme, J. A. Edwards and J. H. Fried, *J. Am. Chem. Soc.* **90**, 5926 (1968).

[6]J. I. DeGraw, P. H. Christie and T. Cairns, *J. Labeled Compounds* **19**, 945 (1982).

[7]C. H. Robinson, L. E. Finckelnor, R. T. Beri, M. Eisler, R. Neri, A. Watnick. P. L. Perlman, P. Holroyol, W. Charney and E. P. Oliveto, *J. Am. Chem. Soc.* **28**, 4611 (1960).

[8]R. E. Beyler, F. Hoffman, R. Moriarty and L. H. Sarett, *J. Org. Chem.* **26**, 2421 (1961).

[9]K. Tsuda, E. Ohki and S. Nozoe, *Ibid.* **28**, 876 (1963).

[10]G. H. Douglas, J. M. Graves, D. Hartley, G. A. Hughes, B. J. McLoughlin, J. Spiddall and H. Smith, *J. Chem. Soc.* 5072 (1963).

[11]S. G. Lavine, N. H. Eudy and C. F. Leffler, *J. Org. Chem.* **31**, 3995 (1966).

[12]M. Perlman, E. Farkas, E. J. Fornefeld, R. Kraay and R. T. Kapala, *J. Am. Chem. Soc.* **82**, 2403 (1960).

[13]B. E. Edwards and P. N. Rao, *J. Org. Chem.* **31**, 324 (1966).

[14]Roussel-UCLAF, *S.A. Brit.* 914,738, 2 January 1963.

[15]L. Velluz, Y. Nomine and Z. Matheiu, *Angew Chem.* **22**, 725 (1960).

[16]G. Stork, H. Loewenthal and P. Mukharji, *J. Am. Chem. Soc.* **78**, 501 (1956).

[17]G. Stork and J. McMurray, *Ibid.* **89**, 5464 (1967).

[18]C. Djerassi and J. Gutzweller, *Ibid.* **88**, 4537 (1966).

[19]M. Heller, R. H. Lenhard and S. Bernstein, *Steroids* **7**, 381 (1966).

[20]H. J. Reich, J. M. Renga and I. L. Reich, *J. Am. Chem. Soc.* **97**, 5434 (1975).

[21]J. Fried and E. F. Sabe, *Ibid.* **79**, 1130 (1957).

[22]J. F. Young, J. A. Osborn, F. H. Jardine and G. Wilkinson, *Chem. Commun.* **131** (1965); F. H. Jardine, J. A. Osborn, G. Wilkinson and J. F. Young, *Chem. Ind.* 560 (1965).