Total Synthesis of Cortisol: Application to Selective Deuteriation at C-1 and C-19

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An 11-step total synthesis of cortisol and its application to selective deuteriation at the 19-methyl and the C-1 positions is described. The dihydroxy acetone group at C-17 of prednisone (**3**) was protected as the bismethylenedioxy derivative (**4**) and degraded to the ring C/D fragment, oxoindanylpropionic acid (**2**), by Birch reduction followed by ozonolysis. The reaction of deuterioisopropenyl anion with compound (**2**) followed by dehydration, cyclisation, and ozonolysis produced [1,1,2,2,4,4,4,19,19,19-$^2H_{10}$]-4,5-seco-17α,20;20,21-bismethylenedioxypregnane-3,5,11-trione [the ^2H-labelled trione (**16a**)]. Cyclisation of (**16a**) in KOH–MeOH afforded the bismethylenedioxy cortisone (**17**), which upon reduction with KBH_4 gave the desired [$^2H_5$]cortisol (**19**).

There has recently been a growing interest in the use of stable isotopically labelled steroids for a variety of chemical, biological and endocrinological studies of steroid hormones.[1] The development of ^{13}C and ^1H n.m.r. and mass spectrometry enables use of these stable isotopes for the structural elucidation and mechanistic studies of the steroid metabolism. However, these studies are, in general, limited because of the high cost and lack of availability of compounds with appropriately labelled positions.

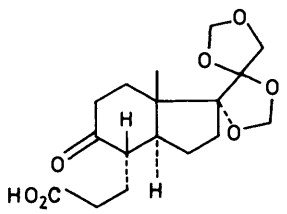
We have successfully used stable isotope dilution mass spectrometry for the pharmacokinetic studies of natural and synthetic steroids, in which stable isotopically labelled analogues serve as ideal internal standards.[2–5] At present, we are interested in applying the stable isotope methodology for selective and accurate quantification of cortisol in human body fluids by g.c.–m.s., procedures which require multiply labelled cortisols containing at least four non-exchangeable stable isotopes with high isotopic purity for use as both biological and analytical internal standards.

Here, we describe the application of the indan skeleton for the synthesis of multiply ^2H-labelled cortisol and for selective deuteriation at C-19 and C-1. The synthesis offers a concise construction of the key indanone intermediate and a convenient method of introducing five ^2H-atoms at carbon atoms from which ^2H is not lost and therefore no primary isotope effects are involved.

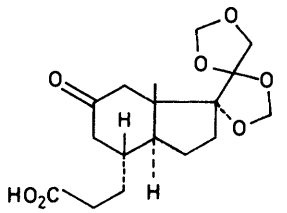
Results and Discussion

The indan skeleton provides a useful C/D ring fragment for the synthesis of steroids, and several methods have been developed for synthesis of androgens[6,7] and estrogens[8] using indan compounds as synthetic building blocks. Application of the indan synthon method is of particular interest for introducing ^{13}C or ^2H in steroid rings A and B. In our attempts to synthesize multiply ^2H-labelled cortisol, a fundamental prerequisite was to prepare a ring C/D synthon which possessed the 11-oxo group and the cortisol side-chain (dihydroxyacetone grouping) at C-17 (steroid numbering).

Dexamethasone multiply labelled with ^{13}C in ring A has been prepared in 18 steps from 16α-methylcorticoid.[9] The route involved intermediary formation of a ring C/D indanone synthon, and required elaborate steps for the introduction of the C-11 oxygen function. Application of this method could lead to the indanone derivative (**1**) with the cortisol side-chain in 9 steps. The transformation of compound (**1**) to the key intermediate 11-oxoindan (**2**) (steroid numbering) would require 6 steps.[10] Stork *et al.*[6,11] developed an efficient synthetic route from 11-oxohydroindan testosterone and progesterone derivatives. It is, however, difficult to introduce the cortisol side-chain at C-17 (steroid numbering) into the required 11-oxohydroindan steroid numbering.[11] Very recently, an 18-step total synthesis of (±)-cortisone has been reported.[12] This method utilises the key 17-isopropenylindanone intermediate (11 steps from a cyclohexenone derivative) which requires a further 7 steps to produce (±)-cortisone by Stork's method.[6]

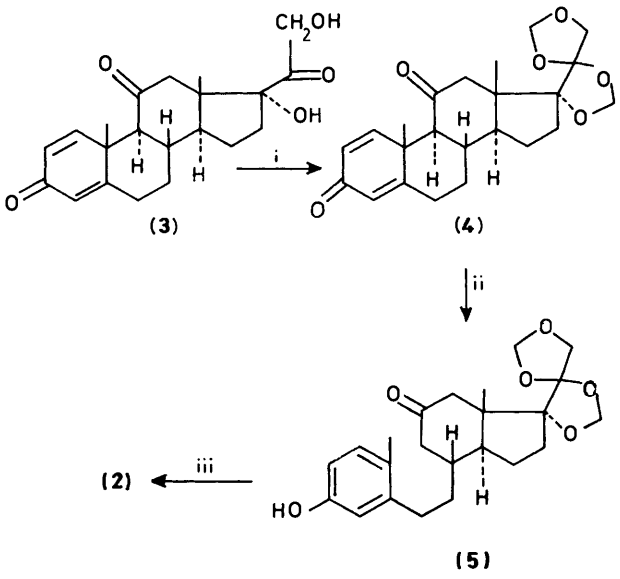


(1)

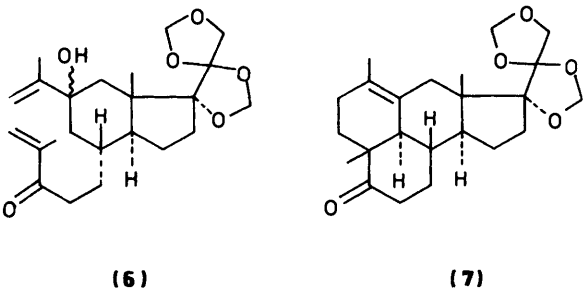


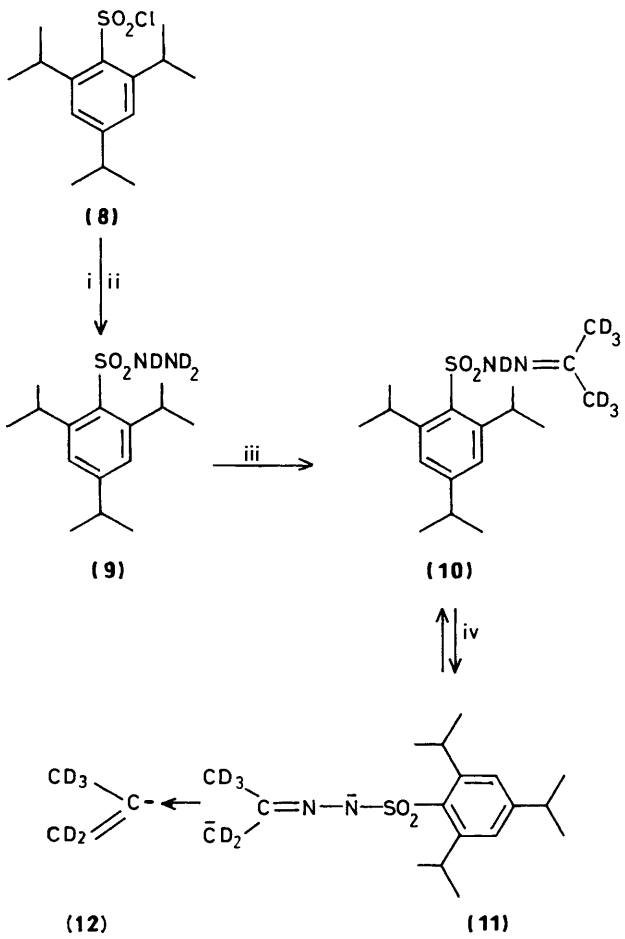
(2)

A characteristic feature of our method was to introduce simultaneously the 11-oxo group and the cortisol side-chain into the key indan intermediate by using an efficient and convenient route. The simple construction of (**2**) was attained in only three steps starting with the readily available prednisone (Scheme 1). The dihydroxy acetone side-chain of prednisone (**3**) was first protected as the bismethylenedioxy (BMD) group by treatment of (**3**) with HCHO–HCl to give (**4**).[13] Treatment of (**4**) with liquid NH_3 in the presence of Li at −78 °C caused the reductive cleavage of C_9–C_{10} bond of (**4**), while the A-ring was aromatised, to give the phenolic acid (**5**) in 51% yield.[14] Ozonolysis of the Birch reduction product (**5**) in EtOAc at −5 °C for 35 min followed by the addition of 30% H_2O_2 gave the desired oxoindanylpropionic acid (**2**) in 41% yield. Conversion of compound (**2**) into the steroid nucleus was then achieved *via* an internal Diels–Alder route.[6] The two-molecular nucleophilic addition of isopropenyl anion[15] to the indanone (**2**) then gave the enone alcohol (**6**).



Scheme 1. *Reagents:* i, HCHO–HCl; ii, Li/NH_3; iii, O_3

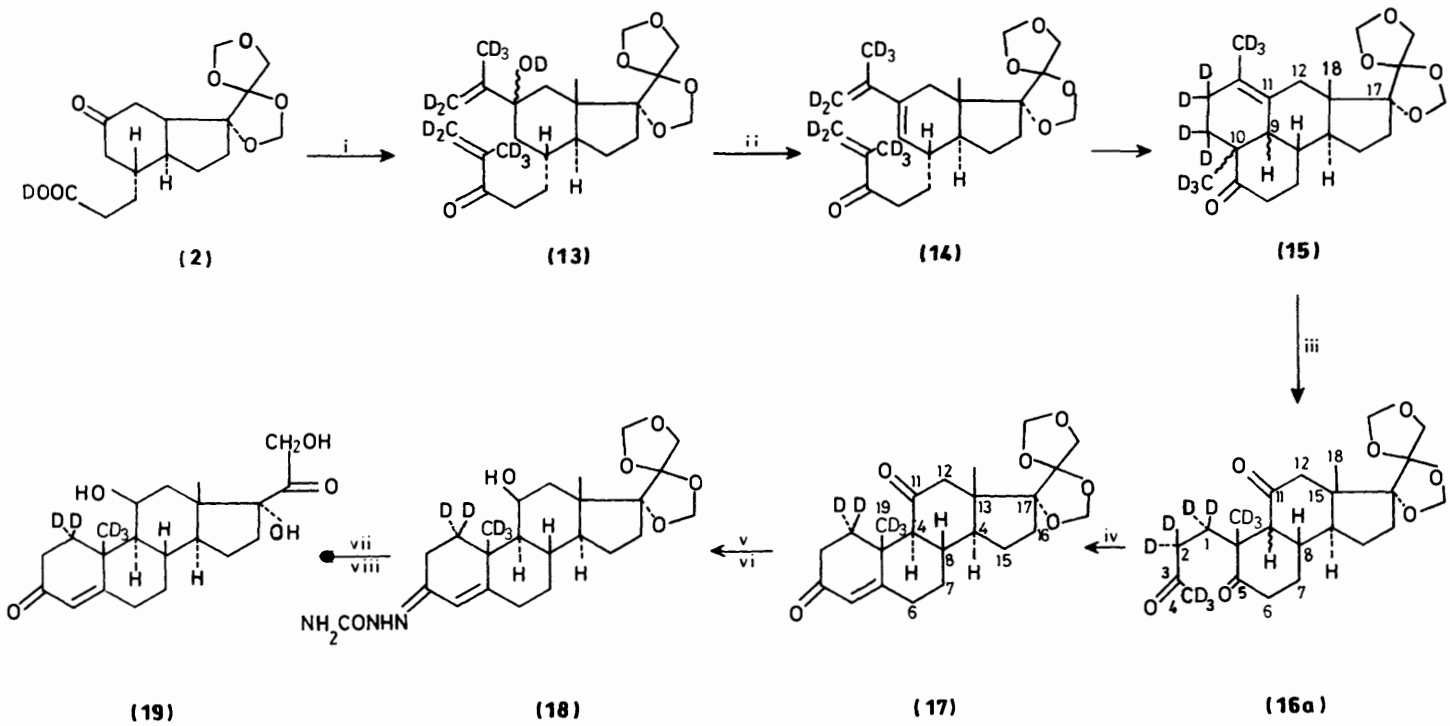




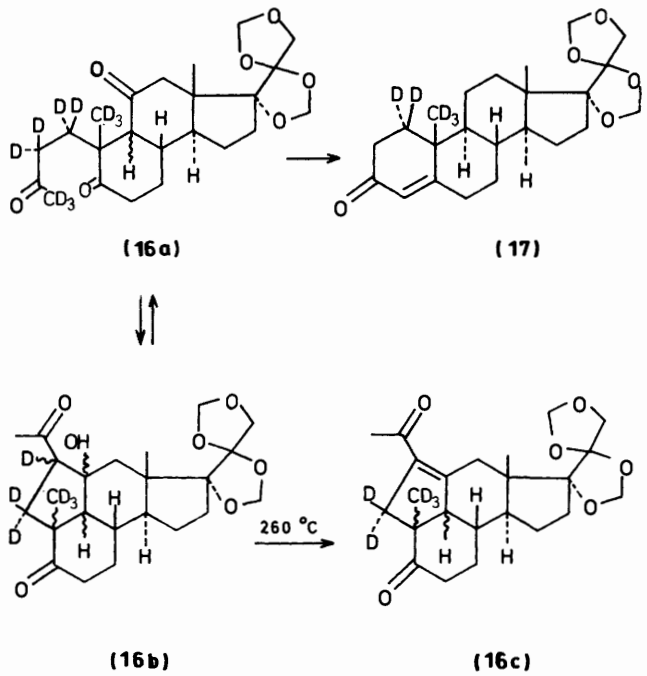
Scheme 2. *Reagents:* i, NH_2NH_2·HCl; ii, D_2O; iii, $(CD_3)_2CO$; iv, BuLi

Treatment of compound (**6**) with CF_3CO_2H gave the cyclised product (**7**), which on ozonolysis followed by cyclisation in KOH–MeOH produced cortisone-BMD. Reduction of cortisone-BMD with KBH_4 by a known route[16,17] gave the desired cortisol. These synthetic procedures were modified to obtain multiply ^2H-labelled cortisol as follows. Acetone enriched with [$^2H_6$]acetone (99.8 atom%) was condensed with 2,4,6-tri-isopropylbenzenesulphonyl trideuteriohydrazide (**9**) under reflux for 30 min to give the labelled hydrazone (**10**) quantitatively.[18] Treatment of compound (**10**) with BuLi reagent (2.2 mol equiv.) in redistilled dimethoxyethane at −78 °C followed by warming to 0 °C generated the deuterioisopropenyl anion (**12**) (Scheme 2). Dropwise addition of the oxoindanylpropionic acid (**2**) to the anion (**12**) thus formed at 0 °C gave the enone alcohol (**13**) quantitatively (Scheme 3). In the anion formation[15] (Scheme 2), the hydrazino hydrogens of (**9**) were displaced completely with deuteriums to exclude the possible exchange of the deuterium atoms of the acetone methyl of (**11**) with the hydrazone proton. Intramolecular cycloaddition of the enone alcohol (**13**) with simultaneous dehydration by treatment with CF_3CO_2D gave the cyclised product (**15**) (Scheme 3). The mass spectrometric results (M^+: *m/z* 398, the corresponding non-labelled reference: *m/z* 388) indicated the incorporation of ten deuterium atoms into the molecule of (**15**). Comparison of the ^1H n.m.r. data between labelled and non-labelled compounds allowed us to assign the two quaternary methyl groups 19-Me (δ_H 1.02) and 18-Me (δ_H 1.03) (steroid numbering). Ozonolysis of compound (**15**) in MeOH–CH_2Cl_2 at −78 °C followed by treatment with Zn–2M AcOH produced the trione (**16a**) in 40% yield after t.l.c. purification.

Heating of (**16a**) in 3.2% KOH–MeOH at 40 °C for 1.5 h gave cortisone-BMD (**17**) and the acetylcyclopentanol (**16b**) in a ratio of 3:5 (Scheme 4). A similar observation was also made by Stork *et al.*[6] with respect to the kinetic conversion of another trione into an acetylcyclopentanol upon treatment with KOH–MeOH. Pyrolysis of non-labelled (**16b**) at 260 °C formed a double bond conjugated to the acetyl carbonyl to give the u.v.-positive (**16c**) (M^+; *m/z* 402), indicating the dehydration of (**16b**). The non-labelled cortisone-BMD synthesized by this route was identical with the authentic cortisone-BMD. This was confirmed by capillary g.c., m.s., and ^1H n.m.r. ^1H N.m.r. of the labelled (**17**) obtained by the present procedures indicated complete disappearance of the 19-Me signal. The mass spectrum showed the molecular ion at *m/z* 407 (the corresponding non-labelled reference, M^+ at *m/z* 402), indicating the incorporation of five deuterium atoms into (**17**). Labelled (**16b**) was converted into (**17**) by re-treating with 3.2% KOH–MeOH. Mass spectra and ^1H n.m.r. data revealed that the five deuterium atoms present at the pro C-2 and C-4 positions of the trione were completely displaced with hydrogens to give cortisone selectively deuteriated at the C-1 methylene and 19-methyl groups. In our preliminary experiment, the non-labelled trione (**16a**) was treated with 3.2% KOD–MeOD and extensive incorporation of deuterium into the cortisone-BMD molecule was observed. The mass spectrum showed the incorporation of eight deuterium atoms into the molecule. Treatment of the non-labelled acetylcyclopentanol (**16b**) with the same basic media (3.2% KOD–MeOD) also gave cortisone-BMD with practically the same extent of deuterium incorporation as in the case of the trione (**16a**). It became apparent from the analysis of n.m.r. and



Scheme 3. *Reagents:* i, $CD_2{=}\bar{C}CD_3$; ii, CF_3CO_2D; iii, O_3; iv, KOH–MeOH; v, $NH_2CONHNH_2$·HCl; vi, KBH_4; vii, CH_3COCO_2H; viii, HF



Scheme 4.

m.s. data that of the eight deuterium atoms incorporated into the cortisone-BMD molecule, five should reside at C-2 (α,β), C-4 and C-6 (α,β). No incorporation was observed at the BMD group. The other three deuterium atoms would be incorporated at C-9 (methine) and C-12 (methylene) adjacent to the C-11 carbonyl function.[19] Under the reaction conditions employed, the 19-Me hydrogens were unaffected. These results indicated that chemically unstable deuterium atoms at the pro C-2 and C-4 positions of the labelled trione (**16a**) were readily removed by back-exchange reaction during the conversion of (**16a**) into (**17**) upon treatment with 3.2% KOH–MeOH.

The conversion of cortisone-BMD into the desired cortisol was achieved by a known route[16,17] in 43% yield as follows. The 3-oxo function of (**17**) was first protected with semicarbazide and the 11-oxo group was then reduced with KBH_4. Removal of the protecting group at C-3 with pyruvic acid followed by hydrolysis of the BMD protecting group with 46% HF afforded the ^{2}H-labelled cortisol (**19**). The non-labelled cortisol synthesized by this route was identical with the authentic compound by m.s., capillary g.c., h.p.l.c., and ^{1}H n.m.r. The molecular ion at *m/z* 367 of the labelled cortisol shifted by five mass units from that of the non-labelled one at *m/z* 362. The 19-methyl signal (δ_H 1.51) observed for the non-labelled cortisol completely disappeared in the labelled analogue. ^{2}H N.m.r. confirmed the incorporation of three deuterium atoms at 19-Me and gave additional information concerning the assignment of two deuterium atoms at the C-1α (δ_H 1.92) and C-1β (δ_H 2.26) positions. There was no evidence of deuterium scrambling in the ^{2}H n.m.r. spectrum. The isotopic purity of the labelled [$^2H_5$]cortisol was high, being 97.94 atom% ([$^2H_5$] 97.27%, [$^2H_6$] 1.14%, [$^2H_7$] 1.59%).

Experimental

All m.p.s were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H N.m.r. spectra were determined on Varian EM-390 90 MHz and Bruker AM-400 400 MHz spectrometers for solutions in $CDCl_3$, CD_3OD, and $(CD_3)_2CO$ (Me_4Si as an internal standard). ^{2}H N.m.r. spectra were determined on a JEOL JNM FX-200 200 MHz spectrometer for a solution in MeOH (CD_3OD as an external standard). E.i. mass spectra were recorded on a Hitachi M-80 mass spectrometer at 70 eV. Tetrahydrofuran (THF),

dimethoxyethane (DME), CH_2Cl_2, and MeOH were redistilled and all other chemicals and reagents were of analytical reagent grade and were used without further purification.

2,4,6-*Tri-isopropylbenzenesulphonohydrazide* (*Trisylhydrazide*) (**9**).—Hydrazine hydrate (99%, 2 ml) was added dropwise to a cold solution (−10 °C) of 2,4,6-tri-isopropylbenzenesulphonyl chloride (**8**) (5.0 g) in dry THF (30 ml), over a period of 10 min and the reaction mixture was stirred at −5 ~ 0 °C for 4 h. D_2O (2 ml) was added, the reaction mixture was transferred into a separating funnel and the lower aqueous layer discarded. The organic layer was washed with D_2O (2 × 5 ml) saturated with NaCl and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure (below 15 °C) gave a white crude product (**9**) used for the following reaction without purification. δ_H(90 MHz; $CDCl_3$) 1.28 (18 H, d, Me of 2′,4′,6′-isopropyl), 2.89 (1 H, sept, –CH– of 4′-isopropyl), 4.16 (2 H, sept, –C– of 2′,6′-isopropyl), and 7.20 (2 H, s, 3′- and 5′-ArH).

Hexadeuterioacetone 2,4,6-*Tri-isopropylbenzenesulphonylhydrazone* (*Trisylhydrazone*) (**10**).—A solution of the trisylhydrazide (5 g) in $(CD_3)_2CO$ (99.8 atom%, 45 ml) was stirred at 20 °C for 30 min. Evaporation of the solution under reduced pressure at room temperature gave the title compound (**10**) [5.0 g, 88% from (**8**)]. The product (**10**) was dried *in vacuo* over P_2O_5 at room temperature for at least 1 day prior to use δ_H(90 MHz; $CDCl_3$) of the non-labelled compound 1.26 (18 H, d, Me of 2′,4′,6′-isopropyl), 1.79 (3 H, s, Me_2CO), 2.88 (1 H, sept, –CH– of 4′-isopropyl), 4.25 (2 H, sept, –CH– of 2′,6′-isopropyl), and 7.17 (2 H, s, 3′- and 5′-ArH). Signals at 1.79 and 1.91 disappeared in the labelled compound.

17α,20;20,21-*Bismethylenedioxypregna*-1,4-*diene*-3,11,20-*trione* (*Prednisone-BMD*) (**4**).—35% HCl (300 ml) and 37% HCHO (300 ml) were added to a suspension of prednisone (**3**) (30.0 g) in $CHCl_3$ (600 ml), and the reaction mixture was stirred at room temperature for 16 h. After the aqueous layer was discarded, the organic layer was washed with saturated $NaHCO_3$, and then with water. The solution was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The resulting material was triturated with ether, filtered, and washed with cold ether to give colourless crystals of prednisone-BMD (**4**) (25.6 g, 77%), m.p. 198—205 °C (lit.,[13] 214—217 °C), δ_H(90 MHz; $CDCl_3$) 0.87 (3 H, s, 18-Me), 1.47 (3 H, s, 19-Me), 3.98 (2 H, s, 21-CH_2), 5.07—5.25 (4 H, m, –OCH_2O–), 6.14 (1 H, s, 4-H), 6.25 (1 H, d, 2-H), and 7.76 (1 H, d, 1-H).

3-*Hydroxy*-9,10-*seco*-17α,20;20,21-*bismethylenedioxypregna*-1(10),2,4-*trien*-11-*one* (*Phenolic acid-BMD*) (**5**).—Lithium plate (1.7 g) in four portions was added to liquid ammonia (500 ml), with stirring at −78 °C, over a period of 10 min. To the blue solution was added dropwise a solution of prednisone-BMD (**4**) (20.0 g) in dry THF (200 ml) over a period of 2 h. The mixture was stirred for a further 3 h at −78 °C and MeOH (*ca.* 5 ml) was added until disappearance of the blue colour. After being left for 30 min at −78 °C the mixture was left overnight at room temperature to remove the excess of ammonia. The solution was concentrated to *ca.* 100 ml under reduced pressure, neutralised with 7% HCl (pH 6—7), then extracted with EtOAc (3 × 100 ml), washed with water (× 2), and dried (Na_2SO_4). The solvent was evaporated to dryness under reduced pressure and the resulting pale yellow crystals were recrystallised from $CHCl_3$–MeOH to give the pure product (**5**) as colourless crystals (10.2 g, 51%), m.p. 229—233 °C (lit.,[19] 235—239 °C); δ_H(90 MHz; $CDCl_3$) 0.85 (3 H, s, 18-Me), 2.22 (3 H, s, 19-Me), 3.99 (2 H, s, 21-CH_2), 5.05—5.23 (4 H, m, –OCH_2O–), 6.63 (1 H, d, 2-H), 6.68 (1 H, s, 4-H), and 7.06 (1 H, d, 1-H); *m/z* 402 (M^+).

Des-A,B-11-*oxo*-17α,20;20,21-*bismethylenedioxypregnane*-8α-*propionic Acid* (*Oxoindanylpropionic Acid*) (**2**).—A solution of the phenolic acid (**5**) (250 mg) in EtOAc (80 ml) was ozonised at −5 °C for 35 min with an ozone–oxygen stream (100 ml min^{-1}, 0.089 mmol O_3 min^{-1}). When reaction was complete, nitrogen was bubbled through the solution for 30 min to drive out the excess of ozone. After addition of H_2O_2 (30%. 3 ml) the mixture was allowed to stand at room temperature. The organic layer was washed with water (3 × 50 ml), dried (Na_2SO_4), and evaporated to dryness to give an oily residue. The residue was purified by silica gel column chromatography using EtOAc–light petroleum–AcOH (60:40:1) as eluant. The combined eluates were washed with water and dried (Na_2SO_4). After evaporation of the solvent, the pure product (**2**) (1.4 g, 41%) was obtained as colourless crystals, δ_H(90 MHz; $CDCl_3$) 0.85 (1 H, s, 18-Me), 3.97 (2 H, s, 21-CH_2), and 5.00—5.19 (4 H, m, OCH_2O); *m/z* 340 (M^+).

[1,1,2,2,4,4,4,19,19,19-^{2}H]-10,11-*Cyclo*-4,5-*seco*-17α,20;20,21-*bismethylenedioxypregn*-3(11)-*en*-5-*one* (**15**).—The oxoindanylpropionic acid (**2**) (200 mg) was dissolved in CH_3OD (99.7 atom%, 10 ml) in order to displace the acidic proton with deuterium. The solvent was immediately evaporated under reduced pressure at room temperature to give the oxoindanylpropionic acid deuteride. The deuteride product (**2**) was dried over P_2O_5 *in vacuo* at room temperature for 2 h prior to use.

A solution of the trisylhydrazone (**10**) (800 mg) in redistilled DME (20 ml) placed in a heat-dried flask was cooled to −78 °C under a stream of argon through H_2SO_4. To this solution was added BuLi (3.2 ml, *ca.* 2.2 mol equiv. as a 1.6mM solution in hexane) dropwise over a period of 5 min when the colour of solution turned to light orange. The mixture was stirred at −78 °C for 20 min and the reaction flask was placed in an ice-bath. When the colour had changed to light yellow and nitrogen evolution had ceased, a solution of the oxoindanylpropionic acid deuteride (**2**) (200 mg) in DME (7 ml) was added over a period of 10 min. The reaction mixture was stirred at 0 °C for 5 h, poured into D_2O (50 ml), and then extracted with CH_2Cl_2 (2 × 50 ml). Evaporation of the solvent under reduced pressure gave the enone alcohol (**13**). To a solution of compound (**13**) (*ca.* 150 mg) in CH_2Cl_2 (6 ml) was added CF_3CO_2D (99.0 atom%, 0.5 ml) dropwise over 5 min at −78 °C. The reaction mixture was left at room temperature for 4 h, then treated with 3% $NaCO_3$, washed with water, and evaporated to dryness. The resulting material was purified by preparative t.l.c. (R_F 0.41, 30% EtOAc in light petroleum as developing solvent) to give the pure compound (**15**) (*ca.* 20 mg, *ca.* 8.5%); δ_H(400 MHz; $CDCl_3$) of the non-labelled compound 1.02 (3 H, s, 19-Me), 1.03 (3 H, s, 18-Me), 1.26 (3 H, s, 4-Me), 3.97 (2 H, s, 21-CH_2), and 5.04—5.20 (4 H, m, OCH_2O). Signals at 1.02 and 1.26 disappeared in the labelled compound; δ_H(400 MHz; $CDCl_3$) of the labelled compound 1.04 (3 H, s, 18-Me), 3.98 (2 H, s, 21-CH_2), and 5.06—5.21 (4 H, m, OCH_2O); *m/z* 398 (M^+, the labelled compound), and *m/z* 388 (M^+, the non-labelled compound).

[1,1,2,2,4,4,4,19,19,19-^{2}H]-4,5-*Seco*-17α,20;20,21-*bismethylenedioxypregnane*-3,5,11-*trione* (**16a**).—A solution of the cyclised product (**15**) (*ca.* 12 mg) in CH_2Cl_2–MeOH (1:1 v/v, 10 ml) was ozonised at −78 °C for 3.5 min with an ozone–oxygen stream (100 ml min^{-1}, 0.089 mmol O_3 min^{-1}). Zinc powder (50 mg) in 2M AcOH (2.5 ml) was added, the mixture was stirred for 1 h at room temperature, and water (10 ml) was added. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 ml), the CH_2Cl_2 extract washed with 3% Na_2CO_3 and water, and the solvent evaporated to dryness to give the crude trione (**16a**). Purification by preparative t.l.c. (R_F 0.25, 35% THF in cyclohexane as developing solvent) gave the pure compound (**16a**) (*ca.* 6 mg, 40%); δ_H(400 MHz; $CDCl_3$) of the non-labelled compound 0.88

(3 H, s, 18-Me), 1.08 (3 H, s, 19-Me), 2.07 (3 H, s, MeCO), 3.95—3.98 (2 H, m, 21-CH_2), and 4.99—5.19 (4 H, m, OCH_2O). Signals at 1.08 and 2.07 disappeared in the labelled compound. δ_H(400 MHz; $CDCl_3$) of the labelled compound 0.90 (3 H, s, 18-Me), 3.95—4.00 (2 H, m, 21-CH_2), and 5.01—5.21 (4 H, m, OCH_2O).

[1,1,19,19,19-^{2}H]-17α,20;20,21-*Bismethylenedioxypregn*-4-*ene*-3,11-*dione* ([$^2H_5$]*Cortisone-BMD*) (**17**).—A solution of the trione (**16a**) (*ca.* 5 mg) dissolved in 3.2% KOH–MeOH (2 ml) was stirred at 40 °C for 1.5 h. The reaction mixture was then diluted with water and extracted with CH_2Cl_2 (3 × 5 ml). Two products {[$^2H_5$]cortisone-BMD (**17**) and acetylcyclopentanol (**16b**)} contained in the extracts were separated by preparative t.l.c. (35% THF in cyclohexane as developing solvent) to give the product (**17**) (*ca.* 1.6 mg, 34%) and the product (**16b**) (*ca.* 2.4 mg, 49%). Product (**17**): δ_H(400 MHz $CDCl_3$) of the non-labelled compound 0.83 (3 H, s, 18-Me), 1.42 (3 H, s, 19-Me), 3.94—4.00 (2 H, d, 21-CH_2), 5.01—5.20 (4 H, m, OCH_2O), and 5.73 (1 H, s, 4-H). A signal at 1.42 disappeared in the labelled compound; δ_H(400 MHz; $CDCl_3$) of the labelled compound 0.83 (3 H, s, 18-Me), 3.94—4.00 (2 H, d, 21-CH_2), 5.01—5.20 (4 H, m, OCH_2O), and 5.73 (1 H, s, 4-H); *m/z* 407 (M^+). Product (**16b**): δ_H(400 MHz; $CDCl_3$) of the non-labelled compound 1.02 (3 H, s, 18-Me), 1.34 (3 H, s, 19-Me), 2.20 (3 H, s, MeCO), 3.93 (2 H, s, 21-CH_2), and 4.95—5.19 (4 H, m, OCH_2O); *m/z* 420 (M^+).

A solution of the acetylcyclopentanol (**16b**) dissolved in 3.2% KOH–MeOH (1.0 ml) was stirred again at 40 °C for 3.5 h to give compound (**17**) (*ca.* 1 mg, 44%).

[1,1,19,19,19-^{2}H]-11β,17α,21-*Trihydroxypregn*-4-*ene*-3,20-*dione* ([$^2H_5$]*Cortisol*) (**19**).—A solution of semicarbazide hydrochloride (10 mg) in water (50 μl) was added to a solution of the [$^2H_5$]cortisone-BMD (**17**) (*ca.* 2.6 mg) in CH_2Cl_2–MeOH–pyridine (10:40:1, 0.5 ml) and the reaction mixture stirred at room temperature for 12 h. The solution was concentrated under reduced pressure, then the mixture extracted with $CHCl_3$ (3 × 3.0 ml), washed with water, and evaporated to dryness to give almost pure [$^2H_5$]cortisone-BMD 3-semicarbazone.

A mixture of [$^2H_5$] cortisone-BMD 3-semicarbazone and KBH_4 (5 mg) in THF–water (5:1, 1.2 ml) was stirred at room temperature for 16 h. After addition of 2% AcOH (1.5 ml) the resulting suspension was extracted with $CHCl_3$ (3 × 2 ml). The extracts were washed with water and evaporated to dryness under reduced pressure to give the crude [$^2H_5$]cortisol-BMD 3-semicarbazone (**18**).

A solution of compound (**18**) in pyruvic acid–water–AcOH (1:1:1, 0.5 ml) was stirred at room temperature for 15 h, then diluted with water (1 ml) and extracted with EtOAc (3 × 3 ml). The extracts were washed with 3.0% $NaHCO_3$ and then with water. Evaporation of the solvent gave the crude [$^2H_5$]cortisol-BMD.

A solution of [$^2H_5$]cortisol-BMD in EtOH–THF (1:1, 0.3 ml) was added dropwise to 46% HF (0.6 ml) in a polyethylene test-tube at 0 °C with stirring and the mixture stirred vigorously for 10 min and then kept at 2 °C for 12 h. The mixture was neutralised to pH *ca.* 7 by careful addition of chilled saturated Na_2CO_3 (6 ml) and then extracted with EtOAc (4 × ml). The extracts were washed with water and the solvent evaporated under reduced pressure to give a crude product. Purification by t.l.c. ($CHCl_3$–MeOH 9:1 as developing solvent) gave the pure [$^2H_5$]cortisol (**19**) (*ca.* 1 mg, 43%) as colourless crystals; δ_H(400 MHz; CD_3OD) of authentic natural cortisol 0.93 (3 H, s, 18-Me), 1.51 (3 H, s, 19-Me), 1.92 (1 H, m, 1-H), 2.26 (1 H, m, 1-H), and 5.70 (1 H, s, 4-H). Signals at 1.51, 1.92, and 2.26 disappeared in the labelled compound; δ_H(400 MHz; CD_3OD) of the labelled compound 0.93 (3 H, s, 18-Me) and 5.70 (1 H, s, 4-H); non-labelled compound *m/z* 362 (M^+), 302, 332, and 344 and labelled compound *m/z* 367 (M^+), 307, 337, and 349.

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