15α-Acetoxy-1,4,6-pregnatriene-3,20-dione (IVe).—15α-Acetoxy-4,6-pregnadiene-3,20-dione (IIIe) (4.15 g.) was dissolved in 40 ml. of purified dioxane together with 2.8 g. of dichlorodicyanoquinone. The solution was refluxed for 2 hr., cooled, and filtered. The residue obtained from the concentration of the filtrate was dissolved in benzene and chromatographed on silica gel. The residue from the combined eluates with 15 and 20% ethyl acetate–benzene was crystallized from acetone–ether to yield 1.95 g. of material. These crystals were dissolved in methylene chloride, washed with a basic solution, then recrystallized from acetone after removal of the solvent. One half of the material was rechromatographed; 0.3 g. of IVe, crystallized from acetone, was obtained from the 20% ethyl acetate–benzene eluates.

15α-Hydroxy-1,4,6-pregnatriene-3,20-dione (IVd).—15α-Acetoxy-1,4,6-pregnatriene-3,20-trione (IVe) (0.7 g.) was hydrolyzed by the method used for 15α-hydroxy-1,4-pregnadiene-3,20-dione. The product was chromatographed on silica gel and the residue from the 30% ethyl acetate–benzene eluates crystallized from acetone to yield 0.2 g. of IVd.

15α-Hydroxy-17α-pregna-1,4-diene-3,20-dione (IX).—The mother liquors from the hydrolysis of 49 g. of 15α-acetoxy-1,4-pregnadiene-3,20-dione (IIe) were concentrated, and the residue so obtained dissolved in benzene for chromatography on 400 g. of silica gel. A yield of 0.9 g. of IX, crystallized in acetone, was found in the combined fractions eluted with 35% ethyl acetate–benzene.

17α-Pregna-1,4-diene-3,15,20-trione (X).—This compound was crystallized in a yield of 0.14 g. from acetone–ether after oxidation[18] of 0.20 g. of the corresponding 15α-hydroxy compound (IX).

Acknowledgment.—We thank Dr. Roy H. Bible, Jr., of these laboratories and referee II for many helpful suggestions concerning the n.m.r. spectra.

Steroids. CCL.[1] The Synthesis of 2-Methylenehydrocortisone[2]

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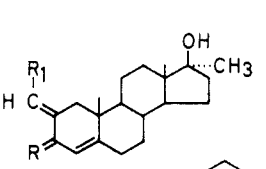
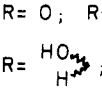
The synthesis of 17α,20;20,21-bismethylenedioxy-11β-hydroxy-2-N-piperidinomethylenepregn-4-en-3-one (IIf) and its conversion to 2-methylenehydrocortisone (IIi) are described. An improved preparation of the bismethylenedioxy derivative of hydrocortisone is reported.

Interest in C-2 substituted cortical hormones stems from the finding by an Upjohn group that introduction of a 2α-methyl substituent into hydrocortisone brought about a 4.5- and 2.6-fold increase, respectively, of its antiinflammatory and mineralocorticoid activities.[3] Subsequent studies in several laboratories have led to the preparation of cortical hormones substituted at C-2 by hydroxyl,[4] cyano,[5] fluoro,[6] hydroxymethylene,[7] and formyl[8] groups. However, none of these modifications were reported to enhance the activity of the parent hormone.

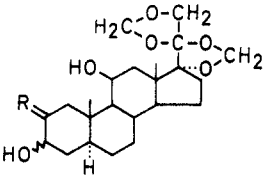
Our interest in hormone analogs having sp^2-hybridized carbon atoms at C-2 and C-3[9] together with our recent work[10] which led to the preparation of 2-methylene-Δ^4-3-ketoandrostenes prompted us to extend the latter study to the cortical hormone series. This communication describes the synthesis of 2-methylenehydrocortisone (IIi).[11]



Ia- R= O; R1= 

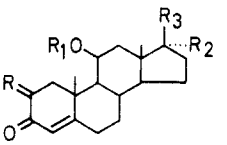
b- R=  ; R1= H

c- R= O; R1= H



IIIa- R= CH_2; with Δ^4 double bond

b- R= α CH_3, β H



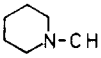
IIa- R= H_2; R1= H; R2= OH; R3= 

b- R= H_2; R1= H; R2,R3= bismethylenedioxy

c- R= H_2; R1= CH_3OCH_2 ; R2,R3= bismethylenedioxy

d- R= H_2; R1= $HOCH_2$; R2,R3= bismethylenedioxy

e- R= CHOH; R1= H; R2,R3= bismethylenedioxy

f- R=  ; R1= H; R2,R3= bismethylenedioxy

g- R= CH_2; R1= H; R2,R3= bismethylenedioxy

h- R= CH_2; R1= H; R2= OH; R3= 

i- R= CH_2; R1= H; R2= OH; R3= 

In our earlier work it was noted that the reduction of 17β-hydroxy-17α-methyl-2-N-piperidinomethylene-androst-4-en-3-one (Ia) with sodium borohydride afforded 17α-methyl-2-methyleneandrost-4-ene-3ξ,17β-

(1) Part CCXLIX: F. Alvarez, *Steroids*, submitted for publication.

(2) This paper constitutes Part VII in the series of ring A modified hormone analogs. Part VI: P. Crabbé, J. Revilla, and A. Bowers, *J. Med. Chem.*, **6**, 182 (1963).

(3) J. A. Hogg, F. H. Lincoln, R. W. Jackson, and W. P. Schneider, *J. Am. Chem. Soc.*, **77**, 6401 (1955); W. W. Byrnes, L. E. Barnes, B. J. Bowman, W. E. Dulin, E. H. Morley, and R. O. Stafford, *Proc. Soc. Exptl. Biol. Med.*, **91**, 67 (1956).

(4) G. Rosenkranz, O. Mancera, and F. Sondheimer, *J. Am. Chem. Soc.*, **77**, 145 (1955).

(5) H. M. Kissman, A. S. Hoffman, and M. J. Weiss, *J. Org. Chem.*, **26**, 2610 (1961); J. C. Orr and A. Bowers, manuscript in preparation.

(6) H. M. Kissman, A. M. Small, and M. J. Weiss, *J. Am. Chem. Soc.*, **82**, 2312 (1960).

(7) H. M. Kissman, A. S. Hoffman, J. F. Poletto, and M. J. Weiss, *J. Med. Pharm. Chem.*, **5**, 950 (1962).

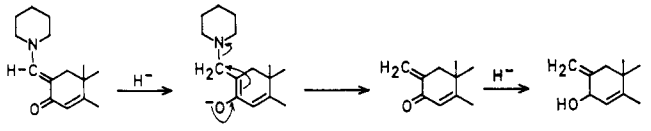
(8) J. A. Edwards, J. C. Orr, and A. Bowers, *J. Org. Chem.*, **27**, 3378 (1962).

(9) A. Bowers, A. D. Cross, J. A. Edwards, H. Carpio, M. C. Calzada, and E. Denot, *J. Med. Chem.*, **6**, 156 (1963), and subsequent papers.

(10) J. A. Edwards, M. C. Calzada, and A. Bowers, *ibid.*, **6**, 178 (1963).

(11) For an alternate route to 2-methylene cortical hormones which led to 2-methylenecortisone 21-acetate *cf.* T. R. Carrington, A. G. Long, and A. F. Turner, *J. Chem. Soc.*, 1572 (1962); see also U. S. Patent 2,847,430 (1958), and P. F. Beal, F. H. Lincoln, and J. A. Hogg, Abstracts, 132nd National Meeting of the American Chemical Society, New York, N. Y., Sept., 1957, p. 12-O.

diol (Ib) in moderate yield.[10] The sequence of reactions leading from Ia to the dienol Ib, which was initiated by attack of hydride on the 2-aminomethylene group, is outlined below. The bisallylic alcohol Ib



was oxidized smoothly by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[12] to 17β-hydroxy-17α-methyl-2-methyleneandrost-4-en-3-one (Ic). On the basis of these experiments it was anticipated that the analogous reduction of 17α,20;20,21-bismethylenedioxy-11β-hydroxy-2-N-piperidinomethylenepregn-4-en-3-one (IIf) would proceed by the same pathway to yield 17α,20;20,21-bismethylenedioxy-2-methylenepregn-4-ene-3ξ,11β-diol (IIIa). Conversion of the latter compound to 2-methylene-11β,17α,21-trihydroxypregn-4-ene-3,20-dione (2-methylenehydrocortisone, IIi) should then be possible.

The required 2-N-piperidinomethylene analog (IIf) of 17α,20;20,21-bismethylenedioxy-11β-hydroxypregn-4-en-3-one (hydrocortisone-BMD, IIb) was obtained from IIb in good yield *via* its corresponding 2-hydroxymethylene derivative IIe.

When the preparation of hydrocortisone-BMD (IIb) was attempted using the reported procedure[13] the desired product was isolated with difficulty and in poor yield. Beyler and co-workers had observed that during the same reaction the initially formed bismethylenedioxy compound was converted in part to a less polar product identified as the 11-methoxymethylene ether derivative IIc.[13b] The formation of this compound was shown to be dependent upon the presence of methanol in the formaldehyde.

It was found that the methyl ether IIc constituted over 30% of the crude reaction mixture and consequently a more reliable procedure was sought for the preparation of hydrocortisone-BMD (IIb).[14] This difficulty was conveniently circumvented by the use of paraformaldehyde as a source of alcohol-free formaldehyde. Thus treatment of a suspension of IIa in pure chloroform with a solution of formalin obtained from paraformaldehyde and hydrochloric acid produced hydrocortisone-BMD (IIb) in 80% yield.[15]

Condensation of the bismethylenedioxy compound IIb with ethyl formate in benzene solution in the presence of sodium methoxide[17] afforded 17α,20;20,21-bismethylenedioxy-11β-hydroxy-2-hydroxy methylenepregn-4-en-3-one (IIe).[8] Brief treatment of IIe with piperidine in benzene solution under reflux[10] furnished 17,20;20,21-bismethylenedioxy-11β-hydroxy-2-N-piperidinomethylenepregn-4-en-3-one (IIf) in nearly quantitative yield. The structures for compounds IIe and IIf followed from their method of preparation and analytical and spectral data.

When aminomethylene steroid IIf was reduced with sodium borohydride in boiling aqueous tetrahydrofuran solution, an amorphous product was obtained which exhibited only low-intensity absorption in the ultraviolet. Oxidation of the crude reduction product with DDQ[12] and purification by chromatography over alumina produced 17α,20;20,21-bismethylenedioxy-11β-hydroxy-2-methylenepregn-4-en-3-one (2-methylenehydrocortisone-BMD, IIg) in low yield. This dienone exhibited a maximum of 261 mμ in the ultraviolet and displayed bands at 1670 and 1620 cm.$^{-1}$ in the infrared in good agreement with spectra reported previously for steroids containing the 2-methylene-Δ^4-3-keto chromophore.[10,11] No attempt was made to isolate the precursor (IIIa) of 2-methylenehydrocortisone-BMD (IIg).

The principal part of the DDQ oxidation product was an amorphous fraction more polar than the dienone IIg. Further purification led to a second crystalline product, m.p. 235–238°, which afforded analytical data in accord with the formula $C_{24}H_{38}O_6$. This substance showed no selective absorption in the ultraviolet and its infrared spectrum was devoid of absorption due to carbonyl and *exo*-cyclic methylene functions. These data are consistent with the structure 17α,20;20,21-bismethylenedioxy-2α-methyl-5α-pregnane-3ξ,11β-diol (IIIb). The formation of this diol requires the reduction of the 2-*exo*-methylene and Δ^4-double bonds of the intermediate 2-methylene-Δ^4-3-ketone IIg by the borohydride reagent. However, analogous 1,4-reductions have been observed previously with α,β-unsaturated keto steroids.[18]

Brief exposure (1 min.)[19] of the 2-methylene-Δ^4-3-ketone (IIg) to boiling 60% formic acid[13] readily removed the bismethylenedioxy protecting groups and afforded a mixture of two substances separable by silica gel chromatography. Ultraviolet and infrared spectroscopy confirmed the presence of the 2-methylene-Δ^4-3-keto groups in both compounds. In addition, the infrared spectrum of the less polar compound exhibited ester absorption bands at 1750 and 1150 cm.$^{-1}$ in accord with its formulation as 2-methylenehydrocortisone 21-formate (IIh). The identification of the second product as 2-methylenehydrocortisone (IIi) follows from its method of preparation, elemental

(12) The selective oxidation of allylic alcohols with DDQ was first demonstrated by D. Burn, V. Petrow, and G. O. Weston, *Tetrahedron Letters*, **9**, 14 (1960).

(13) (a) R. E. Beyler, R. M. Moriarty, F. Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, **80**, 1517 (1958); (b) R. E. Beyler, F. Hoffman, R. M. Moriarty, and L. H. Sarett, *J. Org. Chem.*, **26**, 2421 (1961); (c) U. S. Patents 2,888,456 and 2,888,457 (1959).

(14) The conversion of the more accesible cortisone-BMD to the 11β-hydroxy analog by lithium aluminum hydride reduction and oxidation of the intermediate Δ^4-3,11-diol was reported to be the most efficient route to hydrocortisone-BMD; see ref. 13b.

(15) The crude product obtained after the formaldehyde treatment was a 1:1 mixture of hydrocortisone-BMD (IIb) and a more polar compound which was probably 11;17α,20;20,21-trismethylenedioxyhydrocortisone (IId).[16] The latter substance was converted to IIb in quantitative yield when the mixture was purified by crystallization.

(16) After the completion of the present investigation M. Akhtar, D. H. R. Barton, J. M. Beaton, and A. G. Hortman [*J. Am. Chem. Soc.*, **85**, 1512 (1963)] described the preparation of hydrocortisone-BMD (IIb) by the paraformaldehyde technique. These workers isolated both IIb (in *ca.* 45% yield) and 11;17α,20;20,21-trismethylenedioxyhydrocortisone (IId) (in *ca.* 15% yield) from this reaction.

(17) F. L. Weisenborn, D. C. Remy, and T. L. Jacobs, *ibid.*, **76**, 552 (1954); H. J. Ringold, E. Batres, O. Halpern, and E. Necoechea, *ibid.*, **81**, 427 (1959).

(18) For leading references see footnote 10.

(19) The expected migration of the *exo*-methylene double bond in IIg to the isomeric 2-methyl-$\Delta^{1,4}$-dien-3-one could be detected by prolonging the formic acid hydrolysis. Thus after heating IIg for 12 min., the crude product exhibited maximal absorption at 258 mμ, the hypsochromic shift of 3 mμ being due to the presence of 17α,20;20,21-bismethylenedioxy-11β-hydroxy-2-methylpregna-1,4-dien-3-one, see ref. 20.

(20) The 2-methyl-$\Delta^{1,4}$-3-keto chromophore is characterized by an absorption maximum at 247 mμ. See S. Bernstein, M. Heller, R. Littell, S. M. Stolar, R. H. Lenhard, W. S. Allen, and I. Ringler, *J. Am. Chem. Soc.*, **81**, 1696 (1959).

analysis, the above spectral data, and its chromatographic mobility.

Biological Activity.—Antiinflammatory (cotton pellet implant) and thymolytic assays were carried out with bilaterally adrenalectomized rats (23–26 days old) at three different dose levels. The steroid was administered daily by injection for six consecutive days and the animals were sacrificed on the seventh day. In these tests 2-methylenehydrocortisone had 1.0 and 0.9 times, respectively, the antiinflammatory and thymolytic activities of the standard hydrocortisone.[21]

Experimental[22]

17α,20;20,21-Bismethylenedioxy-11β-hydroxypregn-4-en-3-one (Hydrocortisone-BMD) (IIb).—A mixture of paraformaldehyde (200 g.), water (600 ml.), and concentrated hydrochloric acid (600 ml.) was stirred for 4 hr. and then a suspension of 20 g. of hydrocortisone (IIa) in 1 l. of alcohol-free chloroform was added. Stirring was continued for 3 hr., after which time the aqueous phase was separated. The chloroform layer was washed with a dilute solution of sodium carbonate and water, dried (Na_2SO_4), and evaporated. Crystallization of the resulting solid from acetone–hexane provided 18.1 g. of hydrocortisone-BMD, m.p. 222–225°, identical in all respects with an authentic sample.[13]

17α,20;20,21-Bismethylenedioxy-11β-hydroxy-2-hydroxymethylenepregn-4-en-3-one (IIe).—From a solution of 18 g. of hydrocortisone-BMD (IIb) in 600 ml. of thiophene-free benzene, 50 ml. of solvent was distilled, and after cooling, 18 ml. of ethyl formate and 11 g. of sodium methoxide were added. The reaction mixture was stirred for 20 hr. in a nitrogen atmosphere after which time the precipitated sodium salt was collected and dried. The benzene filtrate was washed with two 300-ml. portions of water which were combined with an aqueous solution (1 l.) of the sodium salt. Addition of an excess of hydrochloric acid precipitated 19 g. of 2-hydroxymethylene compound IIe, m.p. 238–248°. A pure sample of IIe was prepared by acetone–hexane crystallization: m.p. 257–259°; $[\alpha]_D$ −44°; λ_{max} 255 and 308 mμ (log ε 4.03 and 3.74); ν_{max} 1640 and 1590 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{32}O_7$: C, 66.65; H, 7.46; O, 25.90. Found: C, 66.33; H, 7.79; O, 25.55.

17α,20:20,21-Bismethylenedioxy-11β-hydroxy-2-N-piperidinomethylenepregn-4-en-3-one (IIf).—A solution of 5 g. of the 2-hydroxymethylene compound IIe in 5 ml. of piperidine and 150 ml. of benzene was heated under reflux for 15 min. and then concentrated to a small volume under reduced pressure. The solid residue was washed with hexane to yield 5.8 g. of yellow crystals, m.p. 237–240°. The analytical sample, prepared by crystallization from benzene–hexane, melted at 238–240°; $[\alpha]_D$ −231°; λ_{max} 250 and 373 mμ, (log ε 4.23 and 4.17); ν_{max} 1640 and 1540 cm.$^{-1}$.

Anal. Calcd. for $C_{29}H_{41}NO_6$: C, 69.71; H, 8.27; N, 2.80; O, 19.21. Found: C, 70.24; H, 8.28; N, 2.64; O, 19.26.

17α,20;20,21-Bismethylenedioxy-11β-hydroxy-2-methylenepregn-4-en-3-one (2-Methylenehydrocortisone-BMD) (IIg) and 17α,20;20,21-Bismethylenedioxy-2α-methyl-5α-pregnane-3ξ,11β-diol (IIIb).—17α,20;20,21-Bismethylenedioxy-11β-hydroxy-2-N-piperidinomethylenepregn-4-en-3-one (IIf) (4 g.) was dissolved in 180 ml. of a mixture of tetrahydrofuran and water (9:1) and treated with 4 g. of sodium borohydride. This solution was heated under reflux for 7 hr. and then left standing overnight at room temperature. Water was added and the product isolated with methylene chloride. The resulting oil (4.2 g.) was dissolved in 280 ml. of dioxane and oxidized with 3.2 g. of DDQ. After 6 hr., methylene chloride (2 l.) was added and the resulting solution was filtered through a column of 250 g. of alumina. The oil (2.1 g.) eluted from the column was dissolved in 100 ml. of hexane–benzene (3:2) and adsorbed on a column of 84 g. of alumina. Elution with mixtures of benzene–hexane (4:1), benzene, and benzene–ether (9:1) provided 0.73 g. of 2-methylenehydrocortisone-BMD (IIg). After several recrystallizations from acetone–hexane, a sample of dienone IIg melted at 220–222°; $[\alpha]_D$ +53°; λ_{max} 261 mμ (log ε 4.12); ν_{max} 1670 and 1625 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.74. Found: C, 69.09; H, 7.72.

From the amorphous fractions (1.2 g.) eluted with mixtures of benzene–ether and pure ether, there was obtained 0.5 g. of crystals, m.p. 172–175° after rechromatography on alumina and crystallization from acetone–hexane. Four additional crystallizations (acetone–hexane) afforded a pure sample of the diol IIIb, m.p. 235–238°.

Anal. Calcd. for $C_{24}H_{38}O_6$: C, 68.22; H, 9.07; O, 22.72. Found: C, 68.77; H, 8.55; O, 23.06.

Formic Acid Hydrolysis of 17α,20;20,21-bismethylenedioxy-11β-hydroxy-2-methylenepregn-4-en-3-one (IIg).—A solution of 2-methylenehydrocortisone-BMD (IIg) (0.48 g.) in 40 ml. of formic acid was boiled for 1 min. and, after cooling, diluted with water and the product extracted with methylene chloride. This extract was washed with dilute sodium bicarbonate solution and water, dried (Na_2SO_4), and evaporated. The crystalline solid was added to a similarly processed methylene chloride extract obtained from the formic acid hydrolysis of an additional 0.37 g. of the dienone IIg and the resulting solution was adsorbed on a column of 33 g. of silica gel. Elution with 200 ml. of methylene chloride–acetone (6:1) provided 0.15 g. of 2-methylenehydrocortisone 21-formate (IIh) obtained analytically pure after two crystallizations from acetone–hexane, m.p. 233–235°; $[\alpha]_D$ +189° (dioxane); λ_{max} 261 mμ (log ε 4.16); ν_{max} 1750, 1730, 1660, and 1630 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.75; H, 7.19.

Continued elution with 200-ml. portions of methylene chloride–acetone (4:1 and 3:1) afforded 0.45 g. of 2-methylenehydrocortisone (IIi) admixed with traces of the formate IIh. A pure sample of IIi obtained after several crystallizations from acetone–hexane melted at 241–244°; $[\alpha]_D$ +165° (dioxane); λ_{max} 261 mμ (log ε 4.16); ν_{max} 1710, 1650, and 1615 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.29; H, 8.05.

(21) We are indebted to Dr. Ralph I. Dorfman of the Worcester Foundation for Experimental Biology for these assays.

(22) All melting points are uncorrected; they were determined in 1962; all rotations are for chloroform solutions at 16–22°, ultraviolet spectra for ethanol solutions, and infrared spectra for KBr disks, except where stated otherwise. Microanalyses are by Midwest Micro Laboratories, Indianapolis 20, Ind., or by Dr. A. Bernhardt, Mulheim (Ruhr), Germany.