[CONTRIBUTION FROM THE MERCK, SHARP AND DOHME RESEARCH LABORATORIES, DIVISION OF MERCK AND CO., INC.]

17,20;20,21-Bismethylenedioxy Steroids. V. A General Method for Protecting the Dihydroxyacetone Side Chain[1]

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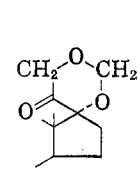
The synthesis of 17,20;20,21-bismethylenedioxy pregnanes (BMD's) from 17α,21-dihydroxy-20-ketopregnanes is reported. This general method for protecting a side chain is discussed, the properties of the bismethylenedioxy grouping are outlined and conditions for formation and reversal are described.

The synthesis of modified adrenocortical steroids has been a subject of major emphasis among steroid chemists during the past several years. These modifications have often involved lengthy syntheses since the activity-enhancing groups had to be inserted at an early stage in a bile acid or sapogenin precursor. Frequently the dihydroxyacetone side chain is not sufficiently stable or inert to the reaction conditions needed to modify the steroid nucleus.

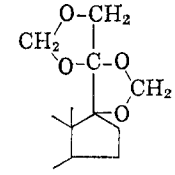
With this fact in mind, several years ago we investigated methods for protecting the sensitive dihydroxyacetone side chain. The initial paper in this series[3] briefly reported the synthesis of five 17,20;20,21-bismethylenedioxy steroids which represented the accomplishment of this objective. The present communication will enlarge upon the details of that work and also present some new information on the bismethylenedioxy (BMD) group.

In the interim period a number of communications from our laboratory[1,4] and from our colleagues at Merck[5] have appeared in which the bismethylenedioxy protecting group was used to synthesize A-, B-, and C-ring modified adrenocortical steroids.

Because of the precedent in the hexose series[6] for preferential formation of 1,3-acetals with formaldehyde, it was hoped that reaction of the dihydroxyacetone side chain with formaldehyde would yield a 17α,21-methylenedioxy compound (I). It was then expected that further protection of the C_{20}-carbonyl group would be required to render the side chain completely inert. Instead, formaldehyde gave a spiroketal system (II) which we have termed the bismethylenedioxy function. This grouping has proven to be remarkably stable to many of the reagents which were later incorporated into the various syntheses that have been reported.[1,4,5]

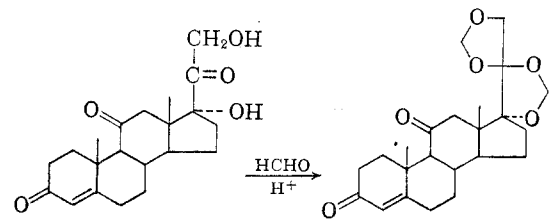


I



II

The first successful experiment in this investigation was conducted with cortisone (III) and employed a two-phase system of chloroform-formalin and concentrated hydrochloric acid. After the reaction mixture had been stirred at room temperature for fifty-two hours, the layers were separated and the organic phase washed with mild base and concentrated. From the crude residue, which contained considerable formaldehyde polymer, a crystalline compound was obtained directly in good yield. Its infrared spectrum did not preclude the presence of a 20-carbonyl group, such as in I, as a band at 5.85 μ could be assigned to the nonresolved 11- and 20-ketones. The ultraviolet spectrum showed that the 3-keto-Δ^4-chromophore was untouched. At this stage the predicted partial structure (I) was an acceptable one. However, elemental analysis of the product conformed more closely to that for introduction of two formaldehyde units than one. When a quanti-



III IV

(1) Paper IV in this series, R. E. Beyler, Frances Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, 82, 178 (1960).

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(3) R. E. Beyler, R. M. Moriarty, Frances Hoffman, and and L. H. Sarett, *J. Am. Chem. Soc.*, 80, 1517 (1958).

(4) (a) Frances Hoffman, R. E. Beyler, and M. Tishler, *J. Am. Chem. Soc.*, 80, 5322 (1958); (b) R. E. Beyler, A. E. Oberster, Frances Hoffman, and L. H. Sarett, *J. Am. Chem. Soc.*, 82, 170 (1960).

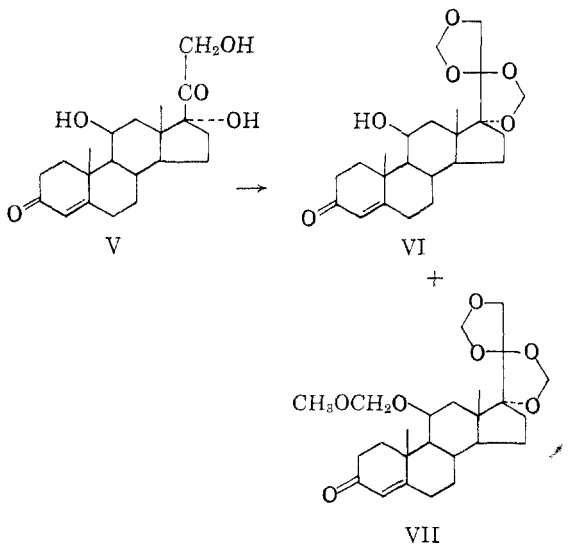
(5) (a) J. H. Fried, G. E. Arth, and L. H. Sarett, *J. Am. Chem. Soc.*, 81, 1235 (1959); (b) N. G. Steinberg, R. Hirschmann, and J. M. Chemerda, *Chem. and Ind.*, 975 (1958); (c) J. H. Fried, G. E. Arth, and L. H. Sarett, *J. Am. Chem. Soc.*, 82, 1684 (1960); (d) R. Hirschmann, G. A. Bailey, R. Walker, and J. M. Chemerda, *J. Am. Chem. Soc.*, 81, 2822 (1959).

(6) S. A. Barker and E. J. Bourne, *Adv. Carbohydrate Chem.*, 7, 177 (1952); S. J. Agyal and G. G. Macdonald, *J. Chem. Soc.*, 686 (1952); J. A. Mills, *Adv. Carbohydrate Chem.*, 10, 1 (1955).

tative methylenedioxy determination was done, using chromotropic acid to measure the formaldehyde generated by acid hydrolysis, the presence of two such groups was confirmed. Thus, the structure IV seemed more probable. Further structure confirmation was obtained by reversal of IV to III (see below). Replacement or cortisone by hydrocortisone in the reaction gave a product without a saturated carbonyl, which proved unequivocally that the 20-ketone was involved in the reaction. Structure IV was then secure. Although stereoisomers at C_{20} in IV are theoretically possible, only one isomer has been isolated in this and all other examples.

The extension of the above findings to other steroids and other aldehydes was next investigated. With respect to the latter it was found that aldehydes other than formaldehyde (butyraldehyde and aryl aldehydes for example) do indeed react to give bisalkylidenedioxy substituents. However, as these products were noncrystalline, formaldehyde remains as the reagent of choice.

When hydrocortisone (V) (or prednisolone) was allowed to react with formalin and acid, an unpredicted side reaction took place. The 11β-hydroxyl function reacted, in part, to form a ketal. The methanol present in commercial formalin and another molecule of formaldehyde gave an 11β-methoxymethyleneoxy substituted bismethylenedioxy compound (VII) as well as the desired bismethylenedioxy hydrocortisone (VI). The structure of VII was assigned on the basis of quantitative methylenedioxy analysis, lack of a hydroxyl band in the infrared spectrum, and acid hydrolysis of VII to hydrocortisone (V).



A mixture of bismethylenedioxy hydrocortisone and the 11-ketal would be useful in syntheses involving A-ring modifications but it would obviously be desirable to form only VI if a multistep synthesis were to be anticipated. Efforts to accomplish this objective were partially successful.

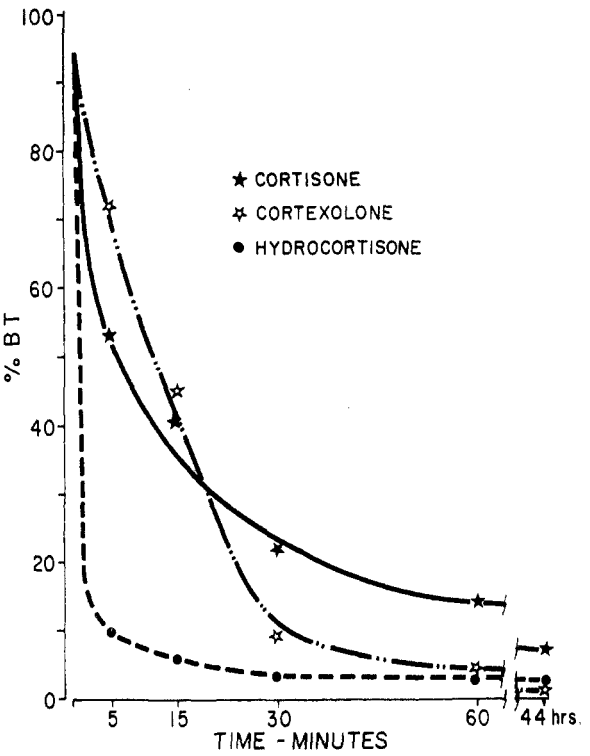


Fig. 1. BMD formation

Because the rate of bismethylenedioxy formation in the 11-hydroxy series is appreciably faster than in the 11-keto series, shorter reaction times were studied. One can avoid formation of most of the 11-ketal (particularly in bismethylenedioxy prednisolone) by shortening the reaction time to an hour or less. However, a more effective method for hydrocortisone involved the use of low-methanol formalin (0.5% methanol) and alcohol-free chloroform or methylene chloride. In this way we were able to prepare bismethylenedioxy hydrocortisone in 50% yield, but the yield was not consistently reproducible. The best way to prepare pure VI is by dioxolanation of bismethylenedioxy cortisone, reduction at C_{11} with lithium aluminum hydride and dioxolane removal.

It is of interest that 11-ketals were not isolated when 9α-fluoro-11β-hydroxy compounds were subjected to bismethylenedioxy-forming conditions. If this reaction does occur, it is only to a minor extent.

Early in our studies it became apparent that the rate of bismethylenedioxy formation was markedly influenced by the substituent at the 11-position. Cortisone was much slower to form a bismethylenedioxy compound, as judged by the disappearance of the blue tetrazoleum (BT) test, than hydrocortisone. As a result of this observation quantitative blue tetrazoleum data on rates of bismethylenedioxy formation were obtained and these are presented in Fig. 1. It can be seen that 50% of the side chain of hydrocortisone is transformed in about one minute whereas cortisone requires about five

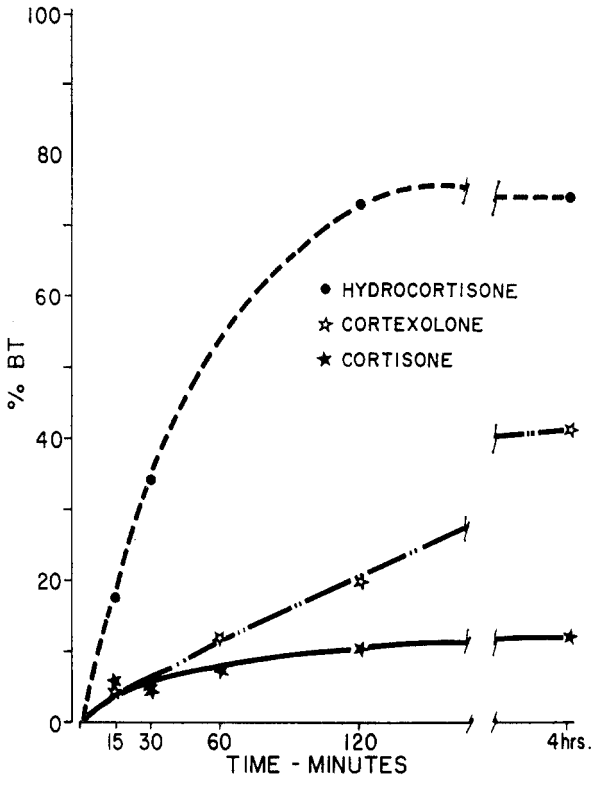


Fig. 2. BMD hydrolysis

minutes and cortexolone (Reichstein's S, no oxygen at C_{11}) about ten minutes. In addition, the final conversion yields differ with the three steroids (*ca.* 1% unchanged cortexolone, *ca.* 2.5% hydrocortisone and *ca.* 6% cortisone after forty-four hours). We do not have a satisfactory explanation for these differences in rate and equilibria. Further, it is noteworthy that in the rate studies, cortexolone was entirely in solution at the beginning of the reaction while hydrocortisone dissolved completely after about five minutes and cortisone did not completely dissolve for about fifteen minutes. The yields of product do not necessarily parallel the blue tetrazoleum data, as one hour is not optimum for preparation of bismethylenedioxy cortisone. Rather is this optimum time more nearly twenty to forty hours.

The hydrolysis of the bismethylenedioxy group to the dihydroxyacetone side chain was first studied with bismethylenedioxy cortisone. Later it was found that the 11-keto compounds were more slowly hydrolyzed than the 11-hydroxy steroids or compounds not substituted at C_{11}. The equilibrium in acid medium also favors the spiroketal in the 11-keto compounds more than in the other two groups of compounds.

The stability to acid hydrolysis is illustrated by the following findings. One part of 10*N* sulfuric acid and nine parts of methanol at reflux for eleven hours gave a product with a weak blue tetrazoleum test. Similarly use of hydriodic acid in methanol or in a two-phase system gave discouraging results (*ca.* 10% "blue tetrazoleum yields") as did also the addition of a "formaldehyde scavenger" (dimedone or chromotropic acid). The use of organic acids (acetic or formic) with or without added mineral acids provided more satisfactory yields. In the case of bismethylenedioxy prednisolone one can obtain 70–75% yield when 60% formic acid is used at 100° for ten minutes.

The rates of bismethylenedioxy-hydrolysis were significantly different for different substituents at C_{11} (Fig. 2). Bismethylenedioxy cortisone was the most sluggish and hydrocortisone the fastest to hydrolyze. Using 50% acetic acid at 100°, the former leveled off at about 10% "blue tetrazoleum yield" after two hours whereas the latter had reached nearly 75% "blue tetrazoleum yield" in this time. Prolonged treatment under these conditions caused the "blue tetrazoleum yield" to diminish, undoubtedly because of side chain destruction under the acid conditions. Again we do not have an entirely satisfactory explanation for the influence of the C_{11}-substituent on the rate of acid hydrolysis. It has been found[7] that variation at even more remote positions of the steroid—as, for example, in the A-ring—can have a marked effect on the rate of bismethylenedioxy-hydrolysis.

This acid stability has been useful in a number of synthetic applications. For instance, a 9,11-oxide was cleaved with hydrochloric acid without damage to the protecting group.[1] Brominations have been carried out on bismethylenedioxy steroids. Dioxolane formations and reversals have been accomplished in the presence of this protecting group.[4,5] Even short-term exposure to catalytic amounts of boron trifluoride etherate has been used.[8] In addition we have prepared 3-enamines of the bismethylenedioxy steroids.

The physical properties of this class of steroids are of interest. In general, they are high melting crystalline solids which can be crystallized from polar or nonpolar solvents. Methanol and ether are generally very satisfactory. In both partition and adsorption chromatography they are markedly less polar than the parent steroid. They always exhibit strong C—O—C absorption at 9.0–9.4 μ in the infrared. The molecular rotation of a large number of bismethylenedioxy compounds shows a levorotatory shift of 400 to 600° from the corresponding 17,21-dihydroxy-20-ketone.

In addition to the bismethylenedioxy compounds reported in our initial communication we have made a number of others, some of which are reported in the experimental section. These include saturated 3-ketones, 4,6-dienones, and various alkylated bismethylenedioxy compounds. It is significant that 16α- and 16β-methyl corticoids

(7) Dr. Ralph Hirschmann, private communication.
(8) R. E. Beyler, Frances Hoffman, L. N. Sarett, and M. Tishler, *J. Org. Chem.*, **26**, 2426 (1961).

also form bismethylenedioxy derivatives satisfactorily. For instance, bismethylenedioxy dexamethasone was prepared in 50% yield in the usual way.

The biological activity of the bismethylenedioxy steroids indicates that the animal is able, in part at least, to remove the bismethylenedioxy group. Diminished but significant activity was found in the biologically active steroid bismethylenedioxy compounds tested.[9] The approximate liver glycogen (glyc.) and granuloma (gran.) activities in terms of hydrocortisone for some of these are as follows: Bismethylenedioxycortisone (glyc. 0.3, gran. 0); bismethylenedioxy hydrocortisone (glyc. 0.25, gran. 0); bismethylenedioxy prednisone (glyc. 1.0, gran. *ca.* 1.9); bismethylenedioxy prednisolone (glyc. 1.0, gran. *ca.* 1.8); bismethylenedioxy-9α-fluorohydrocortisone (glyc. 1.25, gran. 1.8).

EXPERIMENTAL[10]

17α,20;20,21-Bismethylenedioxy-4-pregnene-11β-ol-3-one (hydrocortisone-BMD) (VI). Method A. Two grams of hydrocortisone (V) were dissolved in 100 ml. of chloroform which had been shaken with sulfuric acid, dried over calcium chloride, and distilled. To this mixture was added with cooling 60 ml. of concd. hydrochloric acid and 60 ml. aqueous 37% formaldehyde (containing 0.5% methanol). The reaction mixture was stirred for 7 hr. at room temperature. The chloroform was separated and the aqueous phase extracted with more chloroform. The combined extract was washed with aqueous bicarbonate, dried, and concentrated. From the residue by methanol trituration and washing there was obtained 1.0 g. of bismethylenedioxyhydrocortisone (VI), m.p. 200–220°. Recrystallization from ether and methanol containing a trace of methylene chloride gave the analytical sample, m.p. 220–223°.

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.01; H, 7.97, $[\alpha]_D^{24}$ + 26°. λ_{max} 241.5 mμ, ϵ 15,600. λ_{max}^{Nujol} 2.88, 5.95, 6.10, 9.0 μ.

Method B. 17α,20;20,21-Bismethylenedioxy-3-ethylenedioxy-5-pregnene-11β-ol. To 2.16 g. of bismethylenedioxycortisone-3-dioxolane[1,4b,5a] in 20 ml. of tetrahydrofuran was added 200 mg. of lithium aluminum hydride. The mixture was stirred overnight at room temperature and then refluxed for 1 hr. A small amount of water was carefully added to decompose the excess lithium aluminum hydride and the material filtered through Supercel. Concentration of the filtrate gave 2.06 g. of crystalline residue. Recrystallization from methanol gave 1.50 g. of bismethylenedioxy hydrocortisone-3-dioxolane, m.p. 162–165°, second crop of 204 mg., m.p. 150–160°. One more recrystallization of the first crop from methanol furnished an analytical sample, m.p. 167–169°.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found: C, 67.41, 66.62; H, 8.30, 7.84. λ_{max}^{Nujol} 2.83, 8.9–9.3 μ.

To 200 mg. of the above bismethylenedioxy dioxolane in 4.0 ml. of acetone was added 20 mg. of *p*-toluenesulfonic acid. The mixture was allowed to stand at room temperature for 15 hr. It was poured into saturated aqueous sodium bicarbonate and the acetone distilled off under reduced pressure. It was then extracted with three portions of methylene chloride, dried and concentrated to give 176 mg. of crystalline residue. Recrystallization from methanol yielded 156 mg. in two crops of bismethylenedioxy hydrocortisone, m.p. 222–227°. A mixed m.p. and infrared spectrum proved this compound to be identical with the sample prepared by Method A.

17α,20;20,21-Bismethylenedioxy-4-pregnene-11β-ol-3-one (VI) and 17,20;20,21-bismethylenedioxy-4-pregnene-11β-ol-3-one-11-methoxymethyl ether (VII). Thirty grams of hydrocortisone was combined with 1500 ml. of chloroform and to this solution was added a cooled mixture of 600 ml. of concd. hydrochloric acid and 600 ml. of formalin. The reaction mixture was stirred at room temperature for 1 hr. The two layers were separated, the aqueous layer extracted with chloroform, and the solution combined. The chloroform extract was washed with sodium carbonate, dried, and concentrated *in vacuo*. The entire residue (30.8 g.) was chromatographed on 900 g. of acid washed alumina. Elution of the column with 1:4 petroleum ether (b.p. 40–60°)-ether yielded 7 g. of crude crystalline 17α,20;20,21-bismethylenedioxy - 4 - pregnene - 11β - ol - 3 - one - 11 - methoxymethyl ether (VII). Recrystallization from ether gave 4 g. of analytically pure VII, m.p. 160–165°.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09; CH_2O, 20,0; CH_3O, 6.9; mol. wt., 448.54. Found: C, 67.31; H, 8.11; CH_2O, 20.1; CH_3O, 8.8; mol. wt. (Rast), 475 ± 45. $\lambda_{max}^{CH_3OH}$ 241 mμ, ϵ 15,900. λ_{max}^{Nujol} 6.0, 6.15, 9.0–9.4 μ.

From the ether and ether-chloroform (2:3) effluents there was obtained 10.5 g. of bismethylenedioxy hydrocortisone (VI), m.p. 217–222°.

Acid hydrolysis of a sample of VII, using 50% acetic acid at 100° for 8 hr., acetylation and chromatography yielded *ca.* 25% of hydrocortisone acetate.

17α,20;20,21-Bismethylenedioxy-1,4-pregnadiene-3,11-dione (prednisone-BMD). To a suspension of 500 mg. of prednisone in 25 ml. of chloroform was added a mixture of 10 ml. of formalin and 10 ml. of concd. hydrochloric acid. The two-phase system was stirred at room temperature for 70 hr. The two layers were separated, the aqueous layer extracted with chloroform, and the chloroform extracts combined with the original organic solvent layer. The chloroform was washed with a saturated solution of sodium bicarbonate, dried, and concentrated under reduced pressure to a semicrystalline solid weighing 712 mg. This crude product was triturated with boiling methanol giving 352 mg. of crystalline bismethylenedioxy prednisone m.p. 175–195°. After recrystallization from acetone and methanol, a pure sample of 17α,20;20,21-bismethylenedioxy-1,4-pregnadiene-3,11-dione, m.p. 214–217°, was obtained.

Anal. Calcd. for $C_{23}H_{28}O_6$: C, 68.98; H, 7.05. Found: C, 68.60; H, 7.11. λ_{max} 238 mμ, ϵ 15,300. λ_{max}^{Nujol} 5.87, 6.0, 6.15, 6.2 μ.

17α,20;20,21-Bismethylenedioxy-1,4-pregnadiene-11β-ol-3-one (prodnisolone-BMD) and 17α,20;20,21-bismethylenedioxy - 1,4 - pregnadiene - 11β - ol - 3 - one-11-methoxymethyl ether. Twenty-five grams of prednisolone was suspended in 1250 ml. of chloroform. To this was added a precooled (*ca.* 10°) mixture of 500 ml. of formalin and 500 ml. of concd. hydrochloric acid. The two-phase system was stirred vigorously at room temperature for 20 min., the prednisolone dissolving completely after about 1 min. The layers were separated and the aqueous phase extracted with 500 ml. of chloroform. The combined chloroform extract was washed with water and saturated aqueous sodium bicarbonate, dried, and distilled. The residue was dissolved in 250 ml. of methanol and concentrated to dryness twice to remove most of the formaldehyde polymer. The resultant crystalline product was recrystallized from methanol to give 20.8 g. of crude product, m.p. 230–260°. Recrystallization from methanol–methylene chloride yielded 15.0 g. of bismethylprednisolone m.p. 267–271°. An analytical sample was prepared by recrystallization from ethyl acetate, m.p. 270–274°.

(9) We are indebted to Dr. R. H. Silber of the Merck Institute for Therapeutic Research for the biological test results.

(10) All melting points were determined on a Kofler micro-hot stage, ultraviolet absorption spectra were taken in methanol and rotations were taken in chloroform at approximately 1% concentration unless otherwise specified.

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.57. Found: C, 68.37; H, 7.70. λ_{max} 242 mμ, ϵ 14,600. λ_{max}^{Nujol} 2.90, 6.05, 6.15, 6.2, 9.15 μ $[\alpha]_D$ − 20°.

Concentration of the mother liquors from above gave 4.0 g. of bismethylenedioxy prednisolone-11-methoxymethyl ether, m.p. 210–220°. Recrystallization from acetone gave the analytical sample, m.p. 217–220°.

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found: C, 66.85; H, 7.46. λ_{max}^{KBr} 6.00, 6.14, 6.2, 9.2 μ.

9α-Fluoro-17α,20;20,21-bismethylenedioxy-4-pregnen-11β-ol-3-one(9α-Fluorohydrocortisone-BMD). This preparation has been described previously.[1]

17α,20;20,21-Bismethylenedioxy-4-pregnene-3-one (cortexolone-BMD). To 500 mg. of cortexolone (4-pregnene-17α,21-diol-3,20-dione) in 25 ml. of chloroform was added a mixture of 10 ml. of formalin and 10 ml. of concd. hydrochloric acid. The two-phase system was stirred at room temperature for 44 hr. (Quantitative blue tetrazoleum measurements subsequently indicated the reaction was essentially complete in 1–2 hr.) The two phases were separated and the aqueous phase extracted with two portions of chloroform. The combined chloroform was washed with aqueous sodium bicarbonate and water, dried, and distilled. The residue, 464 mg., was washed with petroleum ether to give 327 mg. of 17α,20;20,21-bismethylenedioxy-4-pregnene-3-one, m.p. 220–245°. A sample was recrystallized from methanol and methylene chloride–ether, m.p. 250–255°.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.76; H, 8.29. λ_{max} 242 mμ, ϵ 16,500. $\lambda_{max}^{CHCl_3}$ 6.0, 6.15, 9.05–9.15 μ.

16α-Methyl-9α-fluoro-17α,20;20,21-bismethylenedioxy-1,4-pregnadiene-11β-ol-3-one (dexamethasone-BMD). Five hundred milligrams of dexamethasone in 25 ml. of chloroform was stirred with 10 ml. of 37% aqueous formaldehyde and 10 ml. of concd. hydrochloric acid for 1 hr. at room temperature. An additional 25 ml. of chloroform was added and the layers separated. The chloroform layer was washed with a saturated solution of sodium bicarbonate, dried and evaporated to dryness *in vacuo*. Twenty-five milliliters of methanol was added to the solid residue and it was again evaporated to dryness. The total residue was recrystallized from methylene chloride–methanol to yield 255 mg. of analytically pure 16α - methyl - 9α - fluoro - 17α,20;20,21 - bismethylenedioxy-1,4-pregnadiene-11β-ol-3-one, m.p. 310–20°.

Anal. Calcd. for $C_{24}H_{31}O_6F$: C, 66.33; H, 7.19. Found: C, 66.43; H, 7.06. λ_{max}^{Nujol} 2.80, 6.0, 6.2, 9.2 μ, λ_{max} 238 mμ, ϵ 14,900.

17α,20;20,21-Bismethylenedioxy-3-pyrrolidyl-3,5-pregnadiene-11-one. Five hundred milligrams of bismethylenedioxy cortisone was dissolved in 10 ml. of ethanol containing a little methylene chloride. This was concentrated to *ca.* 5 ml. to remove methylene chloride. To this was added 0.5 ml. of pyrrolidine and the mixture heated for 1 min. on the steam bath. The resultant precipitate was cooled and filtered, washing with ethanol, to give 540 mg. of light yellow prisms, m.p. 205–212° dec. The compound was analyzed without further purification.

Anal. Calcd. for $C_{27}H_{37}O_5N$: C, 71.18; H, 8.19; N, 3.07. Found: C, 70.57; H, 8.21; N, 3.14. λ_{max} 271 mμ, ϵ 17,000. λ_{max}^{Nujol} 5.90, 6.10, 6.19, 9.0–9.5 μ.

17α,20;20,21-Bisbutyraldioxy-4-pregnene-11β-ol-3-one. To 500 mg. of hydrocortisone, dissolved in 25 ml. of methylene chloride, was added 10 ml. of 40% aqueous butyraldehyde and 10 ml. of concd. hydrochloric acid. The reaction mixture was stirred at room temperature for 6 hr. The solvent layers were separated and the aqueous layer extracted with fresh methylene chloride. The combined methylene chloride extract was washed with water, dried over magnesium sulfate, and concentrated. The residual oil, containing butyraldehyde, was chromatographed on 12 g. of acid washed alumina. From the 4:1 petroleum ether–ether to ether eluates there was obtained 263 mg. of 17α,20;20,21-bisbutyraldioxy-4-pregnene-11β-ol-3-one as a clear glass. λ_{max}^{Nujol} 2.9, 6.0, 6.15, 8.6–9.0 μ. $\lambda_{max}^{CH_3OH}$ 241 mμ, E% 296. Quantitative blue tetrazoleum, 7.3% of hydrocortisone.

The above 263 mg. of product was heated under nitrogen with 50 ml. of 50% acetic acid on the steam bath for 8 hr. The acetic acid was removed by vacuum distillation and the residue purified by extraction into methylene chloride, removal of the organic solvent, and acetylation by heating 10 min. with pyridine–acetic anhydride. The acetylated material (125 mg.) was chromatographed on 5 g. of acid washed alumina. Hydrocortisone acetate was obtained in the 1:4 ether-chloroform eluates, m.p. and mixed m.p. with authentic material 210–217°. The infrared spectrum of this material was essentially identical with that of hydrocortisone acetate.

Bismethylenedioxy group formation time study. Cortisone, hydrocortisone and cortexolone were all treated in exactly the same way as follows: 500 mg. of steroid was dissolved (or suspended) in 25 ml. of chloroform. To this was added a mixture of 10 ml. of formalin and 10 ml. of concd. hydrochloric acid and the mixture immediately stirred at room temperature. This was taken to be "zero time." At time intervals about 1-ml. aliquots of the chloroform layer were withdrawn and washed with saturated aqueous sodium bicarbonate solutions. The chloroform layers were taken to dryness and the residues submitted for ultraviolet and BT analysis.[11] All quantitative blue tetrazoleum results were calculated with reference to the steroid used in the reaction as cortisone, hydrocortisone, and cortexolone give different intensities of absorption at 510 mμ. Because the residues have varying amounts of formaldehyde polymer in them the ultraviolet absorption intensities were used to correct the blue tetrazoleum results with the assumption that the 3-keto-Δ^4-chromophore was not affected by the reaction conditions. These corrected blue tetrazoleum results are plotted in Fig. 1.

Bismethylenedioxy group hydrolyses time study. One hundred twenty milligrams of steroid (cortisone, hydrocortisone, and cortexolone) were heated in 10 ml. of 50% acetic acid at 100°. Aliquots were removed and concentrated to dryness periodically for ultraviolet and blue tetrazoleum assay. There was only about 5% loss of ultraviolet up to 4 hr. but after that the maximum at *ca.* 240 mμ diminished in intensity. Both bismethylenedioxy cortisone and bismethyldioxy hydrocortisone were homogeneous in a minute or less whereas bismethylenedioxy cortexolone required about 4 hr. to dissolve completely. The results of these time studies are presented in Fig. 2.

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