tem 2:3:3:2; petroleum ether (b.p. 90–100°)–ethyl acetate–methanol–water. Hold-back volume two was evaporated to give 330 mg. of semisolid which on treatment with acetone–petroleum ether afforded 220 mg. of Vc; m.p. 228–236°. Three crystallizations from acetone–petroleum ether gave 55 mg.; m.p. 233.5–236°; λ_{max} 238 mμ (ε 13,000); ν_{max} 3490, 3320, 1755, 1733, 1713, 1660, 1620, 1608, and 1245 cm.$^{-1}$; $[\alpha]_D^{25}$ +76.5° (acetone).

Anal. Calcd. for $C_{25}H_{31}O_8F$ (478.49): C, 62.75; H, 6.53; F, 3.97. Found: C, 63.13; H, 6.53; F, 3.62.

9β,11β-Epoxy-16α,17α,21-trihydroxypregn-4-ene-3,20-dione (VII). To a solution of 200 mg. of VI in 20 ml. of methanol, cooled to 0° and flushed with nitrogen, was added a solution of 80 mg. of potassium hydroxide in 5 ml. of methanol. After standing for 1 hr. at room temperature, the solution was neutralized with acetic acid and evaporated *in vacuo* at 35–40°. The residue was dissolved in ethyl acetate, and the solution was washed to neutral with water, dried and evaporated to yield 150 mg. of oil. Trituration with acetone–petroleum ether gave 50 mg.; m.p. 178–200°. Two crystallizations from acetone-petroleum ether raised the m.p. to 207.5–210.5°. A mixed melting point determination with an authentic sample of VII gave no depression. Its infrared spectrum was identical to that of the authentic sample.

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione (VIIIa). To a solution of 160 mg. of Vb in 20 ml. of methanol, cooled to 0°, and flushed with nitrogen, was added a solution of 56 mg. of potassium hydroxide in 5 ml. of methanol. After standing for 1 hr. at room temperature, the solution was neutralized with acetic acid and evaporated *in vacuo* at 35–40°. The crystalline residue was slurried in water, filtered, and washed well with water to yield 50 mg.; m.p. 203–236°. Its infrared spectrum was identical to that of an authentic sample of VIIIa.

The diacetate VIIIb prepared in the usual manner from VIIIa also had an infrared spectrum identical to that of an authentic sample.

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregn-1,4-diene-3,20-dione (VIIIc). To a solution of 240 mg. of Vc in 25 ml. of methanol, cooled to 0°, and flushed with nitrogen, was added a solution of 100 mg. of potassium hydroxide in 5 ml. of methanol. After standing for 1 hr. at room temperature, the solution was neutralized with acetic acid and evaporated *in vacuo* at 35–40°. The residue was treated with acetone–petroleum ether to give a poor-looking solid which was set aside. The mother liquor was evaporated to dryness and submitted to partition chromatography on a Celite[9] column using the system 3:4:3:2; petroleum ether (b.p. 90–100°)–ethyl acetate–methanol–water. Hold-back volumes 6–8 were evaporated to dryness to give a solid. Crystallization from acetone–petroleum ether gave 20 mg., m.p. 265–268°. Mixed melting point determination with an authentic sample of VIIIc showed no depression. The infrared spectra were also identical.

9α-Fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxypregn-1,4-diene-3,20-dione (IX). To a suspension of 14 mg. of VIIIc in 5 ml. of acetone was added 5λ of 72% perchloric acid. Solution became complete immediately. The reaction was allowed to stand for 2 hr. at room temperature and was then treated with 3 ml. of water and 0.4 ml. of saturated sodium bicarbonate solution. Evaporation of the acetone at room temperature gave a solid which was filtered off and washed with water to yield 10 mg., m.p. 277–281°. Its infrared spectrum was identical to that of an authentic sample of triamcinolone acetonide IX.

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PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

The Synthesis of Certain 4-Alkyl Derivatives of Hydrocortisone and 9α-Fluorohydrocortisone

WILLIAM S. ALLEN, CHARLES C. PIDACKS, ROBERT E. SCHAUB, AND MARTIN J. WEISS[1]

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4-Methylhydrocortisone (VI), 4-methyl-9α-fluorohydrocortisone (XI), 4-methyl-9α-fluoroprednisolone (XII), 4-ethyl-9α-fluorohydrocortisone (XVII), and 4,4-dimethyl-9α-fluoro-11β,17α,21-trihydroxy-5-pregnene-3,20-dione (XIX) have been synthesized. The 4-alkyl group was introduced by treatment of a Δ^4-3-ketone with an alkyl iodide in the presence of potassium *t*-butoxide. This alkylation procedure will also cause etherification of a free 21-ol and some oxide ring formation with a ring C fluorohydrin. Introduction of a 4-alkyl group into an active corticoid causes a decrease in thymolytic and glucocorticoid activity and does not reverse the sodium-retaining properties of the 9α-fluoro corticoids.

This paper reports the synthesis of certain 4-alkyl derivatives in the glucocorticoid series. 4-Alkyl, in particular 4-methyl, derivatives of hydrocortisone and its analogs were of considerable interest in view of the important biological effects which result on the introduction of a methyl group at the 2α-,[2] 6α-,[3] 16α-,[4] and 16β-[5] positions of the hydrocortisone molecule.

An attractive procedure for the 4-alkylation of a Δ^4-3-keto system was the method introduced by

(1) To whom inquiries concerning this paper should be addressed.

(2) J. A. Hogg, F. H. Lincoln, R. W. Jackson, and W. P. Schneider, *J. Am. Chem. Soc.*, 77, 6401 (1955).

(3) G. B. Spero *et al.*, *J. Am. Chem. Soc.*, 78, 6213 (1956).

(4) (a) G. E. Arth *et al.*, *J. Am. Chem. Soc.*, 80, 3161 (1958); (b) E. P. Oliveto *et al.*, *J. Am. Chem. Soc.*, 80, 4431 (1958).

(5) E. P. Oliveto *et al.*, *J. Am. Chem. Soc.*, 80, 4428 (1958).

Atwater[6] for the monoalkylation of testosterone, whereby testosterone was treated with potassium *t*-butoxide and a slight excess of alkyl halide in hot *t*-butyl alcohol. Application of this technique to the synthesis of 4-alkyl glucocorticoid derivatives requires blocking of the base-sensitive dihydroxyacetone side chain. At the time, it mistakenly appeared to us that hydrocortisone 20-ethylene ketal (I)[7] was a suitably protected starting compound. Therefore, I was submitted to the Atwater procedure and there was obtained a complex mixture, which on partition chromatography was resolved to give recovered starting material (30%) and three crystalline components (A–C). Two of these products (A and B) had an ultraviolet absorption maximum at 252 mμ, indicating the presence of a 4-methyl-Δ^4-3-keto chromophore. Although the third product (C) appeared to be, on the basis of combustion analysis, a monomethylated derivative of I, the ultraviolet absorption maximum at 242 mμ indicated the absence of a methyl group at C-4.

Compound A, obtained in 12% yield,[8] was presumed to be the desired 4-methylhydrocortisone 20-ethylene ketal (II) in view of the ultraviolet spectrum and the analytical data which indicated a monomethylated derivative. Hydrolysis of the 20-ketal group afforded a product which, on the basis of analytical and spectroscopic data and a positive blue tetrazolium α-ketol test, was assigned the 4-methylhydrocortisone structure (VI). At about the time this synthesis was completed, Steinberg, Hirschmann, and Chemerda reported[9] the synthesis of 4-methylhydrocortisone 21-acetate by another pathway.

Compound B, obtained in 21% yield,[8] on the basis of the analytical data appeared to be a dimethylated product; one of the methyl groups ($\lambda_{max}^{CH_3OH}$ 252 mμ) being substituted at C-4. Hydrolysis afforded a 20-ketone, which failed to react with acetic anhydride in pyridine, gave a negative blue tetrazolium α-ketol test, and by analysis showed the presence of one methoxyl group. The second methyl group in B thus is reasonably assumed to be present as a 21-methoxyl group, and B and its hydrolysis products are therefore the 4-methyl-21-*O*-methyl derivatives II and VII, respectively.

Compound C, which was the major product, obtained in 47% yield,[8] had a normal Δ^4-3-keto chromophore ($\lambda_{max}^{CH_3OH}$ 242 mμ), but analysis indicated a monomethylated derivative of hydrocortisone 20-ketal (I). It was presumably the 21-*O*-methyl derivative IV. This supposition was confirmed by hydrolysis of C to the free 20-ketone. The resulting product (VIII) failed to undergo acetylation, gave a negative blue tetrazolium α-ketol test, and showed the presence of one methoxyl group on combustion analysis.

Thus, the *t*-butoxide-alkyl halide procedure apparently allows preferential etherification of a 21-hydroxyl group in the presence of 17α- and 11β-hydroxyls.[10] The use of this relatively convenient procedure for the preparation of certain 21-*O*-alkyl derivatives is described in another paper.[11] Included therein is a more straightforward synthesis of 21-*O*-methylhydrocortisone (VIII).

As measured by the liver glycogen and thymus involution assays, 4-methylhydrocortisone (VI) had relatively little glucocorticoid activity, which is in agreement with the results reported[9] from the Merck Laboratories for the 21-acetate. However, it still was of some interest to prepare the 4-methyl derivative of 9α-fluorohydrocortisone,[12] with the hope of reversing the sodium-retaining action of this potent compound without at the same time completely negating its strong corticoid activity.

A particularly appropriate starting compound for the synthesis of 4-methyl-9α-fluorohydrocortisone was the bismethylenedioxy (BMD) derivative (V)[13] of 9α-fluorohydrocortisone. Treatment of V with one equivalent of methyl iodide in the presence of potassium *t*-butoxide in refluxing *t*-butyl alcohol gave the anticipated 4-methyl-9α-fluorohydrocortisone bismethylenedioxy derivative (X) (26% yield) and a fluorine-free product (18% yield), presumed to be the 4-methyl 9β,11β-oxide IX, resulting from ring closure of the fluorohydrin under the vigorous basic conditions of this experiment. This preparation of X proved quite capricious, and several attempted repetitions were unsuccessful in that the major or only product that could be isolated was the 4-methyl- 9β,11β-oxide IX. Finally, however, a preparation carried out with rigorously purified V did afford a 41% yield of the desired 4-methylfluorohydrin X. Hydrolysis of X with 60% formic acid[13] then gave 4-methyl-9α-fluorohydrocortisone (XI). In addition to XI, 4-methyl-9α-fluoroprednisolone 21-acetate (XII) was also prepared. This compound was obtained conveniently by 1,2-dehydrogenation of XI 21-acetate with 2,3-dichloro-5,6-dicyanobenzoquinone.[14]

4 - Methyl - 9α - fluorohydrocortisone (XI) was found to have a statistical potency relative to hydrocortisone, as measured by the thymus involu-

(6) N. W. Atwater, *J. Am. Chem. Soc.*, **79**, 5315 (1957); **82**, 2847 (1960).

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

(8) Yield based on unrecovered starting material.

(9) N. G. Steinberg, R. Hirschmann, and J. M. Chemerda, *Chem. & Ind. (London)*, 975 (1958).

(10) Atwater[6] reported that under the conditions of his experiments the 17β-hydroxy group of testosterone did not undergo etherification.

(11) W. S. Allen and M. J. Weiss, *J. Org. Chem.*, **26**, 4153 (1961).

(12) J. Fried and E. F. Sabo, *J. Am. Chem. Soc.*, **79**, 1130 (1957).

(13) R. E. Beyler, R. M. Moriarty, F. Hoffman, and L. H. Sarrett, *J. Am. Chem. Soc.*, **80**, 1517 (1958).

(14) D. Burn, D. N. Kirk, and V. Petrow, *Proc. Chem. Soc.*, 14 (1960).

tion assay,[15] of 2.7 (95% confidence limits: 1.8–4.0) and to be a sodium retainer in an electrolyte assay. 4-Methyl-9α-fluoroprednisolone (XII) had an approximate potency somewhat less than three times hydrocortisone and was also a sodium retainer in the electrolyte assay. When assayed by the same procedure,[15] the parent nonmethylated derivatives, 9α-fluorohydrocortisone and 9α-fluoroprednisolone, had potency ratings relative to hydrocortisone of 5.8 (4.1–8.2) and 9.8 (6.7–14.5), respectively. Both parent compounds were sodium retainers in the electrolyte assay. Thus, 4-methylation not only causes a decrease in thymolytic or glucocorticoid activity as already demonstrated (above and ref. 9) but in addition is unable to reverse the sodium-retaining action of 9α-fluoro corticoids.

On the chance possibility that, at C-4, ethyl substitution might result in compounds of greater biological utility than are produced by methyl substitution, we decide to pursue this course of study further by the preparation of 4-ethyl-9α-fluorohydrocortisone (XVII). Thus, the bismethylenedioxy derivative of 9α-fluorohydrocortisone (V) and ethyl iodide were submitted to the alkylation procedure. The resulting crude product was subjected to partition chromatography to give four crystalline components (D–G). Compound D was by analysis and ultraviolet spectroscopy the desired 4-ethyl derivative XIII. Compound E had the typical 4-alkyl-Δ^4-3-keto chromophore and by analysis lacked fluorine; therefore, it is assigned the 4-ethyl 9β,11β-oxide structure XIV. Compounds F and G did not have the strong ultraviolet absorption characteristic of a conjugated ketone and therefore are presumably 4,4-diethyl[16] derivatives. Compound F has fluorine present and thus is presumed to be the 4,4-diethylfluorohydrin XV. Compound G has no fluorine present, and therefore it is assigned the 4,4-diethyl 9β,11β-oxide structure XVI. Hydrolysis of XIII with 60% formic acid then furnished 4-ethyl-9α-fluorohydrocortisone (XVII). This product, by liver glycogen and thymus involution assays, was a weak corticoid, being substantially less active than the corresponding 4-methyl derivative (VI).

This study was then concluded with the preparation of 9α-fluoro-11β,17α-21-trihydroxy-4,4-dimethyl-5-pregnene-3,20-dione (XIX). The bismethylenedioxy derivative (V) of 9α-fluorohydrocortisone was treated[17] with six equivalents of methyl iodide and three equivalents of potassium *t*-butoxide for nineteen hours in *t*-butyl alcohol at room temperature to give the 4,4-dimethyl derivative XVIII in fair yield. Formic acid hydrolysis then furnished the desired XIX, essentially devoid of glucocorticoid activity (liver glycogen assay).

Finally, an attempt was made without success to extend this alkylation procedure to 4,6-dienones and to 1,4-dienones. Thus, treatment of 6-dehydrotestosterone by the Atwater method resulted in a 63% recovery of starting material and no indication of the presence of any alkylated product.[18] Also, treatment of the bismethylenedioxy derivative of prednisolone gave a 93% recovery of starting material.

EXPERIMENTAL

General. Melting points are uncorrected. Ultraviolet spectra were determined in methanol using a Cary recording spectrophotometer. Infrared spectra were determined in potassium bromide discs on a Perkin-Elmer spectrophotometer (Model 21). Polarimetric data were obtained in chloroform solution unless stated otherwise. Solutions were dried over magnesium sulfate and evaporations were carried out *in vacuo*. The material used for partition chromatography was Celite 545[19] which had been washed with 6*N* hydrochloric acid, distilled water until the washings were neutral, and finally with methanol. It was then dried at 50°. Hold-back volume is defined as the volume of solvent necessary to fill the packed column. All alkylation experiments were carried out under nitrogen.

Methylation of hydrocortisone 20-ethylene ketal (I). *Formation of 4-methylhydrocortisone 20-ethylene ketal* (II, A), *4-methyl-21-O-methylhydrocortisone 20-ethylene ketal* (III, B), *and 21-O-methylhydrocortisone 20-ethylene ketal* (IV, C). A slurry of hydrocortisone 20-ethylene ketal[7] (I) (15.0 g.) in anhydrous *t*-butyl alcohol (300 ml.) was added to a refluxing solution of potassium (2.2 g.) in anhydrous *t*-butyl alcohol (200 ml.). To the hot solution, a solution of methyl iodide (2.3 ml., 5.3 g.) in *t*-butyl alcohol (23 ml.) was added dropwise during 2.5 hr. (a nitrogen atmosphere was maintained throughout the experiment). The solution was cooled, chloroform was added, and the organic layer was washed to neutrality with saturated aqueous saline solution. After drying over magnesium sulfate, the solvent was evaporated to give 16.5 g. of a glass, 15 g. of which in three equal portions was submitted to partition chromatography on Celite diatomaceous earth from the system petroleum ether (b.p. 60–70°)-ethyl acetate–methanol-water (5:2:3:2). Each 5-g. aliquot was dissolved in 50 ml. of the lower phase of the above-described system, and the solution was mixed thoroughly with 100 g. of Celite. This mixture was packed on top of a column (6.5 cm. × 75 cm.) which had been prepared from 700 g. of Celite and 350 ml. of the stationary phase of the solvent system. The column was eluted with the upper phase of this system, and the effluent was allowed to pass through a recording spectrophotometer which had been set at 240 mμ. The holdback volume was 1500 ml. The appropriate fractions from the three columns were pooled for the work-up which is described below.

Three major peaks were apparent. The first peak (last third of the first hold-back volume and first third of second hold-back volume) contained *4-methyl-21-O-methylhydro-*

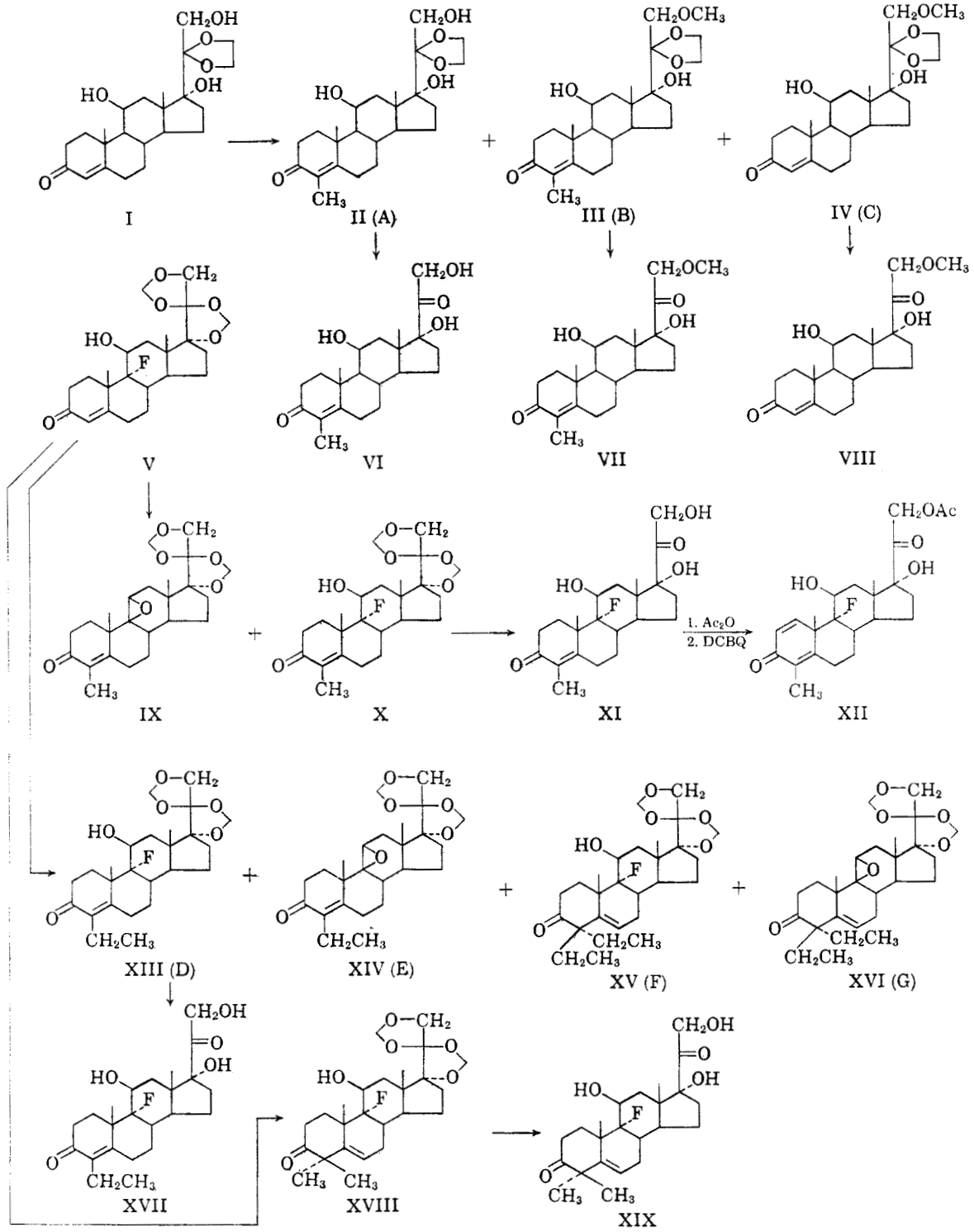
(15) I. Ringler and R. Brownfield, *Endocrinology*, **66**, 900 (1960).

(16) The alkylation of testosterone and 19-nortestosterone by the Atwater technique is reported to afford relatively low yields of 4,4-dialkyl derivative in addition to the 4-monoalkylated products.[6]

(17) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives, and R. B. Kelly, *J. Am. Chem. Soc.*, **76**, 2852 (1954).

(18) An unsuccessful attempt to methylate the 4,6-dienone isoergosterone by the Woodward[17] procedure has been reported by Cooley, Ellis, and Petrow [*J. Chem. Soc.*, 2998 (1955)].

(19) Celite is the trademark of Johns-Manville Corp. for diatomaceous earth.



cortisone 20-ethylene ketal (III, B). Evaporation of solvents gave 2.23 g., 520 mg. of which on recrystallization from acetone–petroleum ether afforded 360 mg. (represents a 21% yield based on nonrecovered starting material) melting at 168–171° [λ_{max} 252 mμ (ε 14,250)]; further recrystallization from the same solvent pair gave product melting at 187–188°; $[\alpha]_D^{25}$ + 97° (0.57%); λ_{max} 252 mμ (ε 14,900); λ_{max} 2.9, 6.02, 6.22 μ.

Anal. Calcd. for $C_{25}H_{38}O_6$ (434.55): C, 69.09; H, 8.81. Found: C, 68.93, 69.21; H, 9.22, 9.16.

The second peak (last third of second hold-back volume and all of third hold-back volume) contained *21-O-methylhydrocortisone 20-ethylene ketal* (IV, C). Evaporation of solvents gave 6.19 g., 4.23 g. of which on recrystallization from acetone afforded 3.16 g. (corresponds to a 47% yield based on unrecovered starting material) melting at 210–212° [λ_{max} 242 mμ (ε 15,400)]. Further recrystallization from acetone raised the m.p. to 212–214°; $[\alpha]_D^{25}$ +100° (0.57%); λ_{max} 242 mμ (ε 16,800); λ_{max} 2.93, 6.04, 6.17 μ.

Anal. Calcd. for $C_{24}H_{36}O_6$ (420.53): C, 68.54; H, 8.63. Found: C, 68.45; H, 8.69.

The third peak (sixth and seventh hold-back volume) contained *4-methylhydrocortisone 20-ethylene ketal* (II, A). Evaporation of solvents afforded 2.10 g., 1.51 g. of which on

recrystallization gave 0.82 g. (represents a 12% yield based on unrecovered starting material) melting at 237–239° [λ_{max} 252 mμ (ε 16,000)]. Further recrystallization from acetone gave material melting at 238–239°; $[\alpha]_D^{25}$ +99.5° (0.51%); λ_{max} 252 mμ (ε 15,200); λ_{max} 2.88, 6.01, 6.22 μ.

Anal. Calcd. for $C_{24}H_{36}O_6$ (420.53): C, 68.54; H, 8.63. Found: C, 68.22; H, 8.68.

Finally, a methanol wash of the column afforded 5.4 g. of material [λ_{max} 243 mμ (ε 12,500), m.p. 205–212°], which after recrystallization from methanol-acetone gave 4.5 g. (30%) of recovered starting material, m.p. 210–216°, (no depression on mixture melting point).

4-Methylhydrocortisone (VI). A methanolic solution (33 ml.) containing 4-methylhydrocortisone 20-ethylene ketal (II, A) (250 mg.) and 8% sulfuric acid (3 ml.) was refluxed for 1 hr. Water was added and the methanol was distilled to give crystalline VI (125 mg.), m.p. 208–212°. Two recrystallizations from acetone–petroleum ether furnished 70 mg., m.p. 214–217°; $[\alpha]_D^{25}$ +122° (0.53% dioxane); λ_{max} 250 mμ (ε 15,100); λ_{max} 2.87, 5.84, 6.07, 6.23 μ.

Anal. Calcd. for $C_{22}H_{32}O_5$ (376.48): C, 70.18; H, 8.57. Found: C, 69.77, 70.19; H, 8.83, 8.94.

4-Methyl-21-O-methylhydrocortisone (VII). This compound was prepared from the corresponding 20-ethylene ketal (III, B) (500 mg.) by the hydrolysis procedure described above for the preparation of 4-methylhydrocortisone (VI). The oil, which was obtained on removal of methanol, was taken up in chloroform. The aqueous layer was separated, and the chloroform layer was washed with saturated sodium bicarbonate solution followed by water, dried, and evaporated. The residual glass was crystallized from acetone–petroleum ether to give 275 mg., m.p. 172–174°. Two recrystallizations from this solvent pair gave material melting at 176–177°; $[\alpha]_D^{25}$ +149° (0.60%); λ_{max} 250 mμ (ε 15,600); λ_{max} 2.86, 5.80, 6.04, 6.22 μ.

Anal. Calcd. for $C_{23}H_{34}O_5$ (390.50): C, 70.74; H, 8.78; OCH_3, 7.95. Found: C, 70.87; H, 8.80; OCH_3, 7.92.

This product gave a negative blue tetrazolium α-ketol test. Attempted acetylation with acetic anhydride in pyridine gave recovered starting material (VII).

21-O-Methylhydrocortisone (VIII). This compound was prepared from the 20-ethylene ketal (IV, C) (500 mg.) by the hydrolysis procedure described above for VI. After evaporation of the methanol, the crystalline product was filtered and dried; yield 450 mg.; m.p. 241–243°. Recrystallization from ethanol afforded VIII with m.p. 252–254°; $[\alpha]_D^{25}$ +147° (0.23% in dioxane); λ_{max} 241 mμ (ε 16,100); λ_{max} 2.91, 3.02, 3.43, 5.85, 6.05, 6.20 μ.

Anal. Calcd. for $C_{22}H_{32}O_5$ (376.48): C, 70.18; H, 8.57; OCH_3, 8.2. Found: C, 69.92; H, 8.88; OCH_3, 8.7.

This product (VIII) gave a negative blue tetrazolium α-ketol test and failed to undergo acetylation with pyridine and acetic anhydride; starting material being recovered.

Methylation of 9α-fluorohydrocortisone bismethylenedioxy derivative (9α-fluoro-11β-hydroxy-17α-20;20,21-bismethylenedioxypregn-4-en-3-one, V). Formation of 4-methyl-9α-fluorohydrocortisone bismethylenedioxy derivative (X) *and 9β, 11β-epoxy-4-methyl-17α,20;20,21-bismethylenedioxypregn-4-en-3-one* (IX). A. A solution of 9α-fluoro-11β-hydroxy-17α,20;20,-21-bismethylenedioxypregn-4-en-3-one (9α-fluorohydrocortisone bismethylenedioxy derivative, V)[13] (3.0 g.) in dry *t*-butyl alcohol (75 ml.) was added to a solution of potassium metal (0.43 g.) in dry *t*-butyl alcohol (25 ml.), and the mixture heated to reflux temperature. At this temperature, a solution of methyl iodide (0.46 ml.) in dry *t*-butyl alcohol (15 ml.) was then added dropwise over a period of 2.5 hr. Refluxing was continued for an additional 30 min. after which the mixture was cooled and neutralized with glacial acetic acid. Chloroform (600 ml.) was then added, and the organic extract washed two times with water, dried over anhydrous magnesium sulfate, and evaporated to dryness to give a semisolid (3.1 g.) which was treated with the lower phase (25 ml.) from the system petroleum ether (b.p. 90–100°), 6: ethyl acetate, 2: methanol, 5: water, 2.A portion (2 g.) of this semisolid would not dissolve and is designated as the insoluble portion.

Crystallization of the insoluble portion from acetone gave 4-methyl-9α-fluoro-11β-hydroxy-17α,20;20,21-bismethylenedioxypregn-4-en-3-one (X) (770 mg.), m.p. 270° dec. Recrystallization from acetone, followed by two recrystallizations from acetone–petroleum ether gave product with m.p. 285° dec.; $[\alpha]_D^{26}$ +14° (0.63%); λ_{max} 248 mμ (ε 15,500); λ_{max} 2.94, 6.05, 6.22, 9.07 μ.

Anal. Calcd. for $C_{24}H_{33}O_6F$ (436.50); C, 66.03; H, 7.62; F, 4.35. Found: C, 65.84; H, 7.87; F, 4.22.

A small amount of additional material [52 mg., m.p. 279° dec.] was obtained from the second chromatography fraction described below; total yield: 822 mg. (26.4%).

The soluble portion was mixed with Celite (50 g.) and packed on top of a column consisting of 500 g. of Celite, impregnated with 250 ml. of the lower phase from the above-described system. The column was eluted with the upper phase of this system, and the effluent was allowed to pass through a recording spectrophotometer which had been set at 240 mμ. Three ultraviolet absorbing fractions were obtained.

Fraction 1 (first two thirds of the first hold-back volume) was evaporated to dryness to give 750 mg. (25.5%) of crude material, which on crystallization from acetone–petroleum ether gave 225 mg. (7.6%) of 9β,11β-epoxy-4-methyl-17α,-20;20,21-bismethylenedioxypregn-4-en-3-one (IX) melting at 232–235°. Four recrystallizations from this solvent pair gave material melting at 243–245°; $[\alpha]_D^{26}$ −105° (1.17%); λ_{max} 252 mμ (ε 14,100); λ_{max} 6.04, 6.22, 9.23 μ.

Anal. Calcd. for $C_{24}H_{32}O_6$ (416.50): C, 69.21; H, 7.74. Found: C, 68.93; H, 8.12; (F, none).

The second fraction (last third of first hold-back volume and first half of second hold-back volume) was evaporated to dryness to give an additional 52 mg. (m.p. 279° dec.) of 4-methyl-9α-fluorohydrocortisone bismethylenedioxy derivative (X).

The third fraction (second half of second hold-back volume and third hold-back volume) contained a small amount of recovered starting material (V) (less than 50 mg.).

B. Several attempts to repeat A were unsuccessful in that the 4-methyl 9β,11β-oxide IX proved to be the major or only product that could be isolated. However, when the experiment was repeated with especially purified 9α-fluorohydrocortisone bismethylenedioxy derivative (V) (in our experience bismethylenedioxy derivatives are often contaminated with formaldehyde polymer) a 41% yield of the 4-methylfluorohydrin X (m.p. 255–270° dec.) was obtained. In this last experiment no attempt was made to isolate the oxide IX which may or may not have been formed.

4-Methyl-9α-fluorohydrocortisone (9α-fluoro-11β,17α,21-trihydroxy-4-methylpregn-4-ene-3,20-dione, XI). A mixture of 9α-fluoro-11β-hydroxy-4-methyl-17α,20:20,21-bismethylenedioxypregn-4-en-3-one (X) (200 mg.) and 60% aqueous formic acid (15 ml.) was heated on a steam bath for 30 min. The mixture was then evaporated to dryness, and the residue was dissolved in acetone and filtered. The filtrate was evaporated to dryness, and the solid residue was crystallized from ethyl acetate–petroleum ether (b.p. 90–100°) to give 63 mg. of 4-methyl-9α-fluorohydrocortisone (XI), m.p. 215–217°.

Two recrystallizations from the same solvent pair gave 37 mg.; m.p. 232–235°; $[\alpha]_D^{25}$ +134° (0.69% in dioxane); λ_{max} 244 mμ (ε 14,600); λ_{max} 2.90, 5.83, 6.03, 6.19 μ (shoulder).

Anal. Calcd. for $C_{22}H_{31}O_5F$ (394.48): C, 66.91; H, 7.91; F, 4.81. Found: C, 67.27; H, 8.24; F, 4.60.

4-Methyl-9α-fluorohydrocortisone 21-acetate (11β,17α,21-trihydroxy-9α-fluoro-4-methylpregn-4-ene-3,20-dione 21-acetate). A solution of 180 mg. of 11β,17α,21-trihydroxy-9α-fluoro-4-methylpregn-4-ene-3,20-dione (XI) in 2 cc. of pyridine and 1 cc. of acetic anhydride was kept at room temperature for 18 hr. The solution was poured into 10 cc. of water and after 1 hr. the gummy solid was collected by filtration. The crude product was recrystallized from acetone–petro-

leum ether to give 100 mg. (50%) of 21-acetate, m.p. 216–218°; λ_{max} 2.85, 5.70 (shoulder) 5.80, 6.06, 6.21 (shoulder), 8.12 μ. This material was used directly for the Δ^1-introduction (below).

4-Methyl-9α-fluoroprednisolone 21-acetate (11β,17α,21-trihydroxy-9α-fluoro-4-methylpregna-1,4-diene-3,20-dione 21-acetate XII). A solution of 11β,17α,21-trihydroxy-9α-fluoro-4-methylpregn-4-ene-3,20-dione 21-acetate (100 mg.) and 2,3-dichloro-4,5-dicyanobenzoquinone[14] (78 mg.) in 10 cc. of dioxane was refluxed for 3 days. The solution was evaporated to dryness. The residue, dissolved in a mixture of ethyl acetate–benzene, was washed several times with cold 5% aqueous sodium hydroxide solution and then with water until neutral, dried, and evaporated to dryness leaving 75 mg. of a glass. Recrystallization of this glass from acetone–petroleum ether gave 38 mg. of XII as white crystals, m.p. 228–230° dec.; λ_{max} 2.90, 5.71, 5.79, 6.01, 6.15, 6.23, 8.01 μ; polarographic assay[20] showed the product to contain 87% $\Delta^{1,4}$-3-one.

Anal. Calcd. for $C_{24}H_{31}FO_6$: C, 66.34; H, 7.19. Found: C, 66.81; H, 7.14.

Ethylation of 9α-fluorohydrocortisone bismethylenedioxy derivative (V). *Formation of 4-ethyl-9α-fluorohydrocortisone bismethylenedioxy derivative (4-ethyl-9α-fluoro-11β-hydroxy-17α,20;20,21-bismethylendioxypregn-4-en-3-one,* XIII, D), *9β,11β-epoxy-4-ethyl-17α,20;20,21-bismethylendioxypregn-4-en-3-one* (XIV, E), *4,4-diethyl-9α-fluoro-11β-hydroxy-17α,-20;20,21-bismethylenedioxypregn-5-en-3-one* (XV, F), *and 9β,-11β,-epoxy-4,4-diethyl-17α,20;20,21-bismethylenedioxypregn-5-en-3-one* (XVI, G). A slurry of 9α-fluorohydrocortisone bismethylenedioxy derivative (V) (10.0 g.) with anhydrous *t*-butyl alcohol (150 ml.) was added to a refluxing solution of potassium metal (1.42 g.) in anhydrous *t*-butyl alcohol (150 ml.). To the refluxing solution was then added dropwise, during 90 min., a solution of ethyl iodide (1.92 ml., 3.70 g.). Refluxing was continued for an additional 15 min. After cooling, the reaction mixture was neutralized with acetic acid, and chloroform (1200 ml.) was added. After washing with saturated sodium bicarbonate solution, followed by saturated saline solution, the organic layer was dried and evaporated to dryness. The residual material (7.8 g.) was extracted with hot methanol. The methanol extract on cooling gave a small amount of yellow precipitate which was discarded. Evaporation of the cooled extracts gave 5.6 g. of material, which was submitted to partition chromatography in two equal portions on Celite with the system heptane:2-methoxyethanol. The sample was dissolved in 30 ml. of the lower phase of this system, and the resulting solution was mixed thoroughly with 60 g. of Celite. The mixture was packed on top of a column (5.5 cm. × 75 cm.) which had been prepared from 500 g. of Celite and 250 ml. of the lower phase of the above-described system. The hold-back volume for this column was 625 ml. The column was eluted with the upper phase of the solvent system, and the effluent was passed through a recording spectrophotometer set at 240 mμ. The appropriate fractions from the two columns were pooled for the work-up which proceeded as follows.

4-Ethyl-9α-fluorohydrocortisone bismethylenedioxy derivative (XIII, D) was obtained on evaporation of hold-back volumes 8–12. There was thus obtained 1.8 g. of product which on recrystallization from acetone–petroleum ether furnished 580 mg. with m.p. 280° dec. A second recrystallization from the same solvent pair gave product with m.p. 270° dec.; $[\alpha]_D^{25}$ 16.2° (0.62%); λ_{max} 248 mμ (ϵ 15,800); λ_{max} 2.87, 6.05, 6.20 μ.

Anal. Calcd. for $C_{25}H_{35}FO_6$ (450.53): C, 66.65; H, 7.83; F, 4.22. Found: C, 66.51, 66.25; H, 8.08, 7.98; F, 4.42.

9β,11β-Epoxy-4-ethyl-17α,20;20,21-bismethylenedioxypregn-4-en-3-one (XIV, E) was obtained on evaporation of the second half of the second hold-back volume and the third hold-back volume. The residual product amounted to 560 mg., which on recrystallization from acetone–petroleum ether gave 300 mg. melting at 170–171°. A second recrystallization from the same solvent pair raised the melting point to 171–172°; $[\alpha]_D^{25}$ −104° (0.61%); λ_{max} 252 mμ (ϵ 14,600); λ_{max} 2.9 (weak), 6.05, 6.21 μ.

Anal. Calcd. for $C_{25}H_{34}O_6$ (430.52): C, 69.74; H, 7.96. Found: C, 69.90; H, 8.24, F, none.

4,4-Diethyl-9α-fluoro-11β-hydroxy-17α,20:20;21-bismethylenedioxypregn-5-en-3-one (XV, F) was obtained on evaporation of hold-back volumes 6 and 7 (nonultraviolet-absorbing). The residual product (220 mg., m.p. 230–234°) on recrystallization from acetone–petroleum ether afforded 177 mg. of product, m.p. 238° dec. Further recrystallization from acetone raised the m.p. to 239° dec.; $[\alpha]_D^{25}$ −103° (0.52%); no significant ultraviolet absorption; λ_{max} 2.9 (strong), 5.9, 6.0 μ.

Anal. Calcd. for $C_{27}H_{39}O_6F$ (478.58): C, 67.76; H, 8.20; F, 3.97. Found: C, 67.43; H, 8.48; F, 4.05.

9β-11β-Epoxy-4,4-diethyl-17α,20;20,21-bismethylenedioxypregn-5-en-3-one (XVI, G) was obtained from the second half of the first hold-back volume. Evaporation of solvent gave 220 mg., m.p. 174–176°, which on recrystallization from acetone–petroleum ether gave 179 mg., m.p. 176–177°. A final recrystallization from this solvent pair gave analytical material melting at 175.5–176.5°; $[\alpha]_D^{25}$ −108° (0.57%); no significant ultraviolet absorption; λ_{max} 5.8, 6.0 μ; no significant absorption at 2.9 μ.

Anal. Calcd. for $C_{27}H_{38}O_6$ (458.6): C, 70.71; H, 8.35. Found: C, 70.86; H, 8.60; F, none.

The *methanol wash* of the columns gave, on evaporation of solvent, 4.0 g. of amorphous material, which could not be crystallized and was probably impure V.

4-Ethyl-9α-fluorohydrocortisone (XVII). A solution of 4-ethyl-9α-fluorohydrocortisone bismethylenedioxy derivative (XIII, D) (400 mg.) in 60% formic acid (30 ml.) was heated for 30 min. on a steam bath. Water was added precipitating solid material. Extraction with chloroform (300 ml.) did not take up all the solids, and the insoluble material was filtered and dried to give 173 mg., m.p. 223–226°, positive blue tetrazolium α-ketol test. Recrystallization from acetone–petroleum ether gave 120 mg., m.p. 222–223°; λ_{max} 248 mμ (ϵ 16,300); λ_{max} 2.85, 5.87, 6.06, 6.23 μ.

Anal. Calcd. for $C_{23}H_{33}FO_5$ (408.49): C, 67.62; H, 8.14; F, 4.65. Found: C, 67.62; H, 8.49; F, 4.66.

9α-Fluoro-11β-hydroxy-4,4-dimethyl-17α,20;20,21-bismethylenedioxypregn-5-en-3-one (XVIII). Potassium metal (860 mg.) was dissolved in refluxing anhydrous *t*-butyl alcohol (50 ml.) under a nitrogen atmosphere. The solution was cooled, and a slurry of 9α-fluorohydrocortisone bismethylenedioxy derivative (V) (3.0 g.) with anhydrous *t*-butyl alcohol (75 ml.) was added. After the addition of methyl iodide (2.76 ml.), dissolved in anhydrous *t*-butyl alcohol (15 ml.), the reaction mixture was stirred at room temperature for 3 hr. All the steroid (V) had not dissolved, and the mixture therefore was heated at reflux for 10 min., cooled, and allowed to stir at room temperature for an additional 13 hr. The mixture was then neutralized with acetic acid, chloroform and water were added, and the organic layer was separated and washed once with saturated sodium bicarbonate solution and twice with water. After drying, the solvent was evaporated and the resulting solid residue was triturated with acetone to give 730 mg. of product (XVIII) melting at 266° dec. and having $E_{1cm}^{1\%}$ 240 mμ (32). Two recrystallizations from chloroform-acetone gave 390 mg. melting at 264° dec. and showing no significant ultraviolet absorption; $[\alpha]_D^{25}$ −107° (0.51% in dioxane); λ_{max} 2.86, 5.89, 5.97 μ.

Anal. Calcd. for $C_{25}H_{35}FO_6$ (450.53): C, 66.65; H, 7.83; F, 4.22. Found: C, 66.29; H, 8.00; F, 4.18.

Concentration of the acetone triturate afforded 620 mg. of material melting at 250° dec. and having $E_{1cm}^{1\%}$ 238 mμ (123).

9α-Fluoro-11β,17α,21-trihydroxy-4,4-dimethylpregn-5-ene 3,20-dione (XIX). A solution of 9α-fluoro-11β-hydroxy-4,4-dimethyl-17α,20;20,21-bismethylenedioxypregn-5-en-3-one

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(XVIII) (290 mg.) in 60% formic acid (70 ml.) was heated for 2.5 hr. on a steam bath. The solvent was distilled, and the residual solid was dissolved in acetone, treated with decolorizing charcoal, and filtered. The product was crystallized on addition of petroleum ether; yield, 98 mg. of material with m.p. 219–222° and giving a positive blue tetrazolium α-ketol test. For analysis, this material was recrystallized twice from acetone–petroleum ether to give material melting at 225–226° after drying *in vacuo* over phosphorous pentoxide at toluene reflux temperature; $[\alpha]_D^{25}$ +3.1° (0.32% in dioxane); λ_{max} 2.95, 5.87, 6.00 (shoulder) μ.

Anal. Calcd. for $C_{23}H_{33}FO_5$ (408.49): C, 67.62; H, 8.14; F, 4.65. Found: C, 67.43; H, 8.50; F, 4.35.

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PEARL RIVER, N. Y.

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Some 21-Substituted Analogs of Cortisone

H. D. BROWN, A. R. MATZUK, D. R. HOFF, AND L. H. SARETT

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A series of analogs of hydrocortisone with various substituents at C-21 has been prepared and tested for biological activity. The compounds prepared included derivatives of cortisone, hydrocortisone and their Δ^1- and 9α-fluoro derivatives. Many of the compounds studied exhibited biological activity in the liver glycogen deposition test and in the local granuloma assay, while systemic granuloma inhibition was found chiefly in the 21-azido analogs.

Some analogs of cortisone with a hetero atom at C-21 have been described in the recent literature, *e.g.*, thiolacetate,[1] some derivatives of compound S and cortisone,[2] and some 21-fluoro analogs from several laboratories.[3]

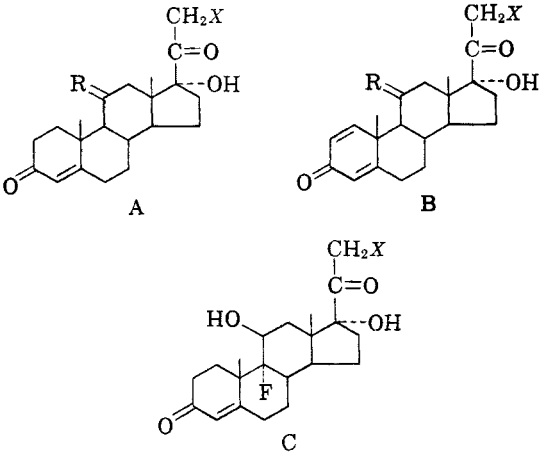
Some hitherto undescribed members of this family containing a C_{21}—N or C_{21}—S bond have been synthesized. The chemical and biological properties of these new steroids are described below. Figure 1 summarizes the principal variations made in this study.

In general the 21-desoxy analogs were secured by the usual bimolecular displacement on the appropriate 21-iodo or mesylate derivative. Thus sodium azide in refluxing acetone smoothly converted the 21-methanesulfonate esters to the corresponding azide. Purification of the azides and especially separation from unchanged mesylate proved to be quite troublesome due to the low solubility of the compounds in nonpolar solvents and to some instability in hot polar solvents (*e.g.*, pyridine and dimethylformamide).

Attempted displacement of the halogen in the 21-iodo derivative of hydrocortisone with sodioacetamide led to the formation of 17,21-oxide[4] and none of the desired acetamido analog was obtained.

Through use of excess mercaptan (as buffer) no difficulty was encountered in preparation of the

Fig. 1. 21-Substituted Analogs of Corticosteroids



A. Derivatives of cortisone and hydrocortisone

R = O	X = —SCH_3	I
	—SC_2H_5	II
	—N_3	III
R = H, OH	X = —$SCH_2C_6H_5$	IV
	—SCN	V
	—N_3	VI
	—$\overset{+}{N}(CH_3)_3CH_3SO_3^-$	VII

B. Derivatives of prednisone and prednisolone

R = O	X = —N_3	VIII
R = —H, —OH	X = —N_3	IX

C. Derivatives of 9α-fluorohydrocortisone

X = —OSO_2CH_3	X
X = —N_3	XI

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