(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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[CONTRIBUTION FROM MERCK SHARP & DOHME RESEARCH LABORATORIES]

Some 21-Substituted Analogs of Cortisone

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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]

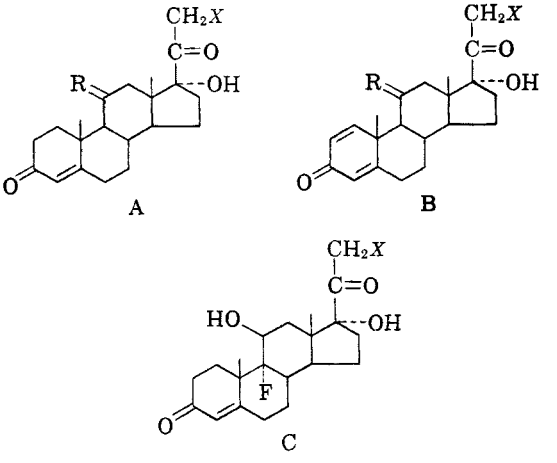
Some hitherto undescribed members of this family containing a C_{21}—N or C_{21}—S bond have been synthesized. The chemical and biological properties of these new steroids are described below. Figure 1 summarizes the principal variations made in this study.

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

(1) C. Djerassi and A. L. Nussbaum, *J. Am. Chem. Soc.*, **75**, 3700 (1953).

(2) P. Bomerang, *Acta Chem. Scand.*, **9**, 587 (1955).

(3) P. Tannhauser, R. J. Pratt, and E. U. Jensen, *J. Am. Chem. Soc.*, **78**, 2658 (1956); J. E. Herz, J. Fried, P. Grabowich, and E. F. Sabo, *J. Am. Chem. Soc.*, **78**, 4812 (1956).

(4) W. S. Allen, S. Bernstein, M. Heller, and R. Littell, *J. Am. Chem. Soc.*, **77**, 4784 (1955); R. F. Hirschmann, G. A. Bailey, G. I. Poos, R. N. Walker, and J. M. Chemerda, *J. Am. Chem. Soc.*, **78**, 4814 (1956).

alkyl and aralkyl mercapto derivatives *via* displacement of the mesylate with sodiomercaptides.

Biological activity. In the local granuloma assay[5] the thio ethers (I, II, and IV) were not appreciably active. On the other hand, the thiocyanate (V) and most of the azido analogs were highly active. The prednisolone azide was equivalent to the corresponding *tert*-butyl acetate.[5] For systemic inhibition of granuloma formation the 21-azido analog of prednisolone (IX) was roughly equivalent to hydrocortisone.

EXPERIMENTAL[6]

17α-Hydroxy-3,11,20-triketo-21-methylmercapto-4-pregnene (I). Using the conditions described below for the ethyl derivative, cortisone-21-mesylate and sodium methylmercaptide yielded a product which melted at 163–166° (from ethyl acetate).

Anal. Calcd. for $C_{22}H_{30}O_4S$: C, 67.67; H, 7.75. Found: C, 67.55; H, 7.61.

17α-Hydroxy-3,11,20-triketo-21-ethylmercapto-4-pregnene (II). Cortisone 21-mesylate (0.877 g., 0.002 mole) was treated with a solution made by adding 1 ml. of 2*N* sodium methoxide (in methanol) to 5 ml. of ethyl mercaptan in 10 ml. of dry dimethoxy ethane. The slightly turbid solution was stirred for 17 hr. at room temperature. Fifty milliliters of chloroform was then added, and the organic phase was washed with water, 1*N* hydrochloric acid, water, and dried. Removal of the solvent left 672 mg. of product which melted at 163–169°; the melt remained slightly turbid (unchanged mesylate?). Chromatography on 21 g. of alumina and elution with ether to 1:1 ether-chloroform gave the desired alkyl mercapto compound. Recrystallization from ethyl acetate sharpened the m.p. to 168°; ultraviolet λ_{max} 237.5 mμ, ε 405.

Anal. Calcd. for $C_{23}H_{32}O_4S$: C, 68.28; H, 7.97. Found: C, 68.28; H, 7.90.

17α-Hydroxy-3,11,20-triketo-21-azido-4-pregnene (III). One-half gram (0.00123 mole) of cortisone-21-mesylate and 0.12 g. (0.00185 mole) of sodium azide were added to 10 ml. of acetone and refluxed for 2 hr. A crystalline precipitate formed during refluxing. The precipitate was filtered and washed with water, acetone, and chloroform. The crude product (0.36 g.) was dissolved in a small amount of hot pyridine and methanol added to turbidity. The mixture was cooled and filtered, m.p. (on at 280°) 294–296° dec. The ultraviolet spectrum showed λ_{max} 238 mμ, ε 413. The infrared spectrum showed hydroxyl at 2.82, azide function at 4.8, 20-carbonyl and 11-carbonyl at 5.82 and 5.90 and conjugate carbonyl at 6.02 and 6.17 μ.

Anal. Calcd. for $C_{21}H_{27}O_4N$: C, 65.43; H, 7.06; N, 10.90. Found: C, 65.65; H, 7.34; N, 10.69.

11β,17α-Dihydroxy-3,20-diketo-21-benzylmercapto-4-pregnene (IV). Six-tenths milliliter of 2*N* sodium methoxide solution was added to 0.3 ml. of benzyl mercaptan in 20 ml. of ethylene glycol dimethyl ether. A white precipitate formed. One-half gram of hydrocortisone-21-mesylate was added and the mixture was heated, with stirring, at 60–65° for 0.5 hr. The reaction mixture was allowed to come to room temperature, with stirring, during 2 hr. It was diluted with chloroform and washed with dilute hydrochloric acid, dilute sodium bicarbonate solution and water. The chloroform layer was dried over anhydrous sodium sulfate and concentrated to dryness under reduced pressure. The residue was dissolved in a little benzene and charged to a column of 15 g. of acid-washed alumina. The fractions eluted with ether-chloroform (1:1 through 3:7) were combined and recrystallized twice from 2 B ethanol to give 41 mg., m.p. (on at 130°) 166–174°. A second crop was obtained from the mother liquors. After recrystallization three times from ethyl acetate–petroleum ether (b.p. 30–60°) the yield was 28 mg., m.p. (on at 130°) 165–169°. Ultraviolet showed λ_{max} 241 mμ, ε 368. A sample for analysis was dried at 78° for 2 hr.

Anal. Calcd. for $C_{28}H_{36}O_4S$: C, 71.77; H, 7.74. Found: C, 72.25; H, 7.73.

Hydrocortisone-21-thiocyanate (V). One-half gram (.00113 mole) of hydrocortisone-21-mesylate and 0.15 g. (0.00155 mole) of potassium thiocyanate in 20 ml. of acetone were refluxed for 2 hr. The precipitate was filtered and the filtrate evaporated to dryness. The residue was combined with the filtered precipitate and washed with water. The crude material was dissolved in excess acetone and concentrated to a small volume. Excess chloroform was added to the concentrate (in which crystals had appeared) and cooled to yield 0.092 g. of V, m.p. (on at 150°) 218–225° dec. Ultraviolet showed λ_{max} at 242 mμ, ε 411. Infrared showed absorption at 2.84, 3.02, 4.72, 5.85, and 6.12 μ.

Anal. Calcd. for $C_{22}H_{29}O_4NS$: C, 65.49; H, 7.25; N, 3.47. Found: C, 65.63; H, 7.18; N, 3.19.

11β,17α-Dihydroxy-3,20-diketo-21-azido-4-pregnene (VI). One-half gram (0.00113 mole) of hydrocortisome-21-mesylate and 0.11 g. (0.00169 mole) of sodium azide in 10 ml. acetone were refluxed for 2 hr. Insoluble material was filtered and washed with water, acetone, and chloroform. The original filtrate was evaporated to dryness, and the residue was washed with water, acetone, and chloroform. Solids were combined, dissolved in a minimum amount of pyridine and excess water was added. Needle-like crystals were obtained by cooling the solution in an ice bath. Drying *in vacuo* at room temperature gave a 0.209 g. yield, m.p. (on at 220°) 228–234° dec. The infrared spectrum showed hydroxyl at 3.0, azide function at 4.82, 20-carbonyl at 5.82, and conjugate carbonyl at 6.1 and 6.19 μ.

Anal. Calcd. for $C_{21}H_{29}O_4N_3$: C, 65.09; H, 7.54; N, 10.85. Found: C, 65.35; H, 7.77; N, 10.90.

Hydrocortisone-21-trimethylammonium methanesulfonate (VII). One-half gram (0.00113 mole) of hydrocortisone-21-mesylate and 25 ml. of 25% trimethylamine in methanol were sealed in a Carius tube and heated in a bomb at 70° for 8 hr. A dark brown solution formed, which was evaporated to dryness. The residue was washed well with acetone, dissolved in methanol, treated with Darco, and filtered. The filtrate was evaporated to dryness. It was washed again with acetone, dissolved in chloroform, and the chloroform concentrated to a small volume. Excess ether was added and allowed to stand for a short time. The precipitate was collected and dried; 29 mg., m.p. 239–244° dec. The infrared spectrum indicated the presence of hydroxyl at 3.0, carbonyl at 5.84, and conjugate carbonyl at 6.0 and 6.15 μ (shoulder). Absorption present in the 8.5-region but not at 7.5 μ suggested ionic methanesulfonate.

Anal. Calcd. for $C_{25}H_{41}O_7NS$: C, 60.10; H, 8.27; N, 2.80. Found: C, 59.58; H, 8.09; N, 3.11.

Prednisone-21-azide-17α-hydroxy-3,11,20-triketo-21-azido-pregnadiene-1,4 (VIII). A solution of prednisone-21-mesylate (0.218 g., 0.0005 mole) and 40 mg. of sodium azide in 25 ml. of acetone was heated under reflux for 1.5 hr. Then the solvent was removed *in vacuo*, and the product was washed successively in a centrifuge tube with water, a little acetone, and ether. There was thus obtained 152 mg. of crystalline material. The crystals vibrated on the hot stage at 160–170° and decomposed at 285–300°. Recrystallization from methanol-pyridine gave a colorless product with the same decomposition point.

(5) C. A. Winter and C. G. Porter, *J. Am. Pharm. Assoc.*, **46**, 515–519 (1957).

(6) All melting points were taken on a Kofler micro hot stage. Ultraviolet absorption spectra were obtained in methanol solution unless otherwise specified. Infrared curves were secured from Nujol mulls (except where noted) using a Baird double beam instrument. Analytical samples were dried at room temperature *in vacuo* unless otherwise indicated.

Anal. Calcd. for $C_{21}H_{25}O_4N_3$: C, 65.78; H, 6.57. Found: C, 66.23; H, 6.75.

Prednisolone-21-azide (IX). Reaction of prednisolone mesylate (650 mg.) and sodium azide (500 mg.) in 25 ml. of refluxing acetone for 3.5 hr. gave 441 mg. of product, after quenching in water. Recrystallization from acetone-water and finally methanol gave a sample with a decomposition point of 230–235°; $[\alpha]_D^{25}$ +214° (*c*, 0.4 in acetone). The infrared spectrum showed bands at 3.1–3.15, (shoulder 2.95–3.0), 4.82 (strong), 5.82, 6.07, 6.28 μ.

Anal. Calcd.: N, 10.90. Found: N, 10.76.

9α-Fluorohydrocortisone-21-mesylate (X). Methanesulfonyl chloride (1 ml.) was added to a stirred, cooled (5°) suspension of 3.4 g. (0.0089 mole) of 9α-fluorohydrocortisone in 40 ml. of pyridine. After 1.5 hr. of cooling and stirring, the mixture was poured into water. The mesylate was collected by filtration; washed with water, dried, and recrystallized from ethyl acetate; yield, 1.6 g., m.p. 212° dec.

9α-Fluoro-4-pregnene-11β,17α-diol-3,20-dione-21-azide (XI). A mixture of 1.39 g. (0.0030 mole) of 9α-fluorohydrocortisone-21-mesylate and 3.3 g. of sodium azide in 150 ml. of acetone was heated under reflux for 2 hr. The reaction mixture was cooled, poured into cold water, and aged 30 min. The azide was collected by filtration, washed with water, dried, and recrystallized from ethyl acetate as needles, m.p. 220–227° dec.; $[\alpha]_D^{26}$ +193° (*c*, 1.025 in acetone); yield, 1.08 g. (88%).

Anal. Calcd. for $C_{21}H_{28}N_3O_4F$: C, 62.20; H, 6.69; N, 10.36. Found: C, 62.25; H, 6.79; N, 10.56.

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RAHWAY, N. J.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. LXVII.[2] Preparation and Stereochemistry of 20α-Hydroxycyclopseudoneosapogenins[3a,b,c]

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Peracid oxidation of pseudotigogenin or pseudosmilagenin (I) gave the known 20α-hydroxycyclopseudoisosapogenins. Similar treatment of pseudosarsasapogenin (V) yielded a mixture of two 20α-hydroxy compounds (VIa and VIb) which differed in configuration at C-22. From the transformations occurring with VIa and VIb a mechanism can be formulated for the reactions of all pseudosapogenins with acids or peracids.

In previous publications we have shown that 20α-hydroxycyclopseudoisosapogenins along with side chain cleavage products could be obtained by chromium trioxide–acetic acid oxidation of the corresponding cyclopseudoisosapogenins[5a,b] whereas similar oxidation of cyclopseudoneosapogenins gave only side chain cleavage products.[5a,6] A more general route to 20α-hydroxy sapogenins involving peracid oxidation of pseudosapogenins seemed possible from the work of Callow and James[7] who obtained a 20α-hydroxy sapogenin by peracid treatment of pseudohecogenin. We first studied the oxidation of pseudotitogenin with peracids, since the structure of the expected product, 20α-hy-

(1) Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

(2) Previous paper in this series, *Appl. Microbiol.*, 8, 345 (1960).

(3)(a) Trivial nomenclature used in this paper is in accordance with that recommended by L. F. and M. Fieser, *Steroids*, Reinhold Publishing Co., New York, 1959, p. 825; (b) Formal nomenclature for steroidal sapogenins is still unsettled. The recommendations for steroid sapogenins in Nomenclature Reports, *J. Am. Chem. Soc.*, November 5, 1960 are obsolete. We have adopted the Tentative Recommendations of the Steroid Nomenclature Sub-Committee, Appendix B, Information Bulletin No. 11, IUPAC (1960) using the system which designates the position of the oxygen atom of ring F with reference to the general plane of the ring, *i.e.* 22α-*O* or 22β-*O*; asymmetry at C-20 is then also designated by reference to the main ring system, *i.e.* 20αH or 20βH while that at C-25 utilizes the α_F, β_F system, 25α_F or 25β_F; (c) Abstracted from a dissertation by Henry A. Walens to be submitted to the Temple University Graduate Council in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(4)(a) Present address: Natural Products Laboratory, Research Triangle Institute, Box 490, Durham, N. C.; (b) Department of Chemistry, Temple University, Philadelphia, Pa.

(5)(a) M. E. Wall and H. A. Walens, *J. Am. Chem. Soc.*, 77, 5661 (1955); (b) M. E. Wall and H. A. Walens, *J. Am. Chem. Soc.*, 80, 1984 (1958).

(6) The side chain cleavage products undoubtedly arise from the chromic acid oxidation of the corresponding pseudosapogenins. R. K. Callow and co-workers, *J. Chem. Soc.*, 1966 (1955), have shown that in acidic media, cyclopseudosapogenins and pseudosapogenins exist in an equilibrium mixture. Since Wall and Serota, *J. Am. Chem. Soc.*, 79, 648 (1957) have demonstrated that cyclopseudoisosapogenins form pseudosapogenins more rapidly than the corresponding cyclopseudoneosapogenins, one would expect that the former should give more side chain cleavage products and less 20α-hydroxylation than the latter. Experimentally, the reverse situation is found. The Fiesers, ref. 3a, p. 828 have attempted to rationalize this situation by suggesting that the 20αH in cyclopseudoneosapogenins is shielded from chromic acid attack by the C-23 methylene group whereas the 20αH in cyclopseudoisosapogenins is in a less hindered environment.

(7) R. K. Callow and V. H. T. James, *Chem. & Ind. (London)*, 112 (1956).