COMMUNICATIONS TO THE EDITOR

16-METHYLATED STEROIDS. I. 16α-METHYLATED ANALOGS OF CORTISONE, A NEW GROUP OF ANTI-INFLAMMATORY STEROIDS

Sir:

During the nine years which have elapsed since the discovery that cortisone was effective in the treatment of rheumatoid arthritis,[1] intense efforts have been expended toward modifying the structure of this hormone in the hope of finding a related compound with superior therapeutic properties. Noteworthy candidates have included $\Delta^{1,4}$ and $\Delta^{1,4,6}$ analogs,[2a,b] C-9 halogenated derivatives,[2c] C_2 and C_6 methylated analogs,[2d,e] C-14[2f] and C-16[2g] hydroxylation products and compounds combining these functions.[2h,i,j,k]

It has been established that in addition to reductions in the A-ring, two pathways of metabolic inactivation of cortisone or hydrocortisone involve reduction at C-20[3,4] to an alcohol and scission of the side chain[4,5] to 17-keto compounds.

On the hypothesis that the side chain could perhaps be stabilized against such catabolic inactivation by an appropriately placed but otherwise chemically inert substituent, some analogs of cortisone containing a 16α-methyl group were synthesized.

Introduction of the methyl group was accomplished by reaction of 16-pregnene-3α-ol-11,20-dione acetate[6] with methylmagnesium iodide[7] to give 16α-methylpregnane-3α-ol-11,20-dione, I, m.p. 155–157°, [α] + 110°,[8] (*Anal.* Found: C, 76.26; H, 10.04). The acetate of I had m.p. 157–158°, [α] + 118°, (*Anal.* Found: C, 74.08; H, 9.12). The 16α-methyl configuration is assigned to the entering group on the basis of rotational data and precedents for α-side attack.[9]

Reaction of I with acetic anhydride–toluenesulfonic acid[10] afforded a mixture of non-crystalline enol acetates from which after oxidation with perbenzoic acid followed by an alkaline hydrolysis, 16α-methylpregnane-3α,17α-diol-3,20-dione (II), m.p. 185–187°, [α] + 60° (*Anal.* Found: C, 73.87; H, 9.38) was obtained. Bromination of II at C-21 was followed by conversion of the resulting bromide to 16α-methyl pregnane-3α,17α,21-triol-11,20-dione 21-acetate (III), m.p. 182–184°, [α] + 75° (*Anal.* Found: C, 68.51; H, 8.41) by means of potassium acetate–sodium iodide in boiling acetone.[11] The corresponding 3-ketone, 16α-methylpregnane-17α,-21-diol-3,11,20-trione 21-acetate, IV, m.p. 238–240° [α] + 76° (tetrahydrofuran), (*Anal.* Found: C, 69.00; H, 8.04) was obtained from III by oxidation with the chromic anhydride–pyridine complex.[12] Bromine in acetic acid was added to a solution of IV in chloroform affording 4ζ-bromo-16α-methylpregnane-17α,21-diol-3,11,20-trione 21-acetate (V), dec. 240–241°, [α] + 84° (tetrahydrofuran), (*Anal.* Found: C, 57.93; H, 6.70; Br, 16.16) and the latter with semicarbazide (*cf.* ref. 10) was converted to 16α-methyl-4-pregnene-17α,-21-diol-3,11,20-trione 21-acetate 3-semicarbazone (VI), dec. 225–228°, λ_{max} 269 mμ,[13] ε 28,400 (*Anal.* Found: C, 63.56; H, 7.31; N, 9.07).

Pyruvic acid treatment of VI provided 16α-methyl-4-pregnene-17α,21-diol-3,11,20-trione 21-acetate (VII), 16α-methylcortisone acetate, m.p. 207–210°, [α] + 181°, λ_{max} 238 mμ, ε 15,400 (*Anal.* Found: C, 69.11; H, 7.57). Dehydrogenation of VII by means of selenium dioxide[14] led to 16α-methyl - 1,4 - pregnadiene - 17α,21 - diol - 3,11,20-trione 21-acetate (VIII), m.p. 210–212°, [α] + 180° (*c*, 0.006),[8] λ_{max} 238 mμ, ε 15,400 (*Anal.* Found: C, 69.42; H, 7.58) in satisfactory yield. Sodium borohydride effected reduction of the C-11 carbonyl functions of the 3,20-bis-semicarbazones[15,16] of VII and VIII whence, after hydrolysis at C-3 and C-20 and acetylation, 16α-methyl-4-pregnene-11β,17α,21-triol-3,20-dione 21-acetate (IX), m.p. 210–212°, [α] + 146°, λ_{max} 242 mμ ε 16,900 (*Anal.* Found: C, 69.04; H, 8.40) and 16α-methyl-1,4-pregnadiene-11β,17α,21-triol-3,20-dione 21-acetate (X), m.p. 145–149°, λ_{max} 242 mμ, ε 15,200, respectively, were isolated.

(1) P. S. Hench, E. C. Kendall, C. H. Slocumb and H. F. Polley, *Proc. Staff Meet. Mayo Clinic*, **24**, 181 (1949).

(2) (a) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Perlman and M. M. Pechet, *Science*, **121**, 176 (1955); (b) D. Gould, E. L. Shapiro, M. J. Gentles, E. B. Hershberg, W. Charney, M. Gilmore, S. Tolksdorf, M. Eisler, P. L. Perlman and M. M. Pechet, THIS JOURNAL, **79**, 502 (1957); (c) J. Fried and E. F. Sabo, *ibid.*, **75**, 2273 (1953); J. Fried and E. F. Sabo, *ibid.*, **76**, 1455 (1954); (d) J. A. Hogg, F. H. Lincoln, R. W. Jackson and W. P. Schneider, *ibid.*, **77**, 6401 (1955); (e) G. B. Spero, J. L. Thompson, B. J. Magerlein, A. R. Hanze, H. C. Murray, O. K. Sebek and J. A. Hogg, *ibid.*, **78**, 6213 (1956); (f) E. J. Agnello, B. L. Bloom and G. D. Laubach, *ibid.*, **77**, 4684 (1955); (g) W. S. Allen and S. Bernstein, *ibid.*, **78**, 1909 (1956); (h) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, *ibid.*, **78**, 5694 (1956); (i) J. E. Herz, J. Fried, P. Grabowich, E. F. Sabo, *ibid.*, **78**, 4813 (1956); (j) G. B. Spero, J. L. Thompson, F. H. Lincoln, W. P. Schneider, J. A. Hogg, *ibid.*, **79**, 1515 (1957); (k) S. Bernstein, M. Heller, R. Littell, S. Stolar, R. Lenhard and W. Allen, *ibid.*, **79**, 4555 (1957).

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(4) (a) D. K. Fukushima, Abstr. of 131st A.C.S. Meeting, Miami, 1957, p. 12-C; (b) E. M. Glenn, R. O. Stafford, S. C. Lyster and B. J. Bowman, *Endocrinology*, **61**, 128 (1957).

(5) S. Burstein, K. Savard and R. I. Dorfman, *ibid.*, **52**, 448 (1953).

(6) W. R. Nes and H. L. Mason, THIS JOURNAL, **73**, 4765 (1951).

(7) *Cf.* R. E. Marker and H. M. Crooks, Jr., *ibid.*, **64**, 1280 (1942).

(8) All rotations were taken in chloroform at 25° unless otherwise noted, concn. = 100 mg. per 10 ml., using the sodium-D line. We are indebted to Dr. D. Williams for determining the rotational dispersion curve of VIII, to Messrs. R. W. Walker for infrared spectra, R. N. Boos and associates for microanalyses and to J. Wittick and associates for ultraviolet spectra.

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(10) *Cf.* T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

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(12) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.* **75**, 422 (1953).

(13) Ultraviolet spectra are of methanolic solutions of the compounds.

(14) *Cf.* Ch. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956).

(15) N. L. Wendler, Huang-Minlon and M. Tishler, THIS JOURNAL, **73**, 3818 (1951).

(16) *Cf.* R. E. Jonesand S. A. Robinson, *J. Org. Chem.*, **21**, 586 (1956).

The biological activities of the compounds herein described are compared with those of other anti-inflammatory steroids in an accompanying Communication.[17]

(17) G. E. Arth, J. Fried, D. B. R. Johnston, D. R. Hoff, L. H. Starett, R. H. Silber, H. C. Stoerk, C. A. Winter, THIS JOURNAL, **80**, 3161 (1958).

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MERCK SHARP & DOHME
LABORATORIES DIVISION
MERCK & CO., INC.
RAHWAY, NEW JERSEY

GLEN E. ARTH
DAVID B. R. JOHNSTON
JOHN FRIED
WILLIAM W. SPOONCER
DALE R. HOFF
LEWIS H. SARETT

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REQUIREMENT OF CYTIDINE TRIPHOSPHATE FOR THE BIOSYNTHESIS OF PHOSPHOPANTETHEINE[1]

Sir:

The enzymes, present in cell-free extracts of *Proteus morganii*, which catalyze reactions 1, 2 and 3

$$\text{pantothenic acid} + \text{ATP}^{2} \longrightarrow \text{4}'\text{-phosphopantothenic acid} + \text{ADP} + \text{P}_i \quad (1)$$

$$\text{4}'\text{-phosphopantothenic acid} + \text{cysteine} + \text{CTP} \longrightarrow \text{4}'\text{-phosphopantothenylcysteine} + \text{CDP}^{3} + \text{P}_i \quad (2)$$

$$\text{4}'\text{-phosphopantothenylcysteine} \longrightarrow \text{4}'\text{-phosphopantetheine} + \text{CO}_2 \quad (3)$$

have been named, respectively: (1) pantothenic acid kinase[4,5]; (2) phosphopantothenic acid–cysteine coupling enzyme[5]; and (3) phosphopantothenylcysteine decarboxylase. A crude extract, prepared from cells ruptured in a Hughes press, was treated with ammonium sulfate and calcium phosphate gel to yield a preparation of the coupling enzyme which was purified 20-fold and was free of the other two enzymes.

The substrates for the reaction catalyzed by the purified coupling enzyme were found to be 4′-phosphopantothenic acid and cysteine. The amount of product formed, 4′-phosphopantothenylcysteine, was determined by measurement of the disappearance of 4′-phosphopantothenic acid by determining the amount of pantothenic acid which could be regenerated by treatment with phosphatase. For this purpose, pantothenic acid assays were performed with *Saccharomyces carlsbergensis* 4228.[6] Unexpectedly, it was found that the purified coupling enzyme did not function unless a small amount of crude extract was also included in the reaction mixture. The activating factor in the crude extract was heat labile and appeared to be an enzyme. Of a large number of compounds which were tested only one, CTP, was able to replace the requirement for this extra enzyme. The activating effects of the crude extract and CTP are shown in Table I. Other nucleoside di- and triphosphates were inactive. Additional experiments have shown that only 0.2 μM of CTP is required to give maximal activity. It seems probable that the extra enzyme required in the absence of added CTP was nucleoside diphosphate kinase, whose function was to replenish the small amount of CTP which was present in the enzyme preparations.

TABLE I

REQUIREMENT OF CTP FOR SYNTHESIS OF 4′-PHOSPHOPANTOTHENYLCYSTEINE

The reaction mixture contained: 0.08 μM 4′-phosphopantothenic acid, 5 μM ATP, 10 μM $MgCl_2$, 80 μM tris-(hydroxymethyl)-aminomethane buffer at *p*H 7.4, 10 μM cysteine, 700 γ of purified coupling enzyme and additions as shown below in a total volume of 2 ml. Incubation was for 3 hr. at 37°, followed by heating for 5 min. at 100° and centrifugation to separate denatured protein. The supernatant solutions were analyzed as described in the text.

Addition	4′-Phosphopantothenylcysteine formed, $\mu M \times 10^2$
None	1.91
ATP, 5μM	1.25
ATP, 5μM + crude extract	4.38
ATP, 5μM + boiled extract	1.91
CTP, 5μM	6.92

Incubation of the purified enzyme with cysteine and CTP gave no detectable cytidine-containing, sulfur-containing compound. Thus it seems likely that the CTP requirement in the reaction is for the activation of the carboxyl group of 4′-phosphopantothenic acid in a manner similar to the way ATP functions in the synthesis of pantothenic acid from pantoic acid and β-alanine.[7]

(1) This investigation was supported by National Science Foundation Grant G4580.

(2) ATP, ADP, CTP and CDP are adenosine and cytidine tri- and diphosphates.

(3) Fragmentary evidence only indicates that CDP and inorganic phosphate (P_i) are products of this reaction.

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DIVISION OF BIOCHEMISTRY
DEPARTMENT OF BIOLOGY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
CAMBRIDGE, MASSACHUSETTS

GENE M. BROWN

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16-METHYLATED STEROIDS. II. 16α-METHYL ANALOGS OF CORTISONE, A NEW GROUP OF ANTI-INFLAMMATORY STEROIDS. 9α-HALO DERIVATIVES

Sir:

Syntheses of the acetates of 16α-methylated analogs of cortisone, hydrocortisone and their 1,2-unsaturated derivatives, I and II, respectively, are reported in an accompanying communication.[1] The enchanced activity and freedom from salt retention characteristic of this group of compounds prompted extension to 9-halogenated analogs.

A dimethylformamide–pyridine solution of 16α-methylhydrocortisone acetate[1] (III) was treated with methanesulfonyl chloride,[2] affording 16α-methyl-4,9(11)-pregnadiene-17α, 21-diol-3,20-dione 21-acetate (IV), m.p. 205–208° λ_{max} 239 mμ, ϵ 17,300, $[\alpha]$ + 93°.[3] (*Anal.* Found: C, 71.96; H, 8.30) which was converted to 16α-methyl-9α-

(1) G. E. Arth, D. B. R. Johnston, J. Fried, W. W. Spooncer, D. R. Hoff and L. H. Sarett, THIS JOURNAL, **80**, 3160 (1958).

(2) Modification of unpublished procedure of E. M. Chamberlain and J. M. Chemerda; *cf.* J. Fried, K. Florey, E. Sabo, J. Herz, A. Restivo, A. Borman and F. Singer, *ibid.*, **77**, 4181 (1955).

(3) Ultraviolet spectra are of methanolic solutions of the compounds. Rotations were determined in chloroform at 25°, concn. = 100 mg./10 ml. using the sodium-D line, unless otherwise noted.