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

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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

pantothenic acid + ATP[2] ⟶
4′-phosphopantothenic acid + ADP + P_i (1)

4′-phosphopantothenic acid + cysteine + CTP ⟶
4′-phosphopantothenylcysteine + CDP[3] + P_i (2)

4′-phosphopantothenylcysteine ⟶
4′-phosphopantetheine + CO_2 (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]

(7) W. K. Maas, *Federation Proc.*, **15**, 305 (1956).

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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, [α] + 93°.[3] (*Anal.* Found: C, 71.96; H, 8.30) which was converted to 16α-methyl-9α-

(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.

(4) G. B. Ward, G. M. Brown and E. E. Snell, *J. Biol. Chem.*, **213**, 869 (1955).

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(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.

TABLE I

Entry	Compound[k]	Liver glycogen activity[a]	Systemic granuloma activity[b]	Sodium retention (Rats)[c]	Sodium retention (Man)[d]	Anti-inflammatory activity (Man)[d]
1	Hydrocortisone	1	1	−	1	1
2	Δ^1-Hydrocortisone	3–4	3–4	−*	<1	3–4
3	2α-Methylhydrocortisone			+		
4	6α-Methyl-Δ^1-hydrocortisone	1.5	5	−*	<1[e]	3–5[e]
5	9α-Fluorohydrocortisone	8	6	+	>1[f]	10[f]
6	9α-Fluoro-Δ^1-hydrocortisone	13	12	+	>1[g]	20[g]
7	9α-Fluoro-16α-hydroxy-Δ^1-hydrocortisone 16α 21-diacetate	12	5	−*	<1[h]	3–5[h]
8	16α-Methylhydrocortisone	2	3	−*	<1[i]	3–4[i,l]
9	16α-Methyl-Δ^1-cortisone	3	13	−*		
10	16α-Methyl-Δ^1-hydrocortisone	5	12	−*	<1[i]	6[i,l]
11	16α-Methyl-9α-fluorohydrocortisone	12	36	−	<1[i]	14[i,l]
12	16α-Methyl-9α-fluoro-Δ^1-hydrocortisone	17	190	−	<1[i,j]	28–40[i,l]

[a] *Cf.* R. M. Reinecke and E. C. Kendall, *Endocrinology*, **31**, 573 (1942). Fasting intact rats are given 5 hourly oral doses of steroids in 1% ethanol–water. [C. C. Porter and R. H. Silber, *ibid.*, **53**, 73 (1953).] [b] Modification of the method of R. Meier, W. Schuler and P. Desaulles, *Experientia*, 6, 469 (1950). Intact male Holtzman rats (*ca.* 125 g.) are dosed orally each day for a week. [c] Adrenalectomized rats maintained for 24 hours after operation on low sodium (0.01%) and potassium diet (0.001%) are injected i.p. with 5 ml. of saline and s.c. with 0.25 ml. 30% ethanol containing 50γ of steroid. Pooled urine (3 rats) collected and analyzed for sodium. A plus indicates sodium retention and an asterisk increased sodium excretion over controls. [d] Hydrocortisone equals 1. [e] E. W. Boland and G. W. Liddle, *Ann. Rheumat. Dis.*, 16, 297 (1957). [f] E. W. Boland, *Ann. N. Y. Acad. Sci.*, **61**, 593 (1955). [g] R. L. Black, K. L. Yielding, R. E. Peterson, G. D. Whedon and J. J. Bunim, *Ann. Rheum. Dis.*, **15**, 76 (1956). [h] R. H. Freyberg, C. A. Berntsen, Jr., and L. Hellman, paper delivered at the 9th International Congress of Rheumatic Diseases, Toronto, Canada, June, 1957. [i] Ref. 6, this paper. [j] Ref. 8, this paper. [k] Entries 8, 9, 10, 11 and 12 were prepared from the corresponding acetates by the sodium methoxide method (*cf.* Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, THIS JOURNAL, 74, 5395 (1952). [l] Based on limited clinical experience.

bromo - 4 - pregnene - 11β,17α,21 - triol - 3,20-dione 21-acetate (V), dec. 173–175°, λ_{max} 244 mμ, ϵ 16,200, [α] + 135°. (*Anal.* Found: C, 58.19; H, 6.50; Br, 16.31) by action of hypobromous acid.[2] By means of potassium acetate in boiling ethanol,[2] V was dehydrobrominated to 9β,11β-oxido-16α-methyl-4-pregnene-17α,21-diol-3,20-dione 21-acetate (VI), m.p. 182–184°, λ_{max} 244 mμ, ϵ 14,600, [α] + 3°. (*Anal.* Found: C, 69.20; H, 7.60). This oxide with hydrogen fluoride in tetrahydrofuran–chloroform[2,4] yielded 16α-methyl-9α-fluoro-4-pregnene-11β,17α,21-triol-3,20-dione 21-acetate (VII), m.p. 219–226°, λ_{max} 239 mμ, ϵ 16,700, [α] + 125° (*c* 0.35). (*Anal.* Found: C, 66.29; H, 7.55). Analogously, VI with a solution of hydrogen chloride in chloroform[4b] afforded 16α-methyl-9α-chloro-4-pregnene-11β,17α,21-triol-3,20-dione 21-acetate (VIII), dec. 210–216°, λ_{max} 241 mμ, ϵ 16,750, [α] + 132° (*c* 0.35). (*Anal.* Found: C, 63.99; H, 7.25). Selenium dioxide[5] effected 1,2-dehydrogenation of VII to 16α-methyl-9α-fluoro-1,4-pregnadiene-11β,17α,21-triol-3,20-dione 21-acetate (IX), m.p. 215–221°, λ_{max} 239 mμ, ϵ 14,900, [α] + 73°. (*Anal.* Found: C, 66.10; H, 6.85).

Neither VII nor IX causes retention of sodium in adrenalectomized rats. Entries 8, 10, 11 and 12 have not caused sodium or water retention in man[6] with the dosages explored, and entry 12,[7] with several times the amount required to achieve suppression of rheumatoid arthritis, does not cause retention of water or sodium or loss of potassium.[8]

Table I shows that anti-inflammatory activity is enhanced by a 16α-methyl substituent. Thus 16α-methyl-9α-fluoro-Δ^1-hydrocortisone, is the most active non-salt-retaining anti-inflammatory steroid for which data sufficient for comparison have been published.

The 16α-methyl group reduces chemical reactivity at C-20, *e.g.*, toward semicarbazide. This relative inertness is paralleled by an increased stability in human plasma *in vitro*, as measured by the phenylhydrazine–sulfuric acid assay.[9]

We are indebted to Dr. J. Van de Kamp and Messrs. S. M. Miller and A. Drucker for the preparation of supplies of these steroids for clinical investigation and for intermediates. Messrs. J. Weijlard and A. Sullivan also provided useful intermediates. We gratefully acknowledge the support and encouragement of Drs. J. Chemerda, K. Pfister, K. Folkers and M. Tishler. We should also like to acknowledge the contributions of Dr.

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

(6) Edward W. Boland and Nathan E. Headley, Abstract of a paper entitled "Preliminary Clinical Observations with a New Series of Synthetic Corticosteroid Compounds in Patents with Rheumatoid Arthritis," presented at the meeting of the American Rheumatism Association, San Francisco, June 20–21, 1958.

(7) Entry 12 has been assigned the generic name *hexadecadrol*. DECADRON is the trademark of Merck & Co., Inc. for *hexadecadrol*.

(8) Joseph J. Bunim, Roger L. Black, Leo Lutwak, Ralph E. Peterson and G. Donald Whedon, Abstract of a paper entitled "Physiologic, Metabolic and Clinical Studies of a New Synthetic Steroid, Hexadecadrol," to be presented at the meeting of the American Rheumatism Association, San Francisco, June 20–21, 1958.

(9) R. H. Silber and R. D. Busch, *J. Clin. Endocrinol. and Metab.*, **16**, 1333 (1956).

Elmer Alpert in coördinating the clinical investigations.

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CONDUCTANCE OF IONOPHORES

Sir:

Recently,[1-3] equations were presented which permit calculation of conductance for 1–1 electrolytes in terms of three arbitrary parameters: Λ_0, the limiting equivalent conductance, a, the center-to-center distance at contact of cation and anion and K_A, the association constant. These equations have since been revised; the final results, given below, are much more convenient for practical computations. The algebraic form of the equations has been rearranged. The dilemma regarding the Stokes radius in the velocity term of the relaxation field has been resolved. A virtual force arising from asymmetry of osmotic pressure has been included. When association is slight, the $c^{3/2}$ terms are negligible for $\kappa a < 0.2$; when association is not negligible, the $c^{3/2}$ term in activity completely swamps the J_2 term; hence the latter has been dropped. In order to save space, all symbols not explicitly defined here will have the meanings given in refs. 1–3.

Define $\Lambda_\eta \equiv (1 + Fc)$ where $Fc = 5\phi/2$. This is the observed conductance, corrected for *volume* viscosity (see first column, p. 3309[3]). This becomes the dependent variable if viscosity data are available; if not, the viscosity effect is approximated by moving $(-F\Lambda_0 c)$ to the right as before. Then for negligible association, $\Lambda_\eta = (\Lambda_0 - Sc^{1/2} + Ec \log c + Jc)$, where $J = (\sigma_1 \Lambda_0 + \sigma_2)$ replaces the former J_1. Here

$$\sigma_1 = (\kappa^2 a^2 b^2/12c)[h(b) + 0.9074 + \ln(\kappa a/c^{1/2})] \quad (1)$$

$$\sigma^2 = \alpha\beta + (11\beta\kappa a/12c^{1/2}) - (\kappa a b\beta/8c^{1/2})[1.0170 + \ln(\kappa a/c^{1/2})] \quad (2)$$

$$h(b) = (2b^2 + 2b - 1)/b^3. \quad (3)$$

Define $\Lambda_\eta' \equiv (\Lambda_\eta + Sc^{1/2} - Ec \log c)$. For the Owen and Zeldes[4] data on potassium halides at 25°, a plot of Λ_η' against c is accurately linear. The intercept at $c = 0$ evaluates Λ_0 and the slope gives J. The $\mathring{a}$ — values found are: KCl, 3.07; KBr, 3.26; KI, 3.50. These values agree well with the sums of the corresponding crystallographic radii.

When association is not negligible, γ_0 is computed as before. Then Λ_η' becomes $(\Lambda_\eta + Sc^{1/2}\gamma_0^{-1/2} - Ec\,\gamma_0 \log c\,\gamma_0)$ where

$$\Lambda_\eta = \Lambda_0 - Sc^{1/2}\gamma_0^{1/2} + Ec\gamma_0 \log c\gamma_0 + Jc\gamma_0 - K_A c\gamma_0 f^2 \Lambda_\eta \quad (4)$$

The quantities $\Delta\Lambda$, y and x are *redefined* as follows: $\Delta\Lambda \equiv (\Lambda_\eta' - \Lambda_0) = (Jc\gamma_0 - K_A c\gamma_0 f^2 \Lambda_\eta)$; $y = \Delta\Lambda/c\gamma_0$ and $x = f^2\Lambda_\eta$. Again trial values of Λ_0 are used until the one is found which linearizes the y–x plot. The slope gives K_A; then from $y(0) = (J - K_A\Lambda_0)$, J and hence a are evaluated. If K_A is known (*e.g.*, by extrapolation of log K_A *vs.* $1/D$), define $\Lambda_J \equiv (\Lambda_\eta' + K_A c\gamma_0 f^2 \Lambda_\eta)$. A plot of Λ_J against $c\gamma_0$ is linear with slope and intercept equal to J and Λ_0, respectively. Alternatively, if a is known, define $\Lambda_K \equiv (\Lambda_\eta' - Jc\gamma_0)$. Then a plot of Λ_κ against $c\gamma_0 f^2 \Lambda_\eta$ determines Λ_0 and K_A. Equation 4 applied to the Mercier and Kraus[5] data for Bu_4NBr in dioxane–water lead to a-values $4.8 \leq a \leq 5.4$. The spread is much less than that reported before; we therefore believe the present equations represent a better approximation. Details will be presented later.

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RETARDATION OF EXCHANGE PROCESSES BY MOLECULAR ASSOCIATION: METHYL ALCOHOL

Sir:

In high resolution nuclear magnetic resonance studies of liquids, exchange processes frequently preclude the observation of spin–spin multiplets.[1] In specific cases one can reduce the rate of exchange by carefully purifying the sample.[2-4] In some instances, however, an alternative procedure can be employed which provides information concerning exchange processes and molecular complexes in liquid systems. This is achieved by adding to the sample a complexing agent which preferentially forms a stable molecular complex with the sample under study and, consequently, decreases the rate of exchange.

As an example, consider the proton magnetic resonance spectrum of methyl alcohol. The spin–spin multiplets which should be observable in the spectrum of this molecule have long eluded detection, presumably because of exchange effects. On the other hand, in solutions containing sufficient quantities of acetone, hydrogen bonding increases the lifetime of –OH group protons in enough molecules to reveal the fine structure. Figure 1 shows the spectrum of methyl alcohol as observed at 40 Mc. in a solution of acetone containing 25% CH_3OH by volume. The theoretical spectrum for the special case of $J/\delta = 0.21$ is added for comparison. The experimental trace gives $J = 4.8$ sec.$^{-1}$ and $\delta = 22.8$ sec.$^{-1}$. It should be noted that two of the lines in the observed –OH multiplet are not predicted theoretically. One of these has been shown to be water; the other is attributed to an additional impurity.

Supplementary experiments with methanol and other molecules have shown that (a) by varying the acetone concentration the internal chemical shift can be changed and the concomitant alterations in fine structure observed and compared

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