gift of neomycin samples, and to Mr. A. D. Argoudelis for counsel and assistance.

(7) Robert F. Carr Fellow, 1957–58.

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A NEW CLASS OF POTENT CORTICAL HORMONES.[1] 6α-CHLOROCORTICOIDS

Sir:

We have found that 6β-chloro substitution among corticoids decreases thymolytic and anti-inflammatory activity and increases sodium retention, 6α-chloro substitution in general not only enhances thymolytic and anti-inflammatory activity but also modifies biological activity toward sodium excretion. Thus 6α-chloroprednisone acetate (A^2 = 4) and 6α-chloroprednisolone (A^2 = 13) exhibit a marked sodium excretion[3] while 6α-chloro-9α-fluoroprednisolone acetate (A = 40) is only a moderate sodium retainer in contrast to the enormous sodium retention of the C-6 unsubstituted compound.[4]

6α - Chloro - 9α - fluoro - 16α - hydroxyhydrocortisone 21-acetate 16,17-acetonide (XI) and the corresponding 1-dehydro compound (XIV), with respective anti-inflammatory activities[2] of 50 and 200 × hydrocortisone exhibit sodium excretion.

The 3-ethyl enol ether[5] of Reichstein's "S" acetate on reaction with N-chloroacetamide in aqueous buffered acetone solution yielded 6β-chloro-"S" acetate (m.p. 193–194°, $[\alpha]_D$ +41°, λ_{max} 240 mμ, log ε 4.15. Found for $C_{23}H_{31}ClO_5$: C, 65.38; H, 7.41; Cl, 8.28) which was inverted to XV by treatment with hydrogen chloride in acetic acid. Selenium dioxide oxidation[6a,b,c,d] followed by saponification and (1) adrenal incubation[7] or (2) fermentation with *Cunninghamella*[8] *bainieri* ATCC 9244 gave 6α-chloroprednisolone (XII). Adrenal or microbiological oxidation of XV followed by acetylation or hydrogen chloride–acetic acid treatment of 5α,6α-oxido - 3,20 - bisethylenedioxyallopregnane - 11β,-17α,21-triol[9] acetate gave VIII which was converted to XII acetate (m.p. 204–205°, $[\alpha]_D$ +68°, λ_{max} 242 mμ, log ε 4.17. Found for $C_{23}H_{29}ClO_6$: C, 63.01; H, 6.68; Cl, 7.99) by selenium dioxide oxidation.

TABLE I

	Compound[b]	Additional substituent	M.P. (dec.) °C.	$[\alpha]_D$ $CHCl_3$	λ_{max} (mμ) EtOH	log ε	Thymolytic[a] activity	Anti-inflamm.[2,a] activity
I	Cortisone Ac.	6β-Chloro-	178–179°	+129°	237	4.16	0.2	0.2
II	Cortisone Ac.	6α-Chloro-	197°	+176°	233	4.16	1.3	1
III	Cortisone Ac.	6β-Chloro-9α-fluoro	221°	+ 80°	234	4.18	1.5	1
IV	Cortisone Ac.	6α-Chloro-9α-fluoro	214°	+132°	230	4.16	15	8
V	Prednisone Ac.	6β-Chloro-	221–222°	+132°	241	4.17	0.3	0.3
VI	Prednisone Ac.	6α-Chloro-	217–218°	+144°	237	4.19	4	4
VII	Prednisone Ac.	6α-Chloro-9α-fluoro	227–229°	+122°	235	4.18	18	14
VIII	Hydrocortisone Ac.	6α-Chloro	174–176°[c]	+ 97	238	4.08	5	11
IX	Hydrocortisone Ac.	6β-Chloro-9α-fluoro	194–195°	+ 44°	238	4.17	0.5	0.5
X	Hydrocortisone Ac.	6α-Chloro-9α-fluoro	134–143°[d]	+ 88°	234	4.11	5	11
XI	Hydrocortisone Ac.	6α-chloro-9α-fluoro-16α-hydroxy-16,17-acetonide	156–157°	+ 90°	234	4.17	100	50
XII	Prednisolone	6α-Chloro	195–196°	+ 61° (Diox.)	242	4.19	14	13
XIII	Prednisolone Ac.	6α-Chloro-9α-fluoro	150°[d,e]	+ 71°	238	4.19	27	40
XIV	Prednisolone Ac.	6α-Chloro-9α-fluoro-16α-hydroxy-16,17-acetonide	294–296°	+ 53°	238	4.17	400	200
XV	"S" Acetate	6α-Chloro	189–190°	+ 87°	237	4.18		
XVI	Δ^1-Dehydro "S" Ac.	6α-Chloro	231–232°	+ 41°	243	4.21		

[a] Thymolytic activity in adrenalectomized rat, oral route, hydrocortisone acetate = 1. Anti-inflammatory assay in immature adrenalectomized rat, cotton pellet implant. Assays by R. I. D. [b] Correct elemental analyses were obtained for all compounds. [c] Double m.p. 105° and 174–176°. [d] Amorphous. [e] Contracts at 110°.

Representative of the synthesis of I, II, III, IV, V, VI, VII, IX, X and XIII is the preparation of XI and XIV. 9α-Fluoro-16α-hydroxyhydrocortisone 16,17-acetonide 21-acetate[10] was converted to the 3-ethyl enol ether (m.p. 214–215°, $[\alpha]_D$ −5°, λ_{max} 241 mμ, log ε 4.31. Found for $C_{28}H_{39}FO_7$: C, 66.23; H, 7.73) which reacted with N-chlorosuccinimide to give the 6β-chloro-Δ^4-3-ketone (m.p. 185–186°, $[\alpha]_D$ +63°, λ_{max} 238 mμ, log ε 4.12. Found for $C_{26}H_{34}ClFO_7$: C, 60.58; H, 6.67; Cl, 6.62). Hydrogen chloride inversion

(1) Paper CVII, J. Iriarte, C. Djerassi and H. J. Ringold, THIS JOURNAL, **81**, in press (1959).

(2) A = Anti-inflammatory activity, oral route, hydrocortisone acetate = I.

(3) Salt assay in adrenalectomized rat without sodium chloride load.

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(6) (a) H. J. Ringold, G. Rosenkranz and F. Sondheimer, *J. Org. Chem.*, **21**, 239 (1956); (b) Ch. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956); (c) S. A. Szpilfogel, T. A. P. Posthumus, M. S. De Winter and D. A. Van Dorp, *Rec. trav. chim.*, **75**, 475 (1956); (d) K. Florey and A. R. Restivo, *J. Org. Chem.*, **22**, 406 (1957).

(7) A. Zaffaroni, H. J. Ringold, G. Rosenkranz, F. Sondheimer, G. H. Thomas and C. Djerassi, THIS JOURNAL, **80**, 1958.

(8) *Cf.* D. H. Peterson, *Record of Chemical Progress*, **17**, 211 (1956), for a review of related microbiological oxidations.

(9) R. Littell and S Bernstein, THIS JOURNAL, **78**, 984 (1956).

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afforded XI which was converted to the $\Delta^{1,4}$-dienone XIV by selenium dioxide oxidation.

(11) The Worcester Foundation for Experimental Biology.

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A MALONIC ACID DERIVATIVE AS AN INTERMEDIATE IN FATTY ACID SYNTHESIS

Sir:

Previous work[1,2] shows that a system of two enzyme fractions catalyzes the synthesis of palmitate from acetyl CoA in presence of Mn^{++}, ATP, TPNH and HCO_3^-. No intermediates could be demonstrated at the level of purity to which these two enzyme fractions (R_{1g} and R_{2g})[2] had been brought. After these fractions were further purified by ion exchange chromatography on cellulose, it became possible to carry out a stepwise synthesis. When R_{1g}, so purified (hereinafter designated as R_{1gc}), was incubated with acetyl CoA in presence of Mn^{++}, ATP and HCO_3^- and then the mixture boiled, a substance was formed which in presence of TPNH and the column-purified R_{2g} fraction (hereinafter referred to as R_{2gc}) was quantitatively converted to long-chain fatty acids (*cf.* Table I). In absence of any one of the four components or of R_{1gc} no intermediate was formed as measured by the extent of TPNH oxidation in the second reaction catalyzed by R_{2gc}.

TABLE I

REQUIREMENTS FOR FORMATION OF THE INTERMEDIATE AND STEPWISE SYNTHESIS OF FATTY ACIDS

Components of incubation mixture	Components added in addition to R_{2gc} and TPNH after heat deprot.	Acetyl CoA incorporation in mμmoles	TPNH oxidation in mμmoles
R_{1gc}, ATP, Mn^{++}, HCO_3^-, AcCoA	None	4.3	9.2
R_{1gc}, Mn^{++}, HCO_3^-, Ac-CoA	ATP	0.0	0.0
R_{1gc}, ATP, HCO_3^-, Ac-CoA	Mn^{++}	0.0	0.0
R_{1gc}, Mn^{++}, ATP, AcCoA	HCO_3^-	0.0	0.0
R_{1gc}, Mn^{++}, ATP, HCO_3^-	AcCoA	0.0	0.0

The complete system was composed of, in μmoles: ATP, 1; $MnCl_2$, 0.5; $KHCO_3$, 4; histidine buffer *p*H 6.5, 20; and 20 mμmoles of Ac-C^{14} CoA (63,000 cpm). Total volume was 0.4 ml.; 0.160 mg. of R_{1gc} was added, and the mixture was incubated for 15 minutes at 38°. Parallel tubes were prepared without one of these components. The reaction was stopped by heat denaturation. The clear filtrate was transferred to a cuvette which contained the missing component indicated above and 30 mμmoles of TPNH. To this mixture 0.3 mg. of R_{2gc} was added, and the reaction was followed spectrophotometrically at 340 mμ. At the end of five minutes the reaction was stopped and palmitate isolated.

The intermediate has these properties: (1) it moves with a different R_f (0.5) from acetyl CoA (0.72) in an ethanol–acetate chromatographic system; (2) it arises from acetyl CoA and CO_2 in equal amount as shown by radioactivity measurements; (3) it can be converted quantitatively to long-chain fatty acids by R_{2gc} in presence of TPNH; and (4) on hydrolysis and subsequent extraction an acid is isolated which contains the whole of the original radioactivity whether derived from C^{14}-acetyl CoA or $HC^{14}O_3^-$. This acid is indistinguishable from malonic acid when chromatographed in pentanol:formic; kerosene:acetic. Malonic acid was isolated in presence of carrier and recrystallized to constant specific activity (m.p. 135°); then converted to the *p*-nitrobenzyl ester which was also recrystallized to a constant specific activity (m.p. 85–86°). The radioactivity of the recrystallized malonic acid and its ester accounted for all the radioactivity of the intermediate.

The above evidence suggests that the first step in fatty acid synthesis is the carboxylation of acetyl CoA to a malonyl derivative catalyzed by the biotin-containing R_{1gc} fraction[2] in presence of ATP and Mn^{++}. The subsequent successive condensation and reductive steps are catalyzed by R_{2gc} in presence of TPNH. Malonic acid as such is not the intermediate.

Addendum.—Since submission of this manuscript a paper by Brady[3] has appeared which suggests that malonyl CoA can be converted to fatty acids in a crude pigeon liver system.

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(3) R. O. Brady, *Proc. Nat. Acad. Sci.*, **44**, 993 (1958).

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THE STEREOCHEMISTRY OF AMARYLLIDACEAE ALKALOIDS DERIVED FROM 5,10b-ETHANOPHENANTHRIDINE

Sir:

Only two stereochemical conformations (II and III) are possible for the alkaloids of the *Amaryllidaceae* derived from 5,10b-ethanophenanthridine (I). Structure II has been favored[1] because several of these alkaloids possess pharmacological properties similar to those of morphine.[2] The alkaloids haemanthamine[3] and haemanthidine,[4] although possessing the 5,10b-ethanophenanthridine nucleus, have been found devoid of such activity. Since these latter alkaloids must possess the nucleus represented by III to permit the formation of apohaemanthamine (IV, R = H) and apohaemanthidine (IV, R = OH), it would appear that phytochemical processes elaborate both stereochemical modifications. We have been able to demonstrate that all alkaloids known to possess the nucleus (I)

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(2) W. C. Wildman, THIS JOURNAL, **78**, 4180 (1956).

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