

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 6 α -CHLOROCORTICOIDS

Sir:

We have found that 6 β -chloro substitution among corticoids decreases thymolytic and anti-inflammatory activity and increases sodium retention, 6 α -chloro substitution in general not only enhances thymolytic and anti-inflammatory activity

tate on reaction with N-chloroacetamide in aqueous buffered acetone solution yielded 6 β -chloro-"S" acetate (m.p. 193-194°, [α]_D +41°, λ_{\max} 240 m μ , log ϵ 4.15. Found for C₂₃H₃₁ClO₆: 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 incubation⁷ or (2) fermentation with *Cunninghamella*⁸ *bainieri* ATCC 9244 gave 6 α -chloroprednisolone (XII). Adrenal or microbiological oxidation of XV followed by acetylation or hydrogen chloride-acetic acid treatment of 5 α ,6 α -oxido-3,20-bisethylenedioxyallopregnane-11 β ,17 α ,21-triol⁹ acetate gave VIII which was converted to XII acetate (m.p. 204-205°, [α]_D +68°, λ_{\max} 242 m μ , log ϵ 4.17. Found for C₂₃H₂₉ClO₆: C, 63.01; H, 6.68; Cl, 7.99) by selenium dioxide oxidation.

TABLE I

Compound ^b	Additional substituent	M.P. (dec.) °C.	[α] _D CHCl ₃	λ_{\max} (m μ) EtOH	log ϵ	Thymo- lytic ^a activity	Anti- inflamm. ^{2,a} activity
I Cortisone Ac.	6 β -Chloro-	178-179°	+129°	237	4.16	0.2	0.2
II Cortisone Ac.	6 α -Chloro-	197°	+176°	233	4.16	1.3	1
III Cortisone Ac.	6 β -Chloro-9 α -fluoro	221°	+80°	234	4.18	1.5	1
IV Cortisone Ac.	6 α -Chloro-9 α -fluoro	214°	+132°	230	4.16	15	8
V Prednisone Ac.	6 β -Chloro-	221-222°	+132°	241	4.17	0.3	0.3
VI Prednisone Ac.	6 α -Chloro-	217-218°	+144°	237	4.19	4	4
VII Prednisone Ac.	6 α -Chloro-9 α -fluoro	227-229°	+122°	235	4.18	18	14
VIII Hydrocortisone Ac.	6 α -Chloro	174-176° ^c	+97	238	4.08	5	11
IX Hydrocortisone Ac.	6 β -Chloro-9 α -fluoro	194-195°	+44°	238	4.17	0.5	0.5
X Hydrocortisone Ac.	6 α -Chloro-9 α -fluoro	134-143° ^d	+88°	234	4.11	5	11
XI Hydrocortisone Ac.	6 α -chloro-9 α -fluoro- 16 α -hydroxy-16,17- acetone	156-157°	+90°	234	4.17	100	50
XII Prednisolone	6 α -Chloro	195-196°	+61° (Diox.)	242	4.19	14	13
XIII Prednisolone Ac.	6 α -Chloro-9 α -fluoro	150° ^{d,e}	+71°	238	4.19	27	40
XIV Prednisolone Ac.	6 α -Chloro-9 α -fluoro- 16 α -hydroxy-16,17- acetone	294-296°	+53°	238	4.17	400	200
XV "S" Acetate	6 α -Chloro	189-190°	+87°	237	4.18		
XVI Δ^1 -Dehydro "S" Ac.	6 α -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°.

but also modifies biological activity toward sodium excretion. Thus 6 α -chloroprednisone acetate (A² = 4) and 6 α -chloroprednisolone (A² = 13) exhibit a marked sodium excretion³ while 6 α -chloro-9 α -fluoroprednisolone acetate (A = 40) is only a moderate sodium retainer in contrast to the enormous sodium retention of the C-6 unsubstituted compound.⁴

6 α -Chloro-9 α -fluoro-16 α -hydroxyhydrocortisone 21-acetate 16,17-acetonide (XI) and the corresponding 1-dehydro compound (XIV), with respective anti-inflammatory activities² of 50 and 200 \times hydrocortisone exhibit sodium excretion.

The 3-ethyl enol ether⁵ of Reichstein's "S" ace-

Representative of the synthesis of I, II, III, IV, V, VI, VII, IX, X and XIII is the preparation of XI and XIV. 9 α -Fluoro-16 α -hydroxyhydrocortisone 16,17-acetonide 21-acetate¹⁰ was converted to the 3-ethyl enol ether (m.p. 214-215°, [α]_D -5°, λ_{\max} 241 m μ , log ϵ 4.31. Found for C₂₈H₃₉FO₇: C, 66.23; H, 7.73) which reacted with N-chlorosuccinimide to give the 6 β -chloro- Δ^4 -3-ketone (m.p. 185-186°, [α]_D +63°, λ_{\max} 238 m μ , log ϵ 4.12. Found for C₂₆H₃₄ClFO₇: C, 60.58; H, 6.67; Cl, 6.62). Hydrogen chloride inversion

(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).

(10) J. Fried, A. Borman, W. B. Kessler, P. Grabowich, E. F. Sabo, *ibid.*, **80**, 2338 (1958).

(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 = 1.

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

(4) J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman, and F. M. Singer, *THIS JOURNAL*, **77**, 4181 (1955).

(5) P. L. Julian, E. W. Meyer, W. J. Karpel and W. Cole, *ibid.*, **73**, 1982 (1951).

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

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 μ moles	TPNH oxidation in μ moles
R_{1gc} , ATP, Mn^{++} , HCO_3^- , AcCoA	None	4.3	9.2
R_{1gc} , Mn^{++} , HCO_3^- , AcCoA	ATP	0.0	0.0
R_{1gc} , ATP, HCO_3^- , AcCoA	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 μ moles: ATP, 1; $MnCl_2$, 0.5; $KHCO_3$, 4; histidine buffer pH 6.5, 20; and 20 μ moles of Ac-C¹⁴ 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 μ moles of TPNH. To this mixture 0.3 mg. of R_{2gc} was added, and the reaction was followed spectrophotometrically at 340 m μ . At the end of five minutes the reaction was stopped and palmitate isolated.

(1) D. M. Gibson, E. B. Titchener and S. J. Wakil, *THIS JOURNAL*, **80**, 2908 (1958).

(2) S. J. Wakil, E. B. Titchener and D. M. Gibson, *Biochim. Biophys. Acta*, **29**, 225 (1958).

measured by the extent of TPNH oxidation in the second reaction catalyzed by R_{2gc} .

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 C¹⁴-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} fraction² 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 Brady³ has appeared which suggests that malonyl CoA can be converted to fatty acids in a crude pigeon liver system.

(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 favored¹ because several of these alkaloids possess pharmacological properties similar to those of morphine.² The alkaloids haemanthamine³ 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)

(1) N. Sugimoto and H. Kugita, *Pharm. Bull.*, **5**, 378 (1957).

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

(3) H. M. Fales and W. C. Wildman, *Chemistry & Industry*, 561 (1958).

(4) S. Uyeyo, H. M. Fales, R. J. Highet and W. C. Wildman, *THIS JOURNAL*, **80**, 2590 (1958).