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.

(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).

(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:

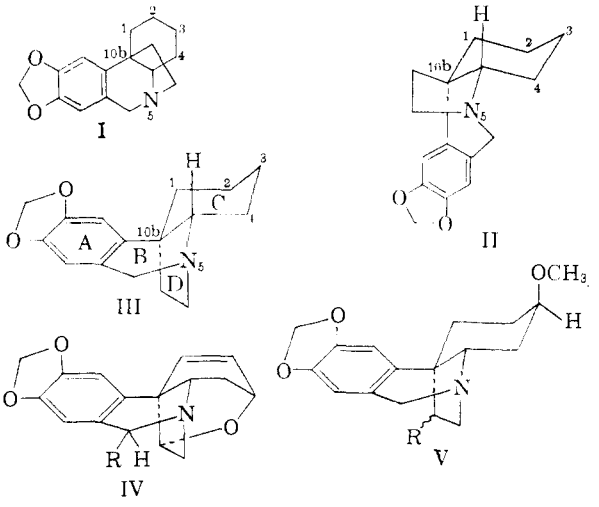
Only two stereochemical conformations (II and III) are possible for the alkaloids of the *Amaryllidaceae* derived from 5,10b-ethanophenanthridine (I). Structure II has been favored[1] because several of these alkaloids possess pharmacological properties similar to those of morphine.[2] The alkaloids 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)

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



are based on the stereostructure III and its mirror image.

Dihydrohaemanthamine[3] (V, R = OH), was treated with thionyl chloride for one hour at 78°. The excess reagent was removed and the residue was refluxed with an excess of lithium aluminum hydride in tetrahydrofuran for 5 hours. The reaction product, desoxydihydrohaemanthamine, isolated in 52% yield, was identical in melting point (95–97°) and infrared spectrum (KBr) with dihydrobuphanisine (V, R = H).[5] A mixture melting point determination was depressed below 70°. The optical rotatory dispersion curves (330–700 mμ) in chloroform of the product ($[\alpha]^{25}D$ +28°) and dihydrobuphanisine ($[\alpha]^{25}D$ −28°) revealed that the two substances were optical antipodes.

This transformation identifies the alkaloids hydroxylated in the 5-membered D ring (haemanthamine, haemanthidine, crinamine[3] and haemultine[6]) with the (+)-crinane nucleus (III) while the alkaloids not hydroxylated in this position, crinine, powelline, buphanidrine, buphanisine, undulatine, and buphanamine, have been shown to contain the enantiomorphic (−)-crinane nucleus.[5,7,8,9] Finally, it seems likely that pharmacological differences between the alkaloids of this nucleus stem from the absolute configurations of the bases rather than the stereochemical differences associated with the fusion of the octahydroindole ring.

(5) H. M. Fales and W. C. Wildman, THIS JOURNAL, **80**, 4395 (1958).

(6) H.-G. Boit and W. Döpke, *Chem. Ber.*, **91**, 1965 (1958).

(7) E. W. Warnhoff and W. C. Wildman, *Chemistry & Industry*, 1293 (1958).

(8) W. C. Wildman, THIS JOURNAL, **80**, 2567 (1958).

(9) These observations should not be interpreted as a general rule, since the occurrence of vittatine, the optical antipode of crinine, has been reported.[10]

(10) H.-G. Boit and H. Ehmke *Chem. Ber.*, **90**, 369 (1957).

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BOOK REVIEWS

Biochemical Preparations. Volume 5. DAVID SHEMIN, Editor-in-Chief. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1957. ix + 115 pp. 15.5 × 23.5 cm. Price, $4.75.

This volume, the fifth in the series, gives comprehensive procedures for the synthesis or isolation of over thirty substances of special interest to biochemists. The excellent style and format, resembling the "Organic Syntheses" series established in previous volumes, are maintained. The utility of the volume is increased by the inclusion of a cumulative subject index, a reference list of compounds of biochemical interest which have appeared in "Organic Syntheses" (through volume 37), a section on the properties and purity of each product and cross references given in the description of each preparation. The reliability and feasibility of each procedure submitted has been checked by an independent expert, whose comments appear as separate notes. The fifth volume of the series is another notable achievement of the objectives of the series as stated in the preface of the first volume: "to provide authoritative, thoroughly checked preparations for substances used in biochemical research and to provide preparations illustrating manipulative techniques and methods that may be useful both to research workers and to students."

A list of the contents of volume five follows: dibenzyl phosphorochloridate, phosphatidyl ethanolamine, sodium phosphocreatine, aldolase, crystalline condensing enzyme, S-succinyl coenzyme A, cytochrome c (addendum) and reduced cytochrome c, separations of nucleotides of ribonucleic acid, separation of 5′-deoxyribonucleotides, nicotinamide mononucleotide (NMN), S-adenosylmethionine (AMe), adenine-8-C^{14}, D-glyceric acid 2-phosphate (trisodium salt), 2-deoxy-D-ribose, L-glutamine and D-glutamine, S-benzyl-DL-homocysteine, S-benzyl-L-homocysteine, and S-benzyl-D-homocysteine, homocystine and homocysteine, cyanomethylimidazole and imidazoleacetic acid hydrochloride, formiminoglycine, formimino-L-aspartic acid, formimino-L-glutamic acid.

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Encyclopedia of Chemical Reactions. Volume VII. Strontium, Sulfur, Tantalum, Technetium, Tellurium, Terbium, Thallium, Thorium, Thulium, Tin and Titanium. Compiled by C. A. JACOBSON, Late Professor of Chemistry, West Virginia University. Edited by CLIFFORD A. HAMPEL, Manager, Chemical Equipment Division, Fansteel Metallurgical Corporation, Editor, "Rare Metals Handbook." Reinhold Publishing Corporation, 430 Park Avenue, New York 22, N. Y. 1958. 479 pp. 16 × 23.5 cm. Price, $12.75.

This seventh volume of the "Encyclopedia of Chemical Reactions" retains essentially the features of its predecessors. The early literature on a large variety of reactions is well covered; the indexes are remarkably complete; the book is printed and bound in a readable and attractive style. That some effort has been made to counter criticisms of earlier volumes is evidenced by the appearance of references as recent as 1957.

A number of the elements included in this volume have been studied intensively during the past fifteen years, and it is unfortunate that more of this new chemistry has not been included. Hydride reactions are omitted almost com-