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).

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