reagent.[8] Table I demonstrates the progression of this reaction with time and demonstrates the requirement for PMS.

TABLE I

CONVERSION OF Δ^4-ESTRENE-3,17-DIONE TO ESTRONE BY Δ^1-DEHYDROGENASE

		μM Phenol (as estrone) Incubation time, min. 0	45	60	90
(1)	Complete system[a]	0.10	0.42	0.48	0.63
(2)	Enzyme omitted[b]	.11	.10	.09	.09
(3)	Estrone synthesized, (1) minus (2)	..	.32	.39	.54
(4)	PMS omitted[c]	.03	.05	.04	.05
(5)	Steroid omitted	.01	.02	.01	.02

[a] The reactions were carried out in air with agitation at 30° in 4 ml. systems containing 115 μM phosphate buffer, *p*H 7.2, and the following additions in the complete system. 0.2 ml. enzyme (supernatant from 105,000 × *g* centrifugation, see text) containing 4.3 mg. protein; 1.84 μM Δ^4-estrene-3,17-dione in 0.1 ml. acetone (or 0.1 ml. acetone only when steroid was omitted); and 3.1 μM phenazine methosulfate (added last). At the end of the reaction the mixture was acidified with 0.3 ml. of concd. HCl, extracted three times with a total of 12 ml. of CH_2Cl_2, the extract dried over Na_2SO_4, and a 5-ml. aliquot evaporated to dryness and analyzed by the Folin reaction.[8] A standard curve was prepared using estrone. [b] A small blank which did not increase on incubation was always found when PMS and steroid were mixed either in the absence of enzyme or in the presence of acid-inactivated enzyme. [c] The sample of Δ^4-estrene-3,17-dione was found to be contaminated with about 1–2% phenol, presumably estrone.

It is believed that at least two and probably three separate enzymes are involved in these dehydrogenating reactions (Δ^1-, Δ^4-5α- and Δ^4-5β-dehydrogenases), and that probably these enzymes are flavoproteins.

This work has been supported by a grant from the American Cancer Society. We are indebted to the U. S. Public Health Service for a fellowship (H.R.L.) and to the American Cancer Society for a Scholarship (P.T.).[9]

(8) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(9) Dr. D. H. Peterson and colleagues have observed the transformation of 19-*nor*-testosterone to estrone and estradiol-3,17β by *Septomyxa affinis* (personal communication).

BEN MAY LABORATORY FOR CANCER RESEARCH
AND DEPARTMENT OF BIOCHEMISTRY H. RICHARD LEVY
THE UNIVERSITY OF CHICAGO PAUL TALALAY
CHICAGO, ILLINOIS

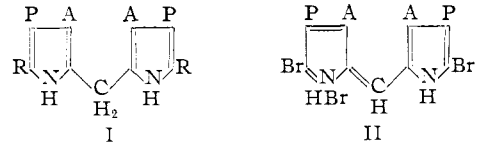
RECEIVED MARCH 18, 1957

DIPYRRYLMETHANES

Sir:

Although dipyrrylmethane itself and nuclear carboxy, acyl, and hydroxy derivatives are known, those without such groups or their vinylogs are not, being presumed to be very unstable.[1] It now appears that some dipyrrylmethanes of the latter type, which may be intermediates in the biosynthesis of porphyrins, may be obtained by and undergo conventional pyrrole reactions without fission or oxidation at the bridge.

For example, I (R = H) forms nearly colorless micro-prisms without visible absorption, m.p. 199° dec., Ehrlich's reaction strongly positive cold. (Calcd. for $C_{19}H_{22}N_2O_8$: C, 56.15; H, 5.46; N, 6.89; eq. wt., 101.6. Found: C, 56.35; H, 5.57; N, 6.80; eq. wt., 103.2.) It was obtained in 80% yield from I (R = COOH)[2] with 10% sodium hydroxide for four hours at 170°, and also from II[2] with sodium amalgam. Diazomethane converted I (R = H) into its tetramethyl ester III, m.p. 105°, (Calcd. for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54; N, 6.06. Found: C, 59.58; H, 6.59; N, 6.10) which gave IV (80%), the ester of I (R = CHO), m.p. 203°, with hydrogen cyanide and hydrogen chloride (Calcd. for $C_{25}H_{30}N_2O_{10}$: C, 57.91; H, 5.83; N, 5.40. Found: C, 57.85; H, 5.73; N, 5.34).



A = CH_2·COOH; P = CH_2CH_2·COOH

Uroporphyrin II[2] was obtained from I (R = H) with formic acid and hydrogen bromide–acetic acid at 100° (~ 20%, methyl ester, m.p. *ca.* 310°, degraded to coproporphyrin II methyl ester, m.p. 284–286°) and also from III with IV in methanolic hydrogen bromide at 20° followed by warming with aqueous sodium hydroxide (~ 25%, methyl ester, m.p. *ca.* 313–315°, degraded to coproporphyrin II methyl ester, m.p. 285–286°). Under these last conditions neither III nor IV separately gave any porphyrin.

(2) S. F. MacDonald and K. H. Michl, *Canad. J. Chem.*, **34**, 1768 (1956).

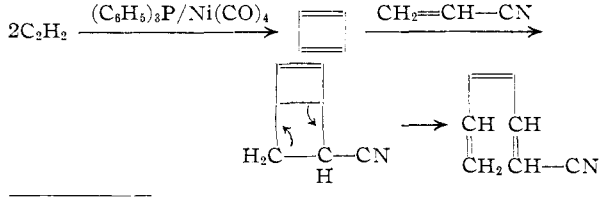
CONTRIBUTION No. 4351
DIVISION OF PURE CHEMISTRY S. F. MACDONALD
NATIONAL RESEARCH COUNCIL OF CANADA
OTTAWA, CANADA

RECEIVED MARCH 18, 1957

A MECHANISM STUDY OF THE 2,4,6-HEPTATRIENENITRILE SYNTHESIS FROM ACRYLONITRILE AND ACETYLENE

Sir:

The existence of transition-metal complexes of cyclobutadiene as intermediates in reactions of acetylene has been suggested recently.[1] For example, it was proposed that the cycloöctatetraene synthesis from acetylene in the presence of nickel cyanide catalyst involves the intermediate complex $Ni(CN)_2 \cdot C_4H_4$. As an extension of this concept, it seemed reasonable to postulate that the heptatrienenitrile synthesis from acrylonitrile and acetylene[2] could be pictured as



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