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

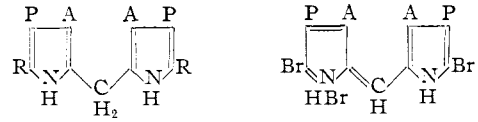
RECEIVED MARCH 18, 1957

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).

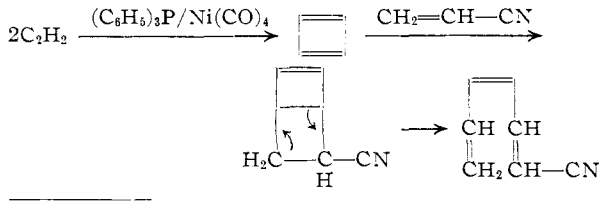
CONTRIBUTION NO. 4351
DIVISION OF PURE CHEMISTRY S. F. MACDONALD
NATIONAL RESEARCH COUNCIL OF CANADA
OTTAWA, CANADA

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



(1) H. C. Longuet-Higgins and L. E. Orgel, *J. Chem. Soc.*, 1969 (1956); private communication with one of the authors.

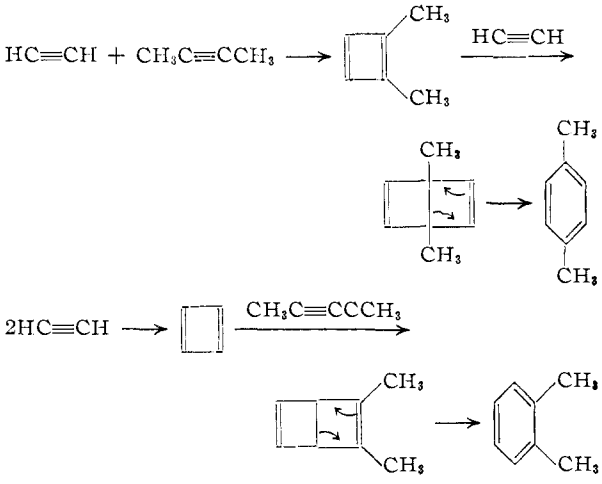
(2) T. L. Cairns, V. A. Engelhardt, H. L. Jackson, G. H. Kalb and J. C. Sauer, THIS JOURNAL, **74**, 5636 (1952).

(1) H. Fischer and H. Orth, "Chemie des Pyrrols," I, 334; II/i, 4. Leipzig, 1934 and 1937.

This reaction sequence necessitates having the terminal methylene group of the heptatrienenitrile derived from the acrylonitrile rather than from the acetylene. A tracer study was carried out to test this hypothesis.

For this purpose, C^{14}-labeled 2,4,6-heptatrienenitrile was prepared by the reaction of labeled acrylonitrile ($\overset{*}{C}H_2=\overset{*}{C}H$-CN) with acetylene in the presence of a nickel carbonyl/triphenylphosphine catalyst. The terminal methylene group in the heptatrienenitrile was then removed by ozonolysis and found to contain virtually no C^{14}. This result affords strong evidence that the terminal methylene group in heptatrienenitrile is derived from acetylene and not from acrylonitrile. Accordingly, it appears that heptatrienenitrile is not formed by a cyclobutadiene mechanism. Similar results were obtained in parallel experiments with labeled methyl 2,4,6-heptatrienoate prepared from acetylene and labeled methyl acrylate.

Another extension of the cyclobutadiene mechanism would suggest that some *p*-xylene, as well as *o*-xylene, should be formed in the cotrimerization of dimethylacetylene with acetylene.



We were, however, unable to detect any *p*-xylene by ultraviolet or infrared analyses.

The labeled acrylonitrile (rel. molar activity 3.8 × 10^5 dis./min.) was prepared by pyrolysis of labeled lactonitrile acetate at 555–560°. The acetate was made by heating labeled vinyl acetate, hydrogen cyanide and potassium cyanide catalyst.[3] Labeled 2,4,6-heptatrienenitrile (rel. molar activity 3.9 × 10^5 dis./min.) was prepared by injecting acetylene into the labeled acrylonitrile.[2] The heptatrienenitrile was ozonized in methylene chloride by the procedure of Clemo and Macdonald.[4] The crude formaldehyde containing other ozonolysis products was converted into the dinitrophenylhydrazone (m.p. 151–158° after recrystallization from methanol (0.19 g., 13%), rel. molar activity 0.4 × 10^5, or a drop in activity of 89.7%). The dinitrophenylhydrazone recovered from the mother liquor (0.14 g., 10%) had a relative molar activity of 0.3 × 10^5, or a drop in activity of 92.3%. The derivative appeared to be essentially pure dinitrophenylhydrazone of formaldehyde based on infrared analysis.

Tagged methyl heptatrienoate (rel. molar activity 60 × 10^4 dis./min.) was prepared from tagged methyl acrylate and acetylene.[2] Ozonolysis gave formaldehyde whose dinitrophenylhydrazone showed a drop in relative molar activity of 98%.

In the dimethylacetylene/acetylene cotrimerization, the reactants were heated with a $Ni(CO)_2/[C_6H_5)_3P]_2$ catalyst in tetrahydrofuran at 80–165° under a bomb gage pressure of 5–15 atm. for 3 hr. Benzene, styrene and *o*-xylene were identified as products. There was no indication of the presence of even small amounts of *p*-xylene (based largely on infrared and ultraviolet spectral data).

CONTRIBUTION NO. 416
CHEMICAL DEPARTMENT
EXPERIMENTAL STATION
E. I. DU PONT DE NEMOURS & CO.
WILMINGTON, DELAWARE
J. C. SAUER
T. L. CAIRNS

RECEIVED APRIL 4, 1957

(3) E. L. Carpenter, British Patent 591,489 (1947).

(4) G. R. Clemo and J. McL. Macdonald, *J. Chem. Soc.*, 1294 (1935).

THE SYNTHESIS OF D-GULOSAMINE

Sir:

A new aminosugar has been isolated recently from streptothricin and streptolin B and the structure of a 2-aminosugar, D-gulosamine, has been proposed for it.[1] This appears to be the first reported isolation of a naturally occurring 2-aminohexose other than the well known D-glucosamine and D-galactosamine. It is also the first isolation of a naturally occurring sugar with the gulose configuration.

We wish to report the synthesis of a 2-aminohexose, possessing the D-gulosamine configuration and also having identical properties to the naturally isolated compound described above. Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside[2] treated with methanesulfonyl chloride in pyridine solution gave an 86% yield of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methylsulfonyl-α-D-galactopyranoside (I), m.p. 219–220°, $[\alpha]^{24}D$ +169° (*c* 1.12, $CHCl_3$). *Anal.* Calcd. for $C_{17}H_{23}O_8NS$: C, 50.86; H, 5.77; S, 7.99. Found: C, 50.93; H, 5.86; S, 7.90. Hydrolysis of I with 60% acetic acid afforded a quantitative yield of methyl 2-acetamido-2-deoxy-3-*O*-methylsulfonyl-α-D-galactopyranoside (II), m.p. 179–180°, $[\alpha]^{22}D$ +132° (*c* 0.88, CH_3OH). *Anal.* Calcd. for $C_{10}H_{19}O_8NS$: C, 38.33; H, 6.11. Found: C, 38.48; H, 6.22. It was characterized by the 4,6-di-*O*-acetyl derivative, m.p. 163–164°, $[\alpha]^{24}D$ +96° (*c* 0.83, $CHCl_3$). *Anal.* Calcd. for $C_{14}H_{23}O_{10}NS$: C, 42.31; H, 5.83. Found: C, 42.22; H, 5.79. A solution of II in methyl cellosolve heated in the presence of sodium acetate[3] gave a product, subsequently acetylated with pyridine and acetic anhydride. After purification by chromatography, a 56% yield of methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-gulopyranoside (III) was obtained;

(1) E. E. Van Tamelen, J. R. Dyer, H. E. Carter, J. V. Pierce and E. E. Daniels, THIS JOURNAL, **78**, 4817 (1956).

(2) P. J. Stoffyn and R. W. Jeanloz, *ibid.*, **76**, 561 (1954).

(3) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, *ibid.*, **76**, 4044 (1954).