TABLE I

OXIDATIVE PHOSPHORYLATION BY BEEF HEART MITOCHONDRIA AND SOLUBLE PREPARATION

Each vessel contained 27.2 μmoles of phosphate buffer, *p*H 7.4, containing P^{32} (1187 c.p.m./μmole phosphorus), 20 μmoles of $MgCl_2$, 10 μmoles of ADP, 50 μmoles of glucose, 6 mg. of hexokinase; and where indicated, 20 μmoles of pyruvate, 5 μmoles of malate, 10 μmoles of TPNH; 0.3 ml. of mitochondria, 0.5 ml. of $S_{25,000}$ enzyme fraction as indicated. Final volume 3.0 ml., incubated 15 minutes at 30°. The reaction was stopped by adding 0.2 ml. of 3 *M* TCA. Inorganic phosphate was measured colorimetrically using a 0.1-ml. aliquot. Differences from the control of 0.3–0.4 optical density units were found, where Δo.d. of 0.20 represents 0.27 μmole of P. Esterified phosphorus was measured using the extraction procedure of Cooper and Lehninger.[3] The amount of phosphorus esterified agreed by the two methods used. The mitochondria were prepared by the method of Hogeboom and Schneider[4] and used after storing for 24 hours at −10°. The $S_{25,000}$ fraction was the supernatant layer obtained after centrifuging a cell homogenate at 0° for three hours at 25,000 × *g*, in two volumes of medium containing 0.9% KCl and 0.001 *M* ethylenediamine tetraacetate ("Versene"). The supernatant fraction was dialyzed against 2 liters of 0.02 *M* tris buffer, *p*H 7.4 for 12 hours. In other experiments not shown, the $S_{105,000}$ fraction was employed with similar results.

Enzyme added	Substrate	μatoms oxygen consumed Expt. I	μatoms oxygen consumed Expt. II	μatoms P esterified Expt. I	μatoms P esterified Expt. II	P/O Expt. I	P/O Expt. II
Mitochondria	Pyruvate + malate	11.8	14.1	23.3	24.8	2.0	1.8
Mitochondria + $S_{25,000}$	Pyruvate + malate	10.7	11	24.4	25.2	2.3	2.3
$S_{25,000}$	Pyruvate + malate	0	...	0	...	0	...
Mitochondria	TPNH	5.4	10.7	0.6	2.0	0.1	0.2
Mitochondria + $S_{25,000}$	TPNH	5.9	9.1	9.7	18.9	1.6	2.1
$S_{25,000}$	TPNH	0	...	0	...	0	...

the present findings may suggest an *in vivo* cooperation between mitochondria and "soluble" enzymes, that could permit utilization of energy from such systems as the pentose cycle.

(3) C. Cooper and A. L. Lehninger, *J. Biol. Chem.*, **219**, 489 (1956).
(4) G. H. Hogeboom and W. C. Schneider, *ibid.*, **194**, 513 (1952).
(5) Postdoctoral Fellow of the United States Public Health Service.

SCIENCE RESEARCH INSTITUTE AND DEPARTMENT OF CHEMISTRY
OREGON STATE COLLEGE
CORVALLIS, OREGON

VERNON H. CHELDELIN
CARL WIDMER[5]
SUDHA JOSHI
R. W. NEWBURGH

RECEIVED NOVEMBER 12, 1956

OXIDATION OF CYCLOÖCTATETRAENE TO THE TROPYLIUM CATION

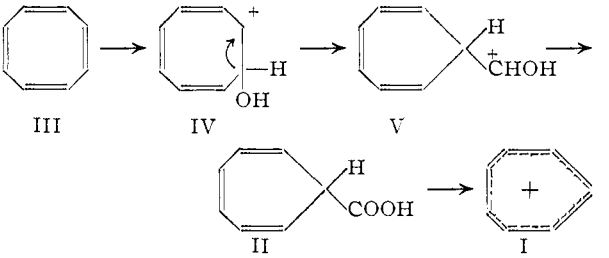
Sir:

In a previous paper[1] it was stated that the tropylium cation (I) was oxidized readily by permanganate to benzaldehyde. During subsequent experiments, designed to see whether this reaction could be used as a specific test for the presence of small amounts of tropylium salts in aqueous solution, we have found that norcaradiene-carboxylic acid (II)[2] is similarly readily oxidized to benzaldehyde. The tropylium cation probably is formed as an intermediate in the latter reaction for we have been able to isolate tropylium salts in yields of up to 30% from the reaction mixture. We have obtained similar results,[3] though with varying yields of tropylium salts, following the oxidation of norcaradiene-carboxylic acid with other oxidants, *e.g.*, ceric salts, $Pb(OAc)_4$, HIO_4, $Na_2S_2O_8$, and we consider that a common two-electron transfer mechanism operates in most of these reactions.

Other oxidation products of norcaradiene-carboxylic acid with permanganate are benzoic acid and benzaldehyde. Terephthalic acid also was obtained when chromic acid in acetic acid was used as the oxidant. There appears to be a close parallel between these results and the oxidation products of cycloöctatetraene (III). Reppe and co-workers report[4] that neutral permanganate oxidizes III to benzoic acid and benzaldehyde, while chromic acid oxidizes it to benzaldehyde and terephthalic acid. It seemed possible therefore that norcaradiene-carboxylic acid might be formed as an intermediate in the oxidation of cycloöctatetraene and, in view of the results described above, tropylium salts also could be formed.[5]

We have now found that appreciable yields (5%) of tropylium salts can be isolated from the reaction products following the oxidation of cycloöctatetraene with permanganate in dilute sulfuric acid–acetone mixtures. To isolate the tropylium salt it is necessary to first extract the other neutral and acidic products of the reaction, then convert the water soluble tropylium sulfate to ditropyl ether, which is then extracted and converted to tropylium bromide with HBr. This method certainly involves losses and the figure of 5% is the minimum yield of tropylium sulfate formed in the reaction. When the oxidation is performed under neutral or alkaline conditions the yield of tropylium salt is negligible.

We consider that the formula scheme shown adequately accounts for the formation of the tropylium cation in this reaction.

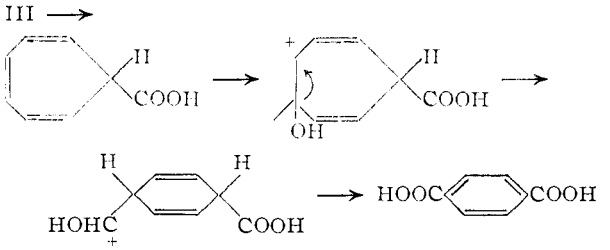


The first step is the formation of a glycol which, in the acid solution used, gives rise to the ion IV. This then undergoes the normal pinacol–pinacolone rearrangement to give the cation V, which is pro-

(1) M. J. S. Dewar and R. Pettit, *J. Chem. Soc.*, 2026 (1956).
(2) The absolute structure of norcaradiene-carboxylic acid is not yet established definitely.[3] A 1,3,5-cycloheptatriene formulation is used in this paper.
(3) Full details to be published elsewhere.
(4) W. Reppe, *et al.*, *Ann.*, **560**, 1 (1948).
(5) W. von E. Doering, *et al.*, THIS JOURNAL, **78**, 5448 (1956).

tonated norcaradienecarboxaldehyde. Oxidation of V gives norcaradienecarboxylic acid II which then forms the tropylium cation by a two electron transfer process as outlined earlier. The tropylium cation is further oxidized to benzaldehyde and benzoic acid.

The formation of terephthalic acid by oxidation of III with chromic acid in acetic acid probably is due to the formation of norcaradienecarboxylic acid as given above, followed by a second similar oxidation-rearrangement, thus



As mentioned previously we have found that chromic acid oxidation of norcaradienecarboxylic acid gave terephthalic acid and no tropylium salts could be isolated.

Acknowledgment.—We wish to thank Professor M. J. S. Dewar for valuable discussion and the D.S.I.R. for a maintenance grant to C.R.G.

CHEMISTRY DEPARTMENT
QUEEN MARY COLLEGE
UNIVERSITY OF LONDON
LONDON, ENGLAND

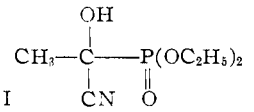
C. R. GANELLIN
R. PETTIT

RECEIVED MARCH 8, 1957

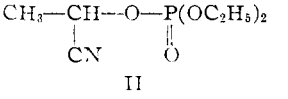
A REARRANGEMENT TO FORM DIETHYL 1-CYANOETHYL PHOSPHATE

Sir:

In a recent report[1] the preparation, some properties and pyrolysis of a compound believed to be diethyl 1-cyano-1-hydroxyethylphosphonate (I)

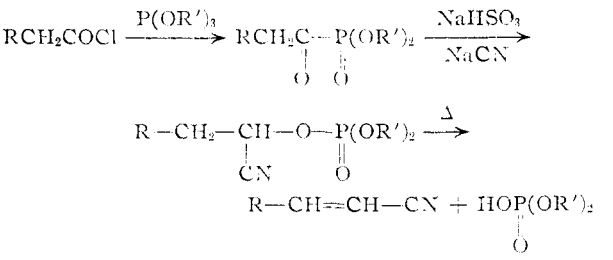


were recorded. More recent analysis by the proton NMR method on a freshly distilled sample of this material, b.p. 91–92° (0.5 mm.), $n^{26}D$ 1.4130, showed a tertiary hydrogen and no hydroxyl hydrogen. The spectrum of the tertiary hydrogen consisted of two superimposed quadruplets which is the proper spectrum for a hydrogen adjacent to a methyl and a phosphate group. This coupled with the earlier observation[1] that the compound would not give an acetyl derivative and that its infrared spectrum showed no absorption in the hydroxyl region leads to the conclusion that the material in question is diethyl 1-cyanoethyl phosphate (II).



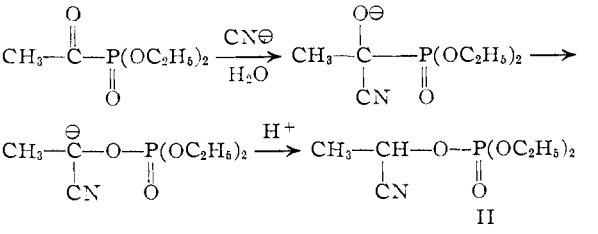
This conclusion is further strengthened by the isolation of acrylonitrile in high (83%) yield upon the pyrolysis of this material.[1] The formation of acrylonitrile would be expected from the pyrolysis of II far more logically than from the pyrolysis of I.

This series of reactions which led to acrylonitrile in excellent yield may demonstrate a general and useful route for the preparation of unsaturated nitriles (at least on a laboratory scale) starting with the readily available acyl halides provided a β-hydrogen atom is present

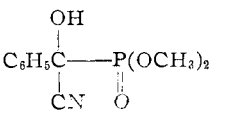


As was reported by earlier workers,[2] the expected product from the reaction of an α-keto phosphonate, sodium bisulfite and sodium cyanide would be a phosphonate, such as I.

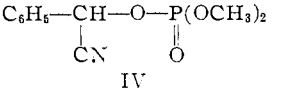
Apparently a rearrangement took place during its preparation under alkaline conditions; for example, as outlined below.



It is believed that the earlier Soviet workers[2] also had phosphate rather than phosphonate materials as products from their reactions. They reported preparation of III which on acid hydrol-



ysis gave mandelic acid, $C_6H_5CHOHCOOH$. It is more likely that they had IV, from which the



formation of mandelic acid upon acid hydrolysis would then be expected.

Recently Barthel, *et al.*,[3] and Lorenz, *et al.*,[3] reported the rearrangement of a hydroxyphospho-

(1) L. A. R. Hall and C. W. Stephens, THIS JOURNAL, **78**, 2565 (1956).

(2) M. I. Kabachnik, P. A. Rossiiskaya and E. S. Shepeleva, *Bull. Acad. Sci. U.R.S.S., Classe Sci. Chim.*, 163 (1947); *C. A.*, **42**, 4133 (1948).

(3) W. F. Barthel, B. H. Alexander, P. A. Giang and S. A. Hall, THIS JOURNAL, **77**, 2424 (1955); W. Lorenz, A. Henglein and G. Schrader, *ibid.*, **77**, 2554 (1955).