—The reaction was carried out by adding gradually the 1,2-quinone to an ethereal solution of diazoethane or 9-diazofluorene or to the benzene solution of diphenyldiazomethane (*cf.* Table II). The final reaction products were obtained by evaporation of the solvent and working up the residues as follows.

The crystals that separated after evaporation of the solvent in the case of IIb, were washed with methyl alcohol.

The oil residue obtained in the case of Ia was triturated with hot methyl alcohol and kept aside in the ice-chest overnight and the brownish solid that separated was then crystallized.

The solid residue obtained in the case of Ic was treated with few ml. of ether, filtered and crystallized.

The yellowish-brown solid deposit in the case of Ib, that was obtained on cooling the reaction mixture, was crystallized.

The solid residue obtained in the case of III was washed with cold benzene and crystallized.

Action of Hydrochloric Acid. (a) **IIb.**—A solution of 0.2 g. of IIb in a mixture of 30 ml. of methyl alcohol and 5 ml. of concentrated hydrochloric acid (d. 1.18) was refluxed for 40 minutes. The reaction mixture was concentrated and the cooled solution was then poured into ice and the crystals that separated after some time were collected and shown to be unchanged IIb (m.p. and mixed m.p.). The yield was almost quantitative.

(b) **Ic.**—A suspension of 0.1 g. of Ic and 12 ml. of concentrated hydrochloric acid was heated (steam-bath) for 20 minutes. The mixture was cooled, filtered off and the solid was crystallized from dilute methyl alcohol in colorless crystals. Ic was recovered almost quantitatively (m.p. 168° and mixed m.p.).

The above experiment was repeated, using 0.25 g. of Ic and 20 ml. of concentrated hydrochloric acid and the reaction mixture was refluxed for four hours. It was filtered while hot and left to cool. The colorless crystals, that separated, were recrystallized from dilute alcohol and identified as 1,2-dihydroxy-4-cyanonaphthalene.[12]

(c) **III.**—The orange solution of 0.2 g. of III in 50 ml. of glacial acetic acid was treated with 1 ml. of concentrated hydrochloric acid. The reaction mixture was heated (steam-bath) for 15 minutes, during which it acquired a deep violet color. The cold reaction mixture was then poured into an ice-water mixture and the blue-violet solid, that separated, was filtered off, washed with alcohol and dried. It was identified as IV by its properties and its transformation into the diacetate of 3,4-dihydroxy-1,2-benzophenazine.[4]

(12) W. Bradley and R. Robinson, *J. Chem. Soc.*, 1484 (1934). We found that 1,2-dihydroxy-4-cyanonaphthalene gives in alcoholic solution a red color with a 15% aqueous titanium trichloride solution (free from iron) (obtained from E. Merck, Darmstadt, Germany).

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COMMUNICATIONS TO THE EDITOR

THE ENZYMATIC FORMATION OF 4-AMINO-5-IMIDAZOLECARBOXAMIDE RIBOTIDE FROM INOSINIC ACID[1]

Sir:

An enzyme system from pigeon liver, inosinic acid transformylase, has been described which incorporates radioactive formate into the 2-position of inosinic acid.[2] A stimulatory effect of leucovorin on this reaction was demonstrated. The postulation was made that the purine ring was cleaved at the 2-position to form 4-amino-5-imidazolecarboxamide ribotide and that this latter compound reacted with radioactive formate to form inosinic acid-2-C^{14}. It was believed that the citrovorum factor acted as a transformylating coenzyme in these reactions.

Evidence is now presented in further support of this hypothesis. Upon precipitation of pigeon liver extract between 15 and 30% ethanol[3] an enzyme system has been isolated which catalyzes the formation of an aryl amine from inosinic acid in the presence of glycine. This aryl amine compound has been absorbed on Norite, eluted with an ethanol–ammonia solution and placed on a Dowex-1-chloride column at *p*H 9. Upon elution of the column with 0.003 *N* HCl a fraction was obtained which gave a positive test for aryl amine (Bratton–Marshall diazo-reaction).[4,5] The identity of this compound as 4-amino-5-imidazolecarboxamide ribotide is indicated by the demonstration that it contains aryl amine:pentose:organic phosphate in the approximate ratio of 1:1:1. The compound shows an ultraviolet absorption peak at 267 mμ, similar to that of 4-amino-5-imidazolecarboxamide.[6]

A study has been made of the factors involved in the accumulation of the ribotide by the enzyme system. It was found that the reaction rate was highly dependent on the concentration of inosinic acid. Saturation of the enzyme system could not be obtained at a substrate concentration of 20 μM./ml. Glycine has also been shown to be an important factor of the reaction system. Increasing concentrations up to 100 μM./ml. resulted in increased amounts of 4-amino-5-imidazolecarboxamide ribotide formation. Neither L-alanine, L-serine, sarcosine, L-methionine, L-phenylalanine, nor L-leucine can substitute for glycine.

The addition of leucovorin[7] results in a two-to-three fold increase in aryl amine formation. Addition of the natural citrovorum factor,[8] at an equimolar level, resulted in the same stimulation. Addition of a boiled juice of pigeon liver results in a further doubling of the aryl amine formation. These results are shown in the table.

(1) Supported by grants from National Cancer Institute, National Institutes of Health, United States Public Health Service, and the Damon Runyon Memorial Fund for Cancer Research, Inc.

(2) J. M. Buchanan and M. P. Schulman, *J. Biol. Chem.*, **202**, 241 (1953).

(3) W. J. Williams and J. M. Buchanan, *ibid.*, **203**, 583 (1953).

(4) A. C. Bratton and E. K. Marshall, Jr., *ibid.*, **128**, 537 (1939).

(5) J. M. Ravel, R. E. Eakin and W. Shive, *ibid.*, **162**, 463 (1946).

(6) M. R. Stetten and C. L. Fox, Jr., *ibid.*, **161**, 333 (1945).

(7) Kindly supplied by Drs. H. P. Broquist and T. H. Jukes.

(8) Kindly supplied by Dr. J. C. Keresztesy.

TABLE I

FORMATION OF ARYL AMINE BY INOSINIC ACID TRANSFORMYLASE OF PIGEON LIVER

The reaction vessels were incubated for 25 minutes at 38° and the reaction stopped by adding 0.5 ml. of 30% trichloroacetic acid. The substrates, where added, were as follows: Na inosinate 10 μM.; glycine 35 μM.; leucovorin 50γ; boiled juice 0.2 ml.; enzyme, 0.5 ml. of 0.1 *M* tris buffer (*p*H 7.4), containing 30 mg./ml. of lyophilized powder; total volume 1.0 ml.

	μM. aryl amine formed
Enzyme + glycine	0.00
Enzyme + inosinate	.00
Enzyme + leucovorin	.010
Enzyme + inosinate + boiled juice	.006
Enzyme + inosinate + glycine	.065
Enzyme + inosinate + glycine + boiled juice	.057
Enzyme + inosinate + leucovorin	.028
Enzyme + inosinate + glycine + leucovorin	.166
Enzyme + inosinate + glycine + leucovorin + boiled juice	.328

Further work with the boiled juice has shown that it may be replaced with an equivalent amount of ashed boiled juice. A search of various inorganic anions and cations has shown that the factor in ashed boiled juice can be replaced by Cu^{++} at a level of $10^{-4}M$. The formation of aryl amine can be completely eliminated by addition of potassium cyanide or Versene to the reaction system.

The importance of 4-amino-5-imidazolecarboxamide ribotide in purine biosynthesis is supported by other work. The riboside has been shown to be formed enzymatically in this Laboratory[9] by the reaction of the free base and ribose-1-phosphate in the presence of purified nucleoside phosphorylase. Greenberg[10] has further stated that the riboside may be converted to the ribotide in the presence of adenosine triphosphate and Mg^{++}, and thence to inosinic acid by formate addition and ring closure.

These results again indicate the probable involvement of the citrovorum factor, either natural or synthetic, as a cofactor of this transformylation. Since *de novo* synthesis of purines does not occur in this system to an appreciable extent, it is possible that glycine exerts its effect on the reaction as a formyl acceptor.

(9) E. D. Korn, F. C. Charalampous and J. M. Buchanan, THIS JOURNAL, **75**, 3610 (1953).

(10) G. R. Greenberg, *Fed. Proc.*, **12**, 211 (1953).

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THE MECHANISM OF THE *PARA*-CLAISEN REARRANGEMENT. EVIDENCE FOR A DIENONE-PHENYL ETHER REARRANGEMENT

Sir:

Two mechanisms for the *para*-Claisen rearrangement have been proposed. The first, a conventional rearrangement of the allyl group to an *ortho*-position followed by a second similar rearrangement to the *para*-position, first advanced by Hurd and Pollack[1] and at nearly the same time by Mumm and Diedericksen,[2] was rejected by these authors because of the reported[2] inversion of the allyl side chain during rearrangement.

Later, Dewar[3] proposed an ion-pair π-complex mechanism.

Since the initiation of the present investigation, the *para*-rearrangement of isotopically labeled phenyl allyl ether has been shown to proceed without inversion of the allyl group,[4,5] and the original report[2] of inversion has been shown by Rhoades, Raulins and Reynolds[6] to be incorrect. As Ryan and O'Connor pointed out,[4] these findings exclude the original Dewar mechanism but do not exclude a modification in which the allyl carbonium ion is unsymmetrically attached to the aryl anion.

Conroy and Firestone[7] have reported the isolation of a maleic anhydride adduct of the cyclohexadienone intermediate in the *para*-rearrangement and showed that it could be made to rearrange to the *p*-allylphenol. Although they concluded from their results that at least a part of the *para*-Claisen rearrangement must proceed through the dienone, the modified Dewar mechanism is excluded only if it can be shown that their dienone did not rearrange to the *p*-allylphenol by reverting to the O-allyl starting material which, in turn, might have rearranged by the modified Dewar mechanism directly to the *p*-allylphenol.

It appears then, that although the evidence above supports the Hurd and Pollack mechanism for the *para*-Claisen rearrangement, it is not compelling.

In a more direct attack on the problem, 1-allyloxy-2,6-dimethallylbenzene (I) and 1-methallyloxy-2-allyl-6-methallylbenzene (II) were prepared and caused to rearrange. Both ethers (I) and (II) (each with b.p. 75° at 0.1 mm., n^{20}D 1.5198) were prepared by a method analogous to that previously used for 1-allyloxy-2,6-diallylbenzene.[8]

Anal. Calcd. for $C_{17}H_{22}O$: C, 84.3; H, 9.1. Found for I: C, 84.3; H, 9.4. For II: C, 84.4; H, 9.2.

The ether (I) when heated in diethylaniline at 200° rearranged in 50% yield to give a mixture of 4-allyl-2,6-dimethallylphenol (III) (59 ± 2%) and 2-allyl-4,6-dimethallylphenol (IV) (41 ± 2%). Similarly, rearrangement of the ether II lead to a mixture of the phenols containing 41 ± 2% of III and 59 ± 2% of IV. The composition of the mixture was determined by catalytic hydrogenation to the mixture of 4-*n*-propyl-2,6-diisobutylphenol (V) and 2-*n*-propyl-4,6-diisobutylphenol (VI) (both having b.p. 83–85° at 0.03 mm.) which were, in turn, analyzed by infrared analysis of 30% solutions in carbon disulfide using the maxima at 812 and 820 cm.$^{-1}$. The authentic samples of phenols (V) and (VI) necessary for the analysis were prepared by rearranging 1-allyloxy-2,6-diisobutylbenzene (b.p. 68–69° at 0.1 mm., n^{20}D 1.4915)

(1) C. D. Hurd and M. A. Pollack, *J. Org. Chem.*, **3**, 550 (1939).

(2) O. Mumm and J. Diedericksen, *Ber.*, **72**, 1523 (1939).

(3) M. J. S. Dewar, "The Electronic Theory of Organic Chemistry," Oxford University Press, London, 1949, p. 229.

(4) J. P. Ryan and P. R. O'Connor, THIS JOURNAL, **74**, 5866 (1952).

(5) H. Schmid and K. Schmid, *Helv. Chim. Acta*, **36**, 489 (1953).

(6) S. J. Rhoades, R. Raulins and R. D. Reynolds, THIS JOURNAL, **75**, 2531 (1953).

(7) H. Conroy and R. A. Firestone, *ibid.*, **75**, 2530 (1953).

(8) L. Claisen, *Ann.*, **418**, 97 (1919).