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).

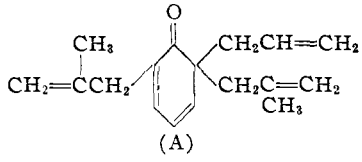
(6) S. J. Rhoades, R. Raulins and R. D. Reynolds, THIS JOURNAL, **75**, 2531 (1953).

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

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

and 1-methallyloxy-2-propyl-6-isobutylbenzene (b. p. 67–68° at 0.05 mm., $n^{20}D$ 1.4915).

The results are consistent with the mechanism proposed by Hurd and Pollack[1] in which (I), for example, rearranges to the intermediate (A) which,



(A)

in turn, undergoes similar internal rearrangement to give the phenols (III) and (IV).

The possibility remains that a species such as (A) is not in the direct path between the ether (I) and the *para*-rearrangement product (III) but that (A) rearranges to (III) *via* (I). The modified Dewar mechanism for the *para*-rearrangement is, therefore, not excluded.

To settle this point, recovered starting material and products were analyzed after incomplete rearrangement of (I). Indeed, after 70% completion, infrared analysis of 10% solutions of the ethers in carbon tetrachloride using the maxima at 1050 and 925 cm.$^{-1}$ showed the recovered ether to contain only 85% of (I) and 15 ± 2% of the *rearranged ether* (II) and when (I) was heated without solvent, analysis of the recovered ether after 50% reaction showed that it had rearranged to (II) to the extent of 48%. It is likely that the (I) rearranges to (A) which can undergo reversion to (I), or rearrangement to (II). When the ether fraction recovered after 30% rearrangement of (I) was examined, however, it contained not more than 5% of the isomeric ether (II) and the composition of the phenolic products was the same as that after complete reaction.

It seems clear that the interconversion of the ethers (I) and (II) cannot provide the explanation of the formation of the large amount of the *p*-methallylphenol (IV) found in the rearrangement of the allyl ether (I). The present results strongly support, therefore, the Hurd and Pollack mechanism for the *para*-Claisen rearrangement and appear to exclude the possibility that the Dewar mechanism plays an important role in this reaction.

The rearrangement of dienone (A) to the methallyl ether (I) which is suggested by the work above, seems to be an example of a rearrangement of a new type and we hope to investigate it further.

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IMPORTANCE OF GLYCOLYTIC AND "OXIDATIVE" PATHWAYS IN GLUCOSE UTILIZATION BY LIVER[1]

Sir:

Bloom and Stetten[2,3] studied the formation of $C^{14}O_2$ from C^{14}-labeled glucose and lactate in tissue slices, and observed that in rat liver the yield of $C^{14}O_2$ from 1-C^{14}-glucose exceeded that from evenly labeled and from 6-C^{14}-glucose. They derived equations relating the $C^{14}O_2$ formed from the evenly and the 1-labeled glucose to that from 1-, 2- and 3-C^{14}-lactates, and on the basis of these equations concluded that glucose catabolism in rat liver does not proceed mainly *via* the Embden–Meyerhof scheme but involves the direct oxidation and decarboxylation of carbon 1 of glucose (hexose monophosphate shunt). They stated that not more than 20–25% (and possibly none) of the CO_2 arising from glucose was formed glycolytically. We have confirmed the observations of Bloom, *et al.*[2] It is shown here, however, that the derivation of their equations is questionable. When the experimental data of Bloom, *et al.*,[2] are applied to revised equations presented here, we find that at least 80% of the CO_2 derived from glucose in liver could have arisen *via* glycolysis.

Let us designate, as in ref. 2, the $C^{14}O_2$ yields from evenly labeled and 1-C^{14}-glucose as a and b, respectively, and the ratio a/b as U. It is assumed, as in ref. 2, that glucose is oxidized to CO_2 by two pathways, namely, glycolysis and the direct oxidative shunt, and that E represents the fraction of the CO_2 formed glycolytically.

If m moles of CO_2 are formed from glucose, mE moles will be formed *via* glycolysis, and $m(1 - E)$ *via* the shunt. The radioactive yield of each fraction can be represented by mE and $m(1 - E)$ multiplied by their respective molar specific activities.

If the molar specific activity of glucose is taken as 1, the molar specific activity of the $C^{14}O_2$ derived from evenly labeled glucose will be 1/6, irrespective of its mode of formation. The molar specific activity of the $C^{14}O_2$ formed from glucose-1-C^{14} by the direct oxidative path will be 1, and that formed glycolytically will be $e/2(c + d + e)$, where c, d and e represent the extent of conversion to $C^{14}O_2$ of the carboxyl, alpha and beta carbons of pyruvate (or lactate) formed from glucose. Let $R = (c + d + e)/3e$ (R is equivalent to the expression $R = (N + T + 1)/3$ in ref. 2), then the molar specific activity of the $C^{14}O_2$ formed glycolytically from glucose-1-C^{14} will be $1/6R$. Thus, the ratio, a/b, *i.e.*

$$\frac{C^{14}O_2 \text{ from evenly labeled glucose}}{C^{14}O_2 \text{ from 1-}C^{14}\text{ labeled glucose}} = \frac{\frac{mE}{6} + \frac{(1 - E)m}{6}}{\frac{mE}{6R} + (1 - E)m} = U$$

$$\text{whence } E = \frac{R(6U - 1)}{U(6R - 1)} \qquad (1)^4$$

By using the experimental values of Bloom, *et al.*,[2] for R and U, namely, 2 and 0.6, respectively, in our equation (1), we find that $E = 0.79$, or that 79% of the CO_2 derived from glucose was formed *via* the classical glycolytic scheme.

(1) Supported by contract with the U. S. Atomic Energy Commission.

(2) B. Bloom, M. R. Stetten and D. Stetten, Jr., *J. Biol. Chem.*, **204**, 681 (1953).

(3) B. Bloom and D. Stetten, Jr., THIS JOURNAL, **75**, 5446 (1953).

(4) Compare with equation (4) of ref. (2), $U = [6RE + (1 - E)]/[6E + 6(1 - E)]$, whence $E = (6U - 1)/(6R - 1)$. E in this equation actually represents the fraction of the CO_2 formed from the *first* carbon of glucose *via* glycolysis, and does not have the meaning designated by Bloom, *et al.*[2] It should be pointed out that, while referring to the same numerical values in a subsequent paper,[3] these authors have defined E as the fraction contributed by glycolysis "to the over-all conversion of glucose to carbon dioxide," which has a different meaning and different numerical value from the E used in ref. 2 and here.