or ferricyanide, the rates of reaction catalyzed by the CN^--treated enzyme are markedly (50–70%) lower when compared to those of an untreated preparation. Full activity can be restored by preincubating the enzyme in 5×10^{-3} *M* $CuSO_4$.

The above experiments indicate that the intra- and intermolecular oxidoreductions mediated by the enzyme may be represented as follows:

$$\begin{tikzcd} \text{Butyryl CoA} \arrow[r, "2e-"] & \underbrace{\text{flavin} \xrightarrow{1e-} Cu^{++}}_{\text{enzyme}} \arrow[r] \arrow[d, "2e-"'] & (Fe^{+++}) \\ & \text{2,6-Dichlorophenolindophenol} & \end{tikzcd}$$

The identification of cupric ion as part of the prosthetic group of this flavoprotein dehydrogenase, together with the preliminary report on the role of molybdenum in xanthine oxidase,[6] possibly suggest a more general involvement of metals in flavoprotein catalyses.

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[6] E. C. DeRenzo, E. Kaleita, P. Heytler, J. J. Oleson, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 753 (1953).

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RECEIVED MAY 27, 1953

3,4-DIHYDROXYPHENYLACETIC ACID—A METABOLITE OF QUERCETIN

Sir:

Rutin, the rhamno-glucoside of quercetin, is being used extensively for therapeutic purposes, alone and in a variety of pharmaceutical formulations. Investigations on the fate of orally administered rutin have yielded contradictory results. Unfortunately, these investigations were concerned with the urinary excretion of rutin instead of the metabolic products of the aglycone quercetin. Ozawa[1] gave the closely related compound, $3^1,4^1$-dihydroxyflavonol, to animals orally and found less than one-tenth of the material excreted in the urine. However, Ozawa found by chromatography three substances of different R_f values in the urine and concluded these substances were metabolites of the compound administered. Because of the unfavorable report of Clark and MacKay[2] on the absorption of orally administered rutin, Haley and Bassin[3] injected rats with rutin subcutaneously. They found the urine contained rutin and unidentified breakdown products conjugated with sulfate and glucuronic acid. The results of Haley and Bassin showed that any rutin or quercetin which might enter the blood stream after oral administration of rutin would be metabolized in part at least.

Evidence obtained in this laboratory during the last four years has shown that oral administration of rutin or its aglycone quercetin to rabbits results in the urinary excretion of appreciable amounts of metabolites of quercetin. One of these breakdown products of quercetin has been isolated recently from rabbit urine in crystalline form, m.p. 127°, and identified as 3,4-dihydroxyphenylacetic acid, (Calcd. for $C_8H_8O_4$: C, 57.54; H, 4.80; neut equiv., 168.1. Found: C, 57.3; H, 4.86; neut equiv. 167.7). Its mixed melting point with an authentic sample was unchanged. The X-ray diffraction pattern of its dimethyl ether was identical with that of a sample of synthetic dimethoxyphenylacetic acid. Crystallographic examination of the compound was confirmatory.

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[1] H. Ozawa, *J. Pharm. Soc. Japan*, **71**, 1191 (1951).

[2] W. J. Clark and E. M. MacKay, *J.A.M.A.*, **143**, 1411 (1950).

[3] T. J. Haley and M. Bassin, *Proc. Soc. Exptl. Biol. Med.*, **81**, 298 (1952).

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RECEIVED MAY 4, 1953

PREPARATION OF CRYSTALLINE 2,3,5-TRI-*O*-BENZOYL-D-RIBOSE FROM D-RIBOSE

Sir:

The procedure developed for the synthesis of benzoylated D-xylofuranose derivatives from D-xylose[1] has now been applied to the D-ribose series. D-Ribose was dissolved in methanol containing 1% hydrogen chloride and the solution left at room temperature until its reducing power had nearly vanished. Pyridine was then added and, after removal of the solvents, the product was benzoylated. The resulting amorphous benzoate, freed of excess reactants, was treated with hydrogen bromide in glacial acetic acid and the crude tri-*O*-benzoyl-D-ribofuranosyl bromide then hydrolyzed in aqueous acetone in the presence of silver carbonate. From aqueous pyridine there was obtained 2,3,5-tri-*O*-benzoyl-D-ribose containing an indefinite amount of pyridine of crystallization. Most of the pyridine was removed by brief drying *in vacuo* over sulfuric acid and the tribenzoate then recrystallized in pure form from alcohol-pentane or ether-pentane. The over-all yield of crystalline solvent-free 2,3,5-tri-*O*-benzoyl-D-ribose varied from 70–81%. The substance melts at 112–113° (cor.) and rotates $[\alpha]^{20}\text{D}$ +68.4° in chloroform (*c* 2.65). *Anal.* Calcd. for $C_{26}H_{22}O_8$: C, 67.52; H, 4.80. Found: C, 67.31; H, 4.91.

The structure of the 2,3,5-tri-*O*-benzoyl-D-ribose was confirmed by the following unequivocal synthesis. D-Ribose was dissolved in benzyl alcohol containing 1% hydrogen chloride and, after the reducing power of the solution had nearly disappeared, the acid was removed with silver carbonate. Concentration of the solution *in vacuo* afforded a crystalline benzyl pentoside [m.p. 95–96° (cor.); $[\alpha]^{20}\text{D}$ −60.5° (H_2O)] which consumed one mole of periodate to give a solution which showed the same rotation as an equivalent quantity of benzyl β-D-glucopyranoside which had been similarly oxidized. These facts showed the substance to be benzyl β-D-ribofuranoside. The corresponding tribenzoate [m.p. 87–88° (cor.); $[\alpha]^{20}\text{D}$ +14.9° ($CHCl_3$)] gave, on hydrogenation over palladium-charcoal, 2,3,5-tri-*O*-benzoyl-D-ribose identical with that prepared directly from D-ribose.

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[1] H. G. Fletcher, Jr., THIS JOURNAL, **75**, 2624 (1953).

While 2,3,5-tri-*O*-benzoyl-D-ribose shows little if any mutarotation in chloroform or aqueous dioxane, methylation studies and comparisons between its rotation and those of some closely related substances (both to be published in the near future) appear to justify the tentative conclusion that it belongs to the β-D-series.

Acetylation of 2,3,5-tri-*O*-benzoyl-D-ribose in pyridine at a low temperature afforded in 88% yield crystalline 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose [m.p. 130–131° (cor.); $[\alpha]^{20}\text{D}$ +44.2° ($CHCl_3$)]. Similarly, benzoylation at low temperature gave in 89% yield D-ribofuranose tetrabenzoate [m.p. 121–122° (cor.); $[\alpha]^{20}\text{D}$ +17.0 ($CHCl_3$)]; the same substance was also obtained through the benzoylation of D-ribose in pyridine at 100° although the yield in this case (11%) was low owing to the difficulty of separating the product from β-D-ribopyranose tetrabenzoate which is formed simultaneously.

2,3,5-Tri-*O*-benzoyl-D-ribose has been used for the synthesis of benzyl β-D-ribofuranoside tribenzoate; it is possible that it may prove of general utility for the synthesis of ribofuranosides.

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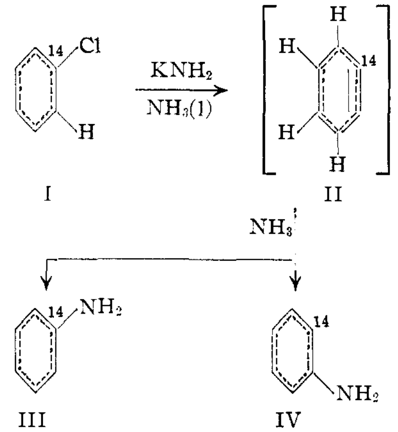
RECEIVED MAY 21, 1953

REARRANGEMENT IN THE REACTION OF CHLOROBENZENE-1-C14 WITH POTASSIUM AMIDE[1]

Sir:

No satisfactory explanation has been published for the rearrangements which often occur in the amination of "non-activated" aryl halides with alkali-metal amides.[2] The pattern of the rearrangements shows a considerable disregard for the influences governing the usual aromatic substitutions and is well illustrated by the products obtained from the amination of the methoxy- and trifluoromethylhalobenzenes. Although the methoxy and trifluoromethyl groups orient oppositely in aromatic nitration, *o*- and *m*-methoxy- and trifluoromethylhalobenzenes with alkali-metal amides yield exclusively *m*-substituted anilines, while the *p*-isomers yield mixtures containing roughly equal amounts of *m*- and *p*-substituted anilines.[3]

Besides the seemingly anomalous influence of substituents any mechanism proposed for the reaction must be in accord with the following observations: (1) the reactions are very rapid, even with chlorobenzene, in liquid ammonia at −33°; (2) the entering amino group has never been found farther than one carbon away from the position occupied by the leaving halogen[3,4]; (3) the starting halides and resulting anilines are not isomerized under the reaction conditions[3d,4]; (4) no reaction occurs in the benzene series with halides (*i.e.*, bromomesitylene,[3d] bromodurene[5] and 2-bromo-3-methylanisole[4]), where a hydrogen is not attached to the position adjacent to that occupied by the leaving halogen. These facts as well as the orientation data for various substituents can be accommodated by an elimination-addition mechanism involving at least transitory existence of an electrically neutral "benzyne" intermediate (II).



As is evident from the above reaction sequence, a critical test of the proposed mechanism would be afforded by the reaction of chlorobenzene-1-C^{14} with potassium amide. If a symmetrical intermediate such as II were involved equal amounts of aniline-1-C^{14} (III) and aniline-2-C^{14} would be formed since C-1 and C-2 become equivalent in II.

We have carried out the reaction of I[6] with potassium amide in liquid ammonia and obtained a 43% yield of C^{14}-labeled aniline. The C^{14} in the product was found to be distributed almost exactly as predicted for intermediate formation of II. While this experiment is not considered to "prove" the "benzyne" mechanism, it strongly indicates formation of an intermediate in which the 1- and 2-positions of the ring are, or can become, equivalent.[7] The only alternative is the occurrence of simultaneous rearranging and non-rearranging displacements in a ratio of almost exactly one to one. The utility of intermediates like II in accounting for the pattern of rearrangements with substituted halobenzenes will be demonstrated in a later paper.

An outline of the tracer experiments follows. The last steps were those developed by Loftfield.[8]

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(1) Supported in part by the program of research of the U. S. Atomic Energy Commission.

(2) The scope of this type of reaction has been investigated principally by Gilman and Bergstrom and their co-workers. For a review, see J. F. Bunnett and R. E. Zahler, *Chem. Revs.*, **49**, 273 (1951).

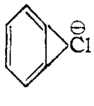
(3) (a) H. Gilman and S. Avakian, THIS JOURNAL, **67**, 349 (1945); (b) H. Gilman and R. H. Kyle, *ibid.*, **70**, 3945 (1948); **74**, 3027 (1952); (c) R. A. Benkeser and R. G. Severson, *ibid.*, **71**, 3838 (1949); (d) C. W. Vaughan, B.S. Thesis, M.I.T., 1951; (e) L. A. Carlsmith, M.S. Thesis, M.I.T., 1953.

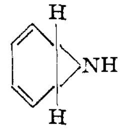
(4) R. A. Benkeser and W. E. Buting, THIS JOURNAL, **74**, 3011 (1952).

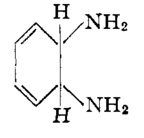
(5) Unpublished experiments by Mr. R. L. Harris.

(6) Obtained from Tracerlab, Inc., on allocation from the U. S. Atomic Energy Commission.

(7) Other possible symmetrical intermediates which would accommodate the C^{14}-tracer experiment and fit the general character of the reaction to a more or less satisfactory degree are:







(8) R. B. Loftfield, THIS JOURNAL, **73**, 4707 (1951).