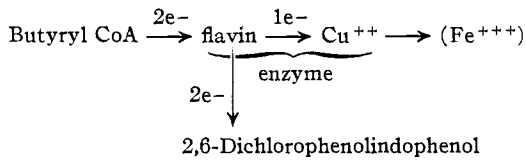
or ferricyanide, the rates of reaction catalyzed by the CN^--treated enzyme are markedly (50–70%) lower when compared to those of an untreated preparation. Full activity can be restored by preincubating the enzyme in 5×10^{-3} *M* $CuSO_4$.

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



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.

(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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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.

(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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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 $[\alpha]^{20}D$ +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.); $[\alpha]^{20}D$ −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.); $[\alpha]^{20}D$ +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.

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