TABLE I

THE INFRARED AND RAMAN SPECTRA AND THEIR INTERPRETATION FOR TETRACHLORODIBORINE

Raman (cm.$^{-1}$)	Infrared (cm.$^{-1}$)	Intensity and polarization	Interpretation
107	..	m	ν_9(e; β-BCl_2)
177	..	m	ν_8(e; β-BCl_2)
291	..	w,p	ν_3(a_1; δ-BCl_2)
347	..	vw	2 × 177 (e) = 354(A_1 + B_1 + B_2)
401	..	vs,p	ν_2(a_1; ν-BCl)
550	..	vvw	107 (e) + 445 (b_2) = 552 (E); 2 × 291(a_1) = 582 (A_1)
..	622	w	177(e) + 445 (b_2) = 622 (E); 730(b_2) − 107(e) = 623 (E)
..	689	w	107(e) + 2 × 291(a_1) = 689 (E)
..	720	s	
..	729	vs	ν_6(b_2; ν-BCl)
..	739	vs	
..	746	s	291(a_1) + 445(b_2) = 736(B_2)*
750	..	vvw	291(a_1) + 445(b_2) = 736(B_2)*
..	760	vw	177(e) + 2 × 291(a_1) = 759 (E)
..	821	ms	107(e) + 729(b_2) = 836(E)
900	..	w	2 × 445(b_2) = 890 (A_1); 177 (e) + 729(b_2) = 906 (E)
..	920	vvs	ν_7(e; ν-BCl)
927	..	w	ν_7(e; ν-BCl)
948	..	w	107(e) + 401(a_1) + 445(b_2) = 953 (E); 2 × 107(e) + 729 (b_2) = 943 (A_1 + A_2 + B_1 + B_2)
..	1029	w	291(a_1) + 729(b_2) = 1020(B_2); 107(e) + 920(e) = 1027(A_1 + A_2 + B_1 + B_2)
..	1057	w	177(e) + 2 × 445(b_2) = 1067 (E)
..	1090	w	177(e) + 920(e) = 1097 (A_1 + A_2 + B_1 + B_2)
1123	..	w,p	ν_1(a_1; ν-$B^{11}B^{11}$)
1150	..	w,p	ν_1(a_1; ν-$B^{11}B^{10}$)
..	1232	w	107(e) + 1123(a_1) = 1230 (E)
1259	1259	vw	107(e) + 1150(a_1) = 1257 (E)
..	1324	w	401(a_1) + 920(e) = 1321 (E)
..	1362	vw	445(b_2) + 920(e) = 1365 (E)
..	2058	w	920(e) + 1123(a_1) = 2043 (E); 920(e) + 1150(a_1) = 2070 (E)

* Shifted by Fermi resonance with ν_5 fundamental.

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BUTYRYL CoA-DEHYDROGENASE, A CUPRO-FLAVOPROTEIN

Sir:

Butyryl CoA dehydrogenase, previously shown to be a flavoprotein of vivid green color,[1] contains copper as part of its prosthetic group. This identification rests on the following observations: (1) a solution of the enzyme of highest purity isolated from a preparative electrophoresis run, and shown to be homogeneous in the analytical ultracentrifuge, was dialyzed exhaustively against 10^{-4} M tris-hydroxymethyl-aminomethane buffer of pH 8.0. Part of the preparation was then denatured by boiling for three minutes and the supernatant flavin solution, the denatured protein and the original preparation were examined by arc and spark spectroscopy.[2] The results summarized in Table I clearly indicate the presence of copper tightly bound to the enzyme protein. (2) Aliquots of two preparations shown to be approximately 22% and 98% pure by analytical electrophoresis (in 0.1 M tris-hydroxymethyl-aminomethane buffer of pH 8.0), and containing 4.7×10^{-5} and 2.6×10^{-5} M flavin,[3] respectively, were washed in platinum crucibles for 1 hour at 1000° F. Manometric copper determination with internal standards[4] showed the presence of 6.9 γ and 3.2 γ of cupric ion per ml. in the two preparations. Thus the mole ratios Cu^{++}/flavin were 2.23/1 and 1.90/1 or approximately equal to 2, at two purity levels of the dehydrogenase.

TABLE I

SPECTROSCOPIC METAL DETERMINATION

	Cu[a]	Mg[a]
Buffer before dialysis	−	−
Buffer after dialysis	−	−
Enzyme after dialysis	++	+
Supernatant of boiled enzyme	−	+
Residue of boiled enzyme	++	−

[a] Only metals found present, all others, specifically Fe, Zn, Mn, Mo, Co, Ca, absent in all samples.

(3) The four-banded spectrum of the enzyme (λ_{max} at 265, 355, 432.5 and 685 mμ) undergoes the following transformations: (a) on treatment with butyryl CoA the peaks at 355, 432.5 and 685 mμ all disappear and the extinctions at these wave lengths are reduced to 0.86, 0.47 and 0.45 times their original value; (b) after dialysis against 5×10^{-3} M KCN,[5] buffered at pH 7.5, for 36 hours, the flavin peaks at 265, 355 and 432.5 are unchanged in both position and extinction while the peak at 685 mμ disappears.

(4) The cyanide-treated enzyme can still be reduced by butyryl CoA as shown by changes in the flavin region (390–480 mμ) of the spectrum, and is capable of catalyzing the interaction between butyryl CoA and dyes such as 2,6-dichlorophenolindophenol, a two-electron change. However, when the enzyme is tested with oxidizing agents mediating one-electron changes such as ferricytochrome c

(1) H. Beinert, R. Bock, D. S. Goldman, D. E. Green, H. R. Mahler, S. Mii, P. G. Stansly and S. J. Wakil, unpublished.

(2) We are indebted to Mr. Rubin Shapiro, Department of Chemistry, University of Wisconsin for performing the spectroscopic analyses.

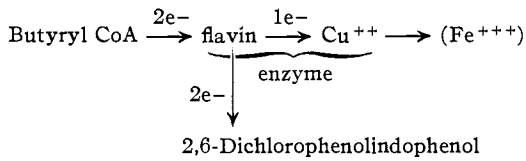
(3) Flavin was determined by assuming a molecular extinction coefficient $\epsilon = 10.6 \times 10^3$ for flavoproteins at their visible maximum (O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150 (1938)). The value so found, was in good agreement with the amount of flavin liberated by boiling the enzyme preparations and calculated assuming an extinction coefficient at 450 mμ $\epsilon = 11.3 \times 10^3$ (E. Dimant, D. R. Sanadi and F. M. Huennekens, THIS JOURNAL, **74**, 5440 (1952)).

(4) O. Warburg and H. A. Krebs, *Biochem. Z.*, **190**, 143 (1927); O. Warburg, *ibid.*, **187**, 255 (1927).

(5) F. Kubowitz, *Biochem. Z.*, **292**, 221 (1937); **299**, 32 (1939).

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).