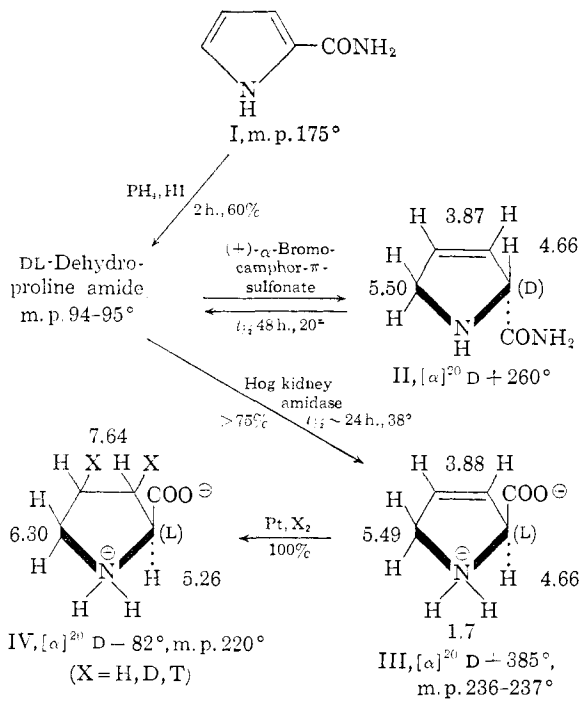

Hog kidney amidase, activated by magnesium ions,[6] hydrolyzed the racemic amide 50% after 24 hours, 75% after 4 days at 38° and addition of fresh enzyme on the third day. Preparations of the L-acid showing rotations as high as $[\alpha]^{20}D$ 385° were 97% optically pure as assayed by D-amino acid oxidase.[7] This leads to a calculated value of $[\alpha]^{20}D \sim -400°$ for pure III, *i.e.*, close to five times that of L-proline, $[\alpha]^{20}D$ −86°.[8] Yields of 100% of III were not obtained because of the formation of gummy side products during the long reaction time. All analyses were satisfactory.

Acknowledgment.—We are greatly indebted to Dr. S. M. Birnbaum for his valuable assistance and coöperation in the enzymatic resolution and assays.

(6) S. M. Birnbaum, "Methods in Enzymology," Academic Press, Inc., New York, N. Y., 1955, Vol. II, p. 397.

(7) L-Amino acid oxidase from snake venom converts III quantitatively to pyrrole-2-carboxylic acid, a reaction which has been used for a new spectrophotometric assay: H. H. Weissbach, A. V. Robertson, B. Witkop and S. Udenfriend, *Anal. Biochem.*, in press.

(8) D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, **193**, 81 (1951).

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A ROLE FOR THYMIDINE NUCLEOTIDES IN THE BIOSYNTHESIS OF L-RHAMNOSE[1]

Sir:

The synthesis of thymidine diphosphate glucose and its conversion to thymidine diphosphate rhamnose has been observed in reaction mixtures of thymidine triphosphate, α-D-glucose-1-phosphate and an enzyme preparation from *Streptococcus faecalis*. The enzyme extract was obtained by Mickle disintegration of *S. faecalis* cells in 0.1 *M* phosphate buffer of *p*H 7.2 containing 0.05 *M* magnesium chloride. The reaction mixtures were prepared by dissolving 20 micromoles of thymidine triphosphate and 30 micromoles of α-D-glucose-1-phosphate in 0.5 ml. of enzyme solution and incubating at room temperature for 1 and 5 hour periods. Examination of these samples by paper chromatography in a solvent system of ethyl alcohol and 1 *M* ammonium acetate of *p*H 7.5 (7:3 by volume) revealed the presence of three new ultraviolet absorbing compounds with R_f values of 0.35, 0.25 and 0.20. A comparison of these values with those for reference compounds in Table I indicates that the compounds with R_f values of 0.25 and 0.20 are probably thymidine monophosphate and thymidine diphosphate, respectively. The compound at R_f value of 0.35 yielded ultraviolet spectra typical of thymidine derivatives and on acid hydrolysis was converted to thymidine monophosphate, inorganic phosphate and reducing sugars. When thymidine triphosphate was incubated with the enzyme extract in the absence of glucose-1-phosphate, small amounts of thymidine monophosphate and thymidine diphosphate were produced but the compound with R_f value of 0.35 was not produced in this reaction mixture.

TABLE I

THE R_f VALUES OF REFERENCE COMPOUNDS IN SOLVENT SYSTEM OF ETHYL ALCOHOL–1 *M* AMMONIUM ACETATE OF *p*H 7.5 (7:3 BY VOLUME)

Compound	R_f value
Thymidine triphosphate	0.11
Thymidine diphosphate	.20
Thymidine monophosphate	.26
Thymidine	.76
Thymine	.70
Uridine diphosphate glucose	.22
Uridine triphosphate	.07
α-D-Glucose-1-phosphate	.18
D-Glucose	.60
L-Rhamnose	.68

The ultraviolet absorbing material at R_f value of 0.35 was isolated by a paper chromatographic method in yields approximating 60% of the original thymidine triphosphate. Hydrolysis in 0.02 *N* hydrochloric acid for 1 hour at 100° converted the product from the 1-hour incubation period to thymidine monophosphate, inorganic phosphate and D-glucose and the product from the 5-hour sample to thymidine monophosphate, inorganic phosphate, D-glucose and L-rhamnose. Quantitative values for these hydrolytic products were obtained by ultraviolet absorption for thymidine monophosphate, by a modified Fiske–SubbaRow method for inorganic phosphate[2] and by the cysteine–sulfuric acid reaction for reducing sugars.[3] Molar ratios of these products are recorded in Table II. The presence of D-glucose and L-rhamnose in the acid hydrolysates was confirmed by R_f values in three different solvent systems, by enzymatic assay with

(1) Published with the approval of the Director as Paper No. 1047 Journal Series, Nebraska Agricultural Experiment Station. Supported in part by a grant from the National Science Foundation.

(2) C. H. Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).

(3) Z. Dische, "Methods of Biochemical Analysis," Vol. II, edited by D. Glick, Interscience Publishers, New York, N. Y., 1955, pp. 313–358.

glucose oxidase[4] and by the characteristic absorption spectra of the reaction product of L-rhamnose in the cysteine–sulfuric acid test.[5] These results indicate that the product from the 1-hour incubation mixture is essentially pure thymidine diphosphate glucose and the product from the 5-hour incubation is apparently a mixture of thymidine diphosphate glucose and thymidine diphosphate rhamnose.

TABLE II

MOLAR RATIOS OF HYDROLYTIC PRODUCTS FROM THYMIDINE DIPHOSPHATE HEXOSE COMPOUNDS FROM THE 1- AND 5-HR. REACTION MIXTURES

Hr.	Thymidine monophosphate	Inorganic phosphate	Total hexose	Rhamnose
1	1.00	0.94	1.05	0.06
5	1.00	0.93	0.96	0.28

That the D-glucosyl unit of thymidine diphosphate glucose is converted to the rhamnosyl unit has been established by the results shown in Table III. In these experiments, samples of 0.8 micromole of thymidine diphosphate glucose in 0.05 ml. of enzyme extract were incubated for 5 hours with 0.15 or 0.3 micromole of the compounds listed in the table. The thymidine diphosphate hexose fraction from each reaction mixture was separated chromatographically and L-rhamnose and D-glucose in the fractions were determined quantitatively.[3] The values in Table III show that the disappearance of glucose was accompanied by a stoichiometric increase in L-rhamnose in the reaction mixture.

TABLE III

MICROMOLES OF L-RHAMNOSE AND D-GLUCOSE IN THE HYDROLYSATES OF THYMIDINE DIPHOSPHATE HEXOSE FRACTIONS PREPARED AS DESCRIBED IN THE TEXT

Compound added	μmoles of L-rhamnose	μmoles of D-glucose	Conversion to L-rhamnose,[a] %
None	0.03	0.79	0
TPNH (0.15 μM.)	.17	.64	17
TPNH (0.3 μM.)	.24	.58	26
DPNH (0.3 μM.)	.18	.64	19
Dialyzed[b] + TPNH (0.3 μM.)	.11	.71	11
Dialyzed + DPNH (0.3 μM.)	.04	.79	1
Glutathione (0.3 μM.)	.05	.73	3
G-1-P (0.3 μM.)	.03	.78	0

[a] The figures have been corrected for the small amount of L-rhamnose initially detected in the TDPG preparation by the colorimetric procedure employed. [b] The enzyme extract was dialyzed for 24 hours in water and for 3 hours in 0.1 *M* phosphate buffer containing 0.05 *M* magnesium chloride.

On the basis of these findings, a reaction sequence suggested for the conversion of D-glucose to L-rhamnose by the enzyme system of *S. faecalis* is outlined in the accompanying equations

$$G + ATP \longrightarrow G\text{-}6\text{-}P \longrightarrow G\text{-}1\text{-}P \quad (1)$$

$$TTP + G\text{-}1\text{-}P \longrightarrow TDPG + P\text{-}P \quad (2)$$

$$TDPG + TPNH \longrightarrow TDPRh + TPN^{+} \quad (3)$$

This reaction sequence is consistent with the observation that specifically labeled glucose is converted to L-rhamnose without randomization of the label[6] and probably accounts for the occurrence of thymidine diphosphate rhamnose in several strains of bacteria.[7,8,9] In this scheme, reaction 1 illustrates the formation of glucose-1-phosphate (G-1-P) from glucose (G) and adenosine triphosphate (ATP) via hexokinase to yield glucose-6-phosphate (G-6-P) and via mutase to yield G-1-P. Reaction 2 indicates the synthesis of thymidine diphosphate glucose (TDPG) by a mechanism similar to that for the synthesis of uridine diphosphate glucose. The enzyme which catalyzes this reaction may be appropriately termed a thymidine diphosphate glucose pyrophosphorylase. Reaction 3 is a diagrammatic representation for a series of reactions resulting in the conversion of the glucosyl moiety of TDPG to the rhamnosyl moiety of thymidine diphosphate rhamnose (TDPRh). These reactions result in epimerizations at positions 3, 4, and 5 and reduction at position 6 of the glucosyl moiety of TDPG. Reduced triphosphopyridine nucleotide (TPNH) is required for this series of reactions and in the crude reaction mixture is probably supplied by other metabolic reactions occurring in the system. The thymidine diphosphate rhamnose probably participates in transglycosylation reactions as a donor of rhamnosyl units in the biosynthesis of heteropolysaccharides.

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(7) J. L. Strominger and S. S. Scott, *Biochim. Biophys. Acta*, **35**, 552 (1959).

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(9) J. Baddiley and N. L. Blumson, *Biochim. Biophys. Acta*, **39**, 376 (1960).

(10) Dow Chemical Co. Fellow, University of Nebraska, 1959–1960.

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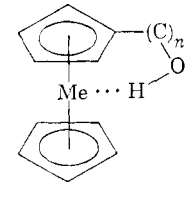
METAL-HYDROGEN BONDING IN METALLOCENE COMPOUNDS[1]

Sir:

Evidence provided by our infrared spectral studies on a wide range of ferrocene and some related metallocene derivatives has established a revealing example of the active electronic role which can be exercised by the metal atom in appropriate reactions of these metallocene systems. Study of the hydrogen bonding characteristics of ferrocene and related derivatives has shown the metal atom to be the site of a substantially strong intramolecular hydrogen bond involving the electrons of the metal atom acting as proton acceptor.



$n = 1, 2$

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