A detailed description of our synthetic methods and their application to the synthesis of other L-arginyl peptides will be presented at a later date.

BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE
PITTSBURGH, PENNSYLVANIA
KLAUS HOFMANN
ALFRED RHEINER
WILLIAM D. PECKHAM

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THE BIOSYNTHESIS OF SUCROSE[1]

Sir:

A previous note[2] reported the formation of trehalose phosphate from UDPG[3] and glucose-6-phosphate. Following the same general procedure, an enzyme has now been found in wheat germ which catalyzes the reaction UDPG + fructose $\rightleftarrows$ sucrose + UDP. The evidence is as follows. The product formed was found to be non-reducing and to behave like sucrose on paper chromatography with two solvents (butanol–acetic acid[4] and ethyl acetate–pyridine[5]). After extraction of the substance from the paper followed by hydrolysis with dilute acid (5 minutes at *p*H 2 at 100°) or with purified invertase, glucose and fructose were detected chromatographically.

As shown in Table I, equal amounts of sucrose and UDP are formed in the reaction. The disappearance of UDPG and the formation of UDP were checked semiquantitatively after separation by paper chromatography with ethanol–ammonium acetate–Versene[6] as solvent.

TABLE I

The complete system contained 0.05 μmole of UDPG, 2 μmoles of fructose and 0.05 ml. of enzyme,[a] 0.1 ml. of 0.1 *M* sodium diethyl barbiturate: final volume, 0.25 ml.; *p*H 8.6; incubated during 10 minutes at 37°. The Δ values represent the difference in μmoles with a non-incubated sample.

	Δ Sucrose[b]	Δ UDP[c]	Δ Inorganic phosphate
Complete system	0.33	0.30	0.05
No UDPG	0	0	0
No fructose	0	0.05	0.04

[a] The enzyme was obtained by extracting wheat germ with three volumes of phosphate buffer 0.05 *M*, *p*H 7. After centrifuging the supernatant was dialyzed overnight cold and centrifuged again. The supernatant was precipitated twice by adding 35 g. of ammonium sulfate per 100 ml. The precipitate was suspended in water, dialyzed for 2 hours and adjusted to *p*H 5. The precipitate was redissolved in water at *p*H 7. The precipitation with acid was repeated three times. The solution contained 40 mg. of protein per ml. [b] Sucrose was estimated by the resorcinol method[7] after destroying the fructose by heating 10 minutes at 100° in 0.01 *N* NaOH. [c] Determined enzymatically.[2]

The same chromatographic procedure was used for studying the reversibility. Starting with UDP and sucrose it was found that UDPG is formed. Its identity was checked by extracting it from the paper and measuring the coenzymic activity on galactowaldenase.[8] The data indicate that the equilibrium is displaced in favor of sucrose synthesis.

No sucrose formation or UDPG disappearance was found to occur if glucose-1-phosphate was added instead of UDPG, or if sorbose, aldoses, arabinose or the 1- or 6-phosphates of fructose or glucose were substituted for fructose.

Although sucrose had been previously obtained by enzymic action, the mechanism of the synthesis in plants remained obscure. The enzyme which Doudoroff and Hassid extracted from *Pseudomonas saccharophyla* catalyzes the formation of sucrose from glucose-1-phosphate and fructose, but it has not been possible to detect such a reaction in plant material.[9] The enzyme described in this paper has been found to be present not only in wheat germ but also in corn and bean germs and in potato sprouts. Tests for UDPG by its coenzymic activity gave positive results on wheat germ extracts.

Moreover, Buchanan, *et al.*[10] have published evidence of the presence of UDPG in other plants. They also suggested that it was involved in sucrose synthesis, probably by reacting with fructose phosphate to give sucrose phosphate. The latter substance can be excluded as an intermediate in the reaction catalyzed by the wheat germ enzyme because the product is all free sucrose and only negligible amounts of inorganic phosphate are released (Table I).

(1) This investigation was supported in part by a research grant (G-3442) from the National Institutes of Health, United States Public Health Service, and by the Rockefeller Foundation.

(2) L. F. Leloir and E. Cabib, THIS JOURNAL, **75**, 5445 (1953).

(3) The abbreviations UDPG for uridine diphosphate glucose, and UDP for uridine diphosphate are used.

(4) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(5) M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, **44**, 402 (1949).

(6) E. Cabib and L. F. Leloir, *J. Biol. Chem.*, in press.

(7) J. H. Roe, *J. Biol. Chem.*, **107**, 15 (1934).

(8) R. Caputto, L. F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.*, **184**, 333 (1950).

(9) W. Z. Hassid, "A Symposium on Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, p. 11.

(10) (a) J. G. Buchanan, *Arch. Biochem. and Biophys.*, **44**, 140 (1953); (b) J. G. Buchanan, V. H. Lynch, A. A. Benson, D. F. Bradley, and M. Calvin, *J. Biol. Chem.*, **203**, 935 (1953).

INSTITUTO DE INVESTIGACIONES BIOQUÍMICAS
FUNDACIÓN CAMPOMAR, J. ALVAREZ 1719
BUENOS AIRES, ARGENTINA
L. F. LELOIR
C. E. CARDINI

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CONFIGURATIONS OF LIGANDS HAVING INTERNAL ROTATION AXES IN COÖRDINATION COMPOUNDS

Sir:

Considerable evidence based on spectroscopic, thermal and electric measurements has been accumulated to indicate that 1,2-disubstituted ethanes, $XH_2C–CH_2X$, exist in *trans* and *gauche* configurations in the gaseous and liquid states and in solutions, but they exist only in *trans* configuration in the solid state.[1] Our infrared measurements on ethylene thiocyanate have also shown that this substance exists in the *trans* and *gauche* configurations in chloroform solutions but it exists only in *trans* configuration in the solid state. The spectrum of the complex $[PtCl_2(CH_2SCN)_2]$ has been found to be quite similar to that of the *gauche* configuration of ethylene thiocyanate but quite different from that of the *trans* configuration. Therefore, the configuration of this chelate ligand in the co-

(1) S. Mizushima, Y. Morino, I. Watanabe, T. Shimanouchi and S. Yamaguchi, *J. Chem. Phys.*, **17**, 591 (1949); S. Mizushima, T. Shimanouchi, I. Nakagawa and A. Miyake, *J. Chem. Phys.*, **21**, 215 (1953); see also S. Mizushima, "The Reilly Lectures," Vol. 5, The University Press of Notre Dame, Notre Dame, Indiana, 1951.