buried in the electron density of the cobalt atom as has been suggested[1] previously whereas these numerically accurate calculations provide no support for a Co-H distance of ~2.0.

DEPARTMENT OF CHEMISTRY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY F. A. COTTON
CAMBRIDGE 39, MASS.

RECEIVED JULY 7, 1958

FORMATION OF GUANOSINE DIPHOSPHATE FUCOSE FROM GUANOSINE DIPHOSPHATE MANNOSE

Sir:

A fucose containing nucleotide, guanosine diphosphate fucose, has been isolated recently from sheep milk[1] and from *Aerobacter aerogenes*.[2] It has now been found that GDPM[3] can be converted to guanosine diphosphate fucose by dialysed crude extracts of *A. aerogenes*[4] in the presence of TPNH.

The conversion was detected as follows: GDPM, prepared from yeast,[5] was incubated with TPNH and crude bacterial extracts obtained by shaking the cells with glass beads[6] followed by centrifugation and dialysis. The guanosine sugar nucleotides were then isolated and purified from the incubation mixtures by charcoal adsorption and paper chromatography.[5] Chromatography of the sugar liberated by 0.01 *N* HCl hydrolysis of the isolated nucleotides revealed, in addition to mannose, the presence of a second compound. This new compound exhibited the characteristic 400 mμ absorption peak when examined by the specific colorimetric assay for 6-deoxyhexose.[7] Upon paper chromatography, the unknown sugar co-chromatographed with authentic fucose using the solvents 2-butanone–acetic acid–saturated boric acid solution,[8] butanol–acetic acid–water,[9] phenol–water[9] or pyridine–ethyl acetate–water.[10] These solvents readily distinguish fucose from rhamnose. Further evidence for the identity of this sugar was indicated by the fact that it was active as a substrate for L-fucose isomerase.[11] The reaction product, presumably L-fuculose, was detected by means of the cysteine–carbazole reaction.[11,12]

The requirement for TPNH is shown in Table I. It is evident from structural considerations that the formation of the L-fucose derivative is a complex reaction which probably involves several steps. The nature of these steps remains to be elucidated.

TABLE I

CONVERSION OF GUANOSINE DIPHOSPHATE MANNOSE TO GUANOSINE DIPHOSPHATE FUCOSE

The reaction mixtures contained 1.0 μmole GDPM, 4 mg. of crude extract protein and additions in 1.0 ml. of 0.05 M tris-(hydroxymethyl)-aminomethane buffer, *p*H 7.8. Incubation was carried out at 37° for 4 hours. The nucleotides were then adsorbed on charcoal and the nucleotide bound sugars liberated by heating for ten minutes at 100° in 0.01 *N* HCl. After deionization with Amberlite MB-3, the fucose in the hydrolysate was estimated colorimetrically[7] or by paper chromatography.

Additions	μmole fucose formed
None	<0.02
2.0 μmoles TPN	0.05
2.0 μmoles TPNH	0.40
2.0 μmoles DPNH	<0.02
2.0 μmoles TPNH and 1.0 μmole GTP in place of GDPM	<0.02

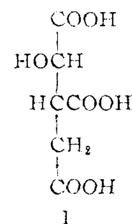
(1) R. Denamur, G. Fauconneau and G. Guntz, *Compt. rend.*, **246**, 2820 (1958).
(2) V. Ginsburg and H. N. Kirkman, THIS JOURNAL **80**, 3481 (1958).
(3) Abbreviations: GDPM, guanosine diphosphate mannose; GTP, guanosine triphosphate; TPNH, reduced triphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide.
(4) Strain A_3S_1 (ATCC 12657).
(5) E. Cabib and L. F. Leloir, *J. Biol. Chem.*, **206**, 779 (1954).
(6) P. M. Nossal, *Australian J. Exptl. Biol.*, **31**, 583 (1953).
(7) Z. Dische and L. B. Shettles, *J. Biol. Chem.*, **175**, 595 (1948).
(8) W. R. Reis and T. Reynolds, *Nature*, **181**, 768 (1958).
(9) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).
(10) M. A. Jermyn and F. A. Isherwood, *ibid.*, **44**, 402 (1949).
(11) M. Green and S. S. Cohen, *J. Biol. Chem.*, **219**, 557 (1956).
(12) Z. Dische and E. Borenfreund, *ibid.*, **192**, 583 (1951).

NATIONAL INSTITUTE OF ARTHRITIS & METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
U. S. PUBLIC HEALTH SERVICE V. GINSBURG
BETHESDA, MARYLAND

RECEIVED JULY 7, 1958

CRYSTALLOGRAPHIC EVIDENCE FOR THE RELATIVE CONFIGURATION OF NATURALLY OCCURRING ISOCITRIC ACID[1]

Recently Greenstein and his co-workers[2] have studied the stereochemistry of the isocitric acids and alloisocitric acids and have concluded that the configuration of the α-carbon atom in the naturally occurring isocitric acid is L_s. Gawron and Glaid,[3] on the basis of *pK* measurements, have concluded that in the isocitric acid lactone the two carboxyl groups are *cis* with respect to the γ-lactone ring while in that of alloisocitric acid the two carboxyls are *trans*. Thus, if the α carbon is in the L configuration, the formula in the Fischer convention for the naturally occurring isocitric acid is I.



Through the kindness of Dr. H. B. Vickery and Dr. D. G. Wilson of the Connecticut Agricultural Experiment Station we have been able to carry out an X-ray structure analysis on excellent crystals of the monopotassium and monorubidium salts of the lactone prepared by them from the isocitric acid occurring in the leaves of *Bryophyllum calycinum*. These salts are isomorphous on the orthorhombic

(1) Supported by a grant (C1253) from the National Cancer Institute, Public Health Service.
(2) (a) J. P. Greenstein, N. Izumiya, M. Winitz and S. M. Birnbaum, THIS JOURNAL, **77**, 707 (1955); (b) M. Winitz, S. M. Birnbaum and J. P. Greenstein, *ibid.*, **77**, 716 (1955).
(3) O. Gawron and A. J. Glaid III, *ibid.*, **77**, 6638 (1955).

space group $P2_12_12_1$ with four molecules (K or Rb)-$C_6O_6H_5$ in cells of dimensions:

	a (Å.)	b (Å.)	c (Å.)	Cell volume (Å³.)	Density (g. cm.⁻³) obs.	calcd.
K	9.059	12.681	6.640	762.8	1.838	1.848
Rb	9.190	12.639	6.825	792.7	2.142	2.166

The standard error of the cell dimensions is about ±0.15% and that of the observed densities about ±0.5%.

The structure was determined from projections on the three principal planes using the method of isomorphous replacement. The three projections for the potassium salt have been refined by difference Fourier maps and then by least squares using the full matrix to account for the overlap of atoms. At the present stage of refinement the R values for the $hk0$, $0kl$, and $h0l$ projections are 7.6, 11.5, 12.6%, respectively, the unobserved reflections being included at one-half their estimated upper limit.

The structure which we have obtained for the lactone ion confirms the conclusion of Gawron and Glaid that the carboxyl groups are *cis* with respect to the lactone ring. We also find that the metal atom is coördinated with eight oxygen atoms. All oxygen atoms including that in the lactone ring take part in this coördination. The structure clearly explains the pronounced cleavage of these crystals parallel to the b face.

Complete details of the structure analysis and a discussion of the bond lengths and bond angles will be presented elsewhere. We also hope to determine the absolute configuration of the rubidium salt by the method of Bijvoet and his co-workers.[4]

(4) J. M. Bijvoet, A. F. Peerdeman and A. J. vau Bommel, *Nature* **168**, 271 (1951).

INSTITUTE FOR CANCER RESEARCH
PHILADELPHIA 11, PA.

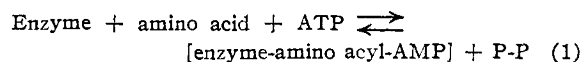
JENNY PICKWORTH GLUSKER
A. L. PATTERSON
WARNER E. LOVE
MARILYN L. DORNBERG

RECEIVED JUNE 7, 1958

REVERSIBILITY OF AMINO ACID INCORPORATION INTO RIBONUCLEIC ACID

Sir:

The incorporation of C^{14}-labeled amino acids into ribonucleic acid[1] has been reported[2] and confirmed.[3] That a specific amino acid-activating enzyme is required for the incorporation into ribonucleic acid me-amino of the amino acid it activates,[3a,b] suggests these reactions



Enzyme + amino acid + ATP ⇄ [enzyme-amino acyl-AMP] + P-P (1)

[Enzy acyl-AMP] + RNA ⇄ amino acid-RNA + enzyme + AMP (2)

(1) These abbreviations are used: RNA, ribonucleic acid; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; TCA, trichloroacetic acid; GMP, guanosine 5′-monophosphate; CMP, cytidine 5′-monophosphate; P-P, pyrophosphate.

(2) M. B. Hoagland, P. C. Zamecnik and M. L. Stephenson, *Biochim. et Biophys. Acta*, **24**, 215 (1957).

(3) (a) R. S. Schweet, F. C. Bovard, E. H. Allen and E. Glassman, *Proc. Natl. Acad. Sci.*, **44**, 173 (1958); (b) P. Berg and E. J. Ofengand, *ibid.*, **44**, 78 (1958); (c) K. Ogata and H. Nohara, *Biochim. et Biophys. Acta*, **25**, 659 (1957).

The reversibility of Reaction 1 has been shown previously.[4] Indirect evidence suggesting reversal of the overall reaction has been reported.[5] Reversal has been followed directly here by measuring the cleavage of isolated amino acid-RNA and incorporation of AMP into ATP.

Cleavage of isolated threonine-RNA and leucine-RNA, and incorporation of C^{14}-labeled AMP into ATP, dependent on amino acid-RNA, is shown in Table I. No ATP or free amino acid was added in

TABLE I

CLEAVAGE OF AMINO ACID-RNA AND INCORPORATION OF AMP INTO ATP

Constituents	Starting with threonine-RNA: Counts/min. of C^{14}-AMP found in ATP	Counts/min. of C^{14}-threonine-RNA remaining	Starting with leucine-RNA: Counts/min. of C^{14}-AMP found in ATP	Counts/min. of C^{14}-leucine RNA remaining
(1) Complete mixture[a]	408	168	526	416
(2) Zero time control	114	983	84	860
(3) As (1), but RNA in place of amino acid-RNA[b]	108	...	64	...
(4) As (1), but boiled enzyme	100	887	...	...
(5) As (1), but P-P omitted	128	316	100	820
(6) As (1), but AMP omitted	...	805	...	804

[a] The complete reaction mixture for AMP incorporation into ATP contained 0.5 ml. of activating enzyme, approximately 0.5 mg. of RNA or C^{12}-amino acid-RNA; 100 μmoles of Tris buffer, pH 7.5; 2 μmoles of C^{14}-AMP (Schwarz Laboratories), containing 60,000 counts/min./μmole; 2 μmoles of magnesium chloride; 2 μmoles of P-P; and water to make 1.8 ml. Mixtures were incubated at 37° for 15 minutes; then 0.7 mg. of casein, 1.8 ml. of 7% TCA and 10 μmoles of ATP were added and the ATP isolated and counted (see Holley[5]). Incubation conditions for studying cleavage of amino acid-RNA were similar, but using C^{12}-AMP and C^{14}-amino acid-RNA, and counting residual amino acid-RNA (precipitated by perchloric acid). Separate activating enzyme fractions for leucine or threonine activation, free of RNA, were prepared from guinea pig liver.[6] Labeled amino acid-RNA compounds were prepared by phenol extraction from the usual reaction mixture for incorporation,[3a] and contained 1 mμmole of C^{14}-amino acid (2500 c.p.m.) per mg. of RNA. [b] RNA was prepared by phenol extraction and was active for amino acid incorporation.[7] In this experiment 0.5 mμmole of C^{12}-threonine or leucine was added. The dash indicates "experiment omitted."

these experiments. Both amino acid-RNA compounds were cleaved in the presence of AMP plus P-P (complete mixture), although the quantitative importance of P-P depends on the particular amino-acid-RNA and enzyme fraction used. In other experiments, the amount of threonine-RNA split depended on the amount of AMP added, GMP or CMP did not replace AMP; Mg ion was essential; and leucine-RNA was not split by threonine-activating enzyme.

(4) M. B. Hoagland, E. B. Keller and P. C. Zamecnik, *J. Biol. Chem.*, **218**, 345 (1956); J. A. De Moss, S. M. Genuth and G. D. Novelli, *Proc. Natl. Acad. Sci.*, **42**, 325 (1956); P. Berg, *Fed. Proc.*, **16**, 152 (1957).

(5) R. W. Holley, THIS JOURNAL, **79**, 658 (1957); M. B. Hoagland, M. L. Stephenson, J. F. Scott, L. I. Hecht and P. C. Zamecnik, *J. Biol. Chem.*, **231**, 241 (1958); J. Mager and F. Lipmann, *Proc. Natl. Acad. Sci.*, **44**, 305 (1958).

(6) E. H. Allen, E. Glassman and R. S. Schweet, in preparation.

(7) K. S. Kirby, *Biochem. J.*, **64**, 405 (1956).