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]

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 |

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.

---

[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).

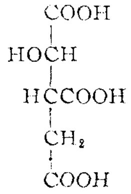
NATIONAL INSTITUTE OF
ARTHRITIS & METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
U. S. PUBLIC HEALTH SERVICE V. GINSBURG
BETHESDA, MARYLAND

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.



$$\begin{array}{c} \mathrm{COOH} \\ | \\ \mathrm{HOCH} \\ | \\ \mathrm{HCCOOH} \\ | \\ \mathrm{CH_2} \\ | \\ \mathrm{COOH} \end{array}$$

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).