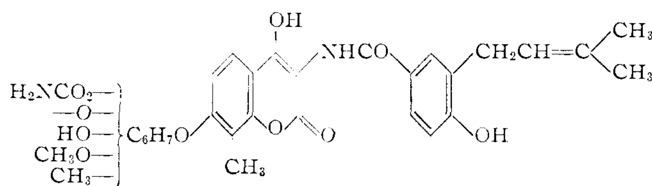
These data permit the following partial structure for novobiocin:



We acknowledge with gratitude the contributions of Drs. M. Calvin, H. E. Carter, D. J. Cram, J. L. Johnson and E. C. Olson, Mrs. A. E. Fonken and Mr. W. A. Struck to this work.

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RECEIVED FEBRUARY 9, 1956

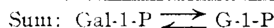
DIPHOSPHOPYRIDINE NUCLEOTIDE, A COFACTOR FOR GALACTO-WALDENASE[1]

Sir:

The interconversion of Gal-1-P and G-1-P in galactose adapted yeast[2,3] and in mammalian tissue[4] has been found to occur according to the equations

$$\text{Gal-1-P} + \text{UDPG} \rightleftharpoons \text{UDPGal} + \text{G-1-P} \quad (1)$$

$$\text{USPGal} \rightleftharpoons \text{UDPG} \quad (2)$$



$$\text{Sum: Gal-1-P} \rightleftharpoons \text{G-1-P}$$

Reaction (1), catalyzed by a specific uridyl transferase[3,4] is a reversible transfer of the uridyl group from G-1-P to Gal-1-P. Reaction (2) is an inversion at the C4 of the monosaccharide moiety catalyzed by galacto-waldenase.[3]

Galacto-waldenase has now been purified about 30-fold from a water extract of calf liver acetone powder. The extract was adjusted to *p*H 5.5 and acetone (−10°) was added to a final concentration of 25%. The precipitate was dissolved in glycine buffer, *p*H 8.0, and fractionated with alkaline ammonium sulfate (active fraction 45–65% saturation). This precipitate was dissolved in water and ammonium sulfate was added to 35% saturation. The active protein was then precipitated by adjusting the *p*H to 4.9. Further purification was obtained by absorption and elution from calcium phosphate gel followed by a final alkaline ammonium sulfate fractionation.

The activity of the fractionated preparation of galacto-waldenase was measured by the rate of DPN reduction when UDPGal[4] was added as substrate and UDPG dehydrogenase[5,6] and DPN were present as indicators of the inversion.

$$\text{UDPGal} \longrightarrow \text{UDPG} \quad (3)$$

$$\text{UDPG} + 2\text{DPN}^+ \longrightarrow \text{UDPGA} + 2\text{DPNH} + 2\text{H}^+ \quad (4)$$

When purified galacto-waldenase was incubated with UDPGal in the absence of the indicator system, the inversion reaction did not proceed. It could be initiated, however, by adding catalytic amounts of DPN. This is illustrated in Table I. That the cofactor is DPN and not some impurity in the preparation is borne out by experiments using purified *Neurospora*, DPNase.[7] DPN preincubated with DPNase until it was no longer active when assayed with alcohol dehydrogenase was not active in the galacto-waldenase system. A control with DPN similarly preincubated with heat inactivated DPNase was active. DPNH was inactive in the system unless it was oxidized by preincubation with acetaldehyde and alcohol dehydrogenase. DPN could not be replaced by TPN.

TABLE I

EFFECT OF DPN ON GALACTO-WALDENASE REACTION

Reaction mixture consisted of 0.065 μmole of UDPGal containing 0.009 μmole UDPG as impurity, 4.0 μmoles of cysteine, purified galacto-waldenase (12 μgm. protein), and DPN or TPN as indicated in a total volume of 0.5 ml. of 0.1 *M* glycine buffer, *p*H 8.7. The reaction mixture was inactivated after 15 min. incubation at room temperature by heating at 100° for 20 sec.; 200-μl. aliquots were analyzed for UDPG with DPN and UDPG dehydrogenase.[6] UDPGal remaining was determined in the same cuvette by the subsequent addition of galacto-waldenase. A blank of 0.009 μmole due to UDPG originally present in the UDPGal has been subtracted.

Nucleotide added, μmole		UDPG formed, μmole	UDPGal utilized, μmole
	None	0.000	0.000
DPN	0.0005	.006	.005
DPN	.002	.012	.012
DPN	.02	.026	.023
DPN	.016 (DPNase treated)	.000	.002
DPN	.016 (Heated DPNase treated)	.024	.023
DPNH	.014	.000	.000
DPNH	.014 (enzy. reoxidized)	.022	.021
TPN	.02	.002	...
TPN	.20	.004	.005

The marked effect of catalytic amounts of DPN suggests that the inversion occurs by an oxidation and subsequent reduction at the C4 of the carbohydrate. The structure of the hypothetical oxidized intermediate remains to be determined. Attempts to accumulate such an intermediate are in progress.

Acknowledgment.—The author wishes to express sincere appreciation to Dr. Herman M. Kalckar, in whose laboratory these experiments were performed, for his constant and valuable advice.

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RECEIVED FEBRUARY 3, 1956

(1) The following abbreviations have been used: Gal-1-P for α-D-galactose-1-phosphate, G-1-P for α-D-glucose-1-phosphate, UDPG for uridine diphosphoglucose, UDPGal for uridine diphosphogalactose, DPN for diphosphopyridine nucleotide, DPNH for reduced diphosphopyridine nucleotide, TPN for triphosphopyridine nucleotide; UDPGA for uridine diphosphoglucuronic acid.

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