Biosynthesis of Branched Chain Deoxysugars

IV. ISOLATION OF CYTIDINE DIPHOSPHATE 6-DEOXY-3-C-METHYL-2-O-METHYL-4-O-(O-METHYLGLYCOLYL)-L-ALDOHEXOPYRANOSONIDE FROM AZOTOBACTER VINELANDII*

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SUMMARY

A new cytidine nucleotide was found in Azotobacter vinelandii strain O. On the basis of chemical analyses, mass spectra, infrared spectra, nuclear magnetic resonance spectra, periodate oxidation studies, and a series of degradation studies followed by comparison with authentic samples, it is proposed that the new compound is cytidine-5'-diphospho-6-deoxy-3-C-methyl-2-O-methyl-4-O-(O-methylglycolyl)-L-aldohexopyranoside. The compound is therefore an O-methylglycolic acid ester of cytidine-5'-diphospho-vinelose previously found in the same microorganism.

EXPERIMENTAL PROCEDURES AND RESULTS

Two nucleotides present in extracts of Azotobacter vinelandii strain O have been shown to be derivatives of cytidine 5'-diphosphate (1, 3). In one of these (CDP-X) the pyrophosphate group is attached to an O-methylated, branched chain sugar, designated vinelose, and in the other (CDP-Y) it is attached to a carboxylic acid ester of O-methylated, branched chain sugar.

The structure of these nucleotidyl sugars is of interest, particularly in view of the extensive investigations (see the recent review by Grisebach (4)) on biosynthesis of branched chain sugars in connection with the participation of nucleotides in the branch formation. Interest in these compounds also arises from earlier observations (for a review of the literature, see References 4 and 5) that most of branched chain sugars as well as their carboxylic acid ester and methyl ether derivatives were found in antibiotic glycosides produced by microorganisms. It seems possible that these nucleotides would function in either transformations or transfer processes involving the sugar residues at some stage in the biosynthetic route leading to this type of bacterial glycoside.

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Fig. 1. Summary of reactions of CDP-(O-methylglycolyl)-vinelose, and its derivatives. The 4-O-(O-methylglycolyl)-vinelose residue is drawn as having a β-L-talose configuration.

Although this is believed to be so on the basis of the evidence presented in the text, the possibility that the sugar has other configurations could not be excluded.

The following paper chromatographic solvent systems were used generally by descending chromatography on Toyo No. 51A paper: Solvent A, 95% ethanol-1 M ammonium acetate, pH 7.2 (15:6); Solvent B, isobutyric acid-1 N ammonia (5:3); Solvent C, 1-butanol-ethanol-water (52:32:16); Solvent D, ethyl acetate-acetic acid-water (3:1:3); Solvent E, 1-butanol-pyridine-water (6:4:3); Solvent F, 1-butanol-ethanol-water (4:1:6); Solvent G, 95% ethanol-1 M ammonium acetate, pH 3.8 (15:6); Solvent H, ethanol-2-butanone-0.5 M morpholinium tetraborate, pH 8.6, in 0.01 M EDTA (70:20:30); Solvent I, pyridine-ethanol-acetic acid-water (1:3:1:15); Solvent J, 1-butanol-pyridine-0.05 M morpholinium tetraborate, pH 8.6 (7:5:2); Solvent K, 1-pentanol-acetic acid-water (4:1:5); Solvent L, 1-octanol-formic acid-water (75:25:75); Solvent M, 1-butanol-benzene-water (1:1:1); Solvent N, isobutyric acid saturated with water; and Solvent O, 95% ethanol containing 5% ammonium-pyridine-water (3:1:13).

Paper electrophoresis was carried out on 60-cm strips of Toyo No. 51A paper in the apparatus described by Markham and Smith (12) at a potential gradient of 30 volts per cm for 70 min. The buffers used for these procedures were: Buffer A, 0.05 M sodium acetate-acetic acid, pH 4.8; and Buffer B, 0.1 M sodium borate, pH 9.5.

Nucleotides were examined on paper chromatograms or electrophoretograms under ultraviolet light and phosphoric esters were detected by the perchloric acid-molybdate reagent (13). To reveal carboxylic acid hydroxamates, papers were sprayed with 95% ethanol containing 1% FeCl₃ and 0.1% HCl. The hydroxamate spots gave an intense red-purple color.

The following instruments were used: a Jasco photoelectric spectrometer, model ORD/UV-5, for the measurement of optical rotation and optical rotatory dispersion; a Hitachi grating infrared spectrometer, model EMG, for the infrared spectra; a Varian nuclear magnetic resonance spectrometer, model HA-100, for the nuclear magnetic resonance spectra; and a Hitachi mass spectrometer, model RMU-6C, for the mass spectra. The authors would like to express their appreciation to Dr. T. Murachi, Nagoya City University, Nagoya, Japan, for assistance with the measurement of optical rotation and optical rotatory dispersion, Dr. Y. Asahi, Takeda Chemical Industry, Osaka, Japan, for assistance with the determination of nuclear magnetic resonance spectra, and Dr. K. Sasaki in this department for assistance with the determination of mass spectra.

Isolation and General Properties of CDP-Y—With 25 kg of the frozen bacteria as starting material, a purification of CDP-Y as far as the stage for paper chromatography in Solvent A was done (see Reference 3). The sample (for Rf value see Table I) was eluted from the papers with water and then screened successively by paper chromatography in Solvent B, paper chromatography in Solvent C, and paper electrophoresis in Buffer A essentially as described in the previous paper (3) for the purification of CDP-vinelose. The nucleotide was eluted from the electro-
phoretograms with water. After the eluates were lyophilized, the compound was further purified by precipitating it as the sodium salt with acetone from aqueous methanol solution. The yield was 146 mg (185 μmoles, if calculated from ultraviolet light absorption measurements with the use of 13.0 as a mili-
molar extinction coefficient); \([\alpha]_{280}^{20} +12.5^\circ \text{ (c, 1.55, in H}_2\text{O)}\).

The absorption spectrum in the ultraviolet region from 220 to 310 mp was identical with that of highly purified 5'-CMP. The millimolar extinction coefficient in 0.01 n HCl at 280 μm was 13.0, based on absorbance per 2 moles of total phosphate. This value is also identical with that of 5'-CMP. The ratio of total phosphate to acid labile phosphate to pentose (as ribose) to methoxyl group was 2.00:1.01:0.92:1.90. The occurrence of two methoxyl groups distinguishes CDP-Y from CDP-vinlose.

Formation of CDP-vinlose from CDP-Y by Alkaline Hydrolysis—The presence of an ester linkage in CDP-Y was first indicated by its positive reaction with the hydroxylamine-FeCl₃ reagent (14) for carboxylic acid ester. In support of the result of the color reaction, it was further shown that CDP-Y is susceptible to hydrolysis with weak alkali. A complete degradation of CDP-Y under the mildest possible condition was found to occur on treating in 1 n ammonia at 35° for 2 hours. Under this condition, an ultraviolet light absorbing product was obtained and tentatively identified as CDP-vinlose by paper chromatography in Solvents A, B, G, and H and by paper electrophoresis in Buffers A and B (Table I).

To obtain a substantial amount of the nucleotide fragment (CDP-deacyl-Y; Compound II, Fig. 1) for further characterization, 88 mg of CDP-Y, dissolved in 3 ml of 1 n ammonia, were placed in a water bath at 35° for 2 hours. The nucleotide product after hydrolysis was recovered from the mixture by the charcoal method (15). The resulting eluate (84 ml) from charcoal (25 mg) was reduced to 0.4 ml on a rotating evaporator, from which the nucleotide was precipitated as white powder with methanol. The nucleotide was recovered from the mixture by the charcoal method (15). The resulting eluate (84 ml) from charcoal (25 mg) was reduced to 0.4 ml on a rotating evaporator, from which the nucleotide was precipitated as white powder with acetone. The yield was 48 mg (65 μmoles as cytidine); \([\alpha]_{280}^{20} +12.5^\circ \text{ (c, 1.55, in H}_2\text{O)}\).

With this preparation, a series of identification procedures was undertaken. All of the evidence so far obtained indicates that CDP-deacyl-Y is, in fact, CDP-vinlose. The evidence may be outlined as follows.

1. The nucleotide exhibited an ultraviolet light absorption spectrum typical of a cytidine derivative. The ratio of cytidine to total phosphate to labile phosphate to pentose (as ribose) to methoxyl group was 1.00:1.09:0.96:0.91:1.05. The reducing value, determined by ferriyamid reduction (16) after hydrolysis with 0.01 n HCl for 5 min at 100° and expressed relative to a glucose standard, was 0.26. With this method, the reducing value of authentic vinlose is 0.27 relative to glucose.

2. Enzymatic hydrolysis of the nucleotide with snake venom pyrophosphatase yielded two products, which were separated from each other by ion exchange chromatography on Dowex 1-chloride. The separation method was essentially the same as that used previously for the separation of pyrophosphatase digest of CDP-vinlose (3).

One of the products, which was eluted first from the column, was recovered and concentrated by the charcoal method and precipitated as the barium salt with ethanol from water; 25 mg of crystalline material were obtained from 46 mg of CDP-deacyl-Y. Its ultraviolet absorption spectrum, infrared absorption spectrum, and optical rotatory dispersion spectrum were all identical with the corresponding spectra of 5'-CMP. Inorganic phosphate was liberated from the sample when it was exposed to the action with 5'-nucleotidase. It is concluded therefore that the first product is 5'-CMP.

The second product was likewise recovered and concentrated to amorphous solids. The yield from 40 mg (65 μmoles as cytidine) of CDP-deacyl-Y was 55 μmoles, as estimated from the phosphate content; \([M]_{280}^{20} +4540^\circ \text{ (in H}_2\text{O)}\). The substance was shown by paper chromatography and paper electrophoresis (Table I) as well as by the measurement of nuclear magnetic resonance spectrum (Fig. 2, Table II) and optical rotatory dispersion (300 to 650 mp) to be indistinguishable from vinose-1-P. The phosphate ester from CDP-deacyl-Y is referred to as deacyl-Y-1-P (Compound III, Fig. 1).

3. Deacyl-Y-1-P is susceptible to degradation by E. coli alkaline phosphatase. One of the cleavage products was shown to be inorganic phosphate with the Lowry-Lopez reagent (17) and the other (deacyl-Y; Compound IV, Fig. 1) to be vinose by means of paper chromatography (Table III), infrared absorption spectroscopy (Fig. 3), and mass spectrometry. Since the mass spectrum of deacyl-Y is essentially identical with the mass spectrum of vinose presented in the previous paper (3), it is not shown here.

All of the results described above support the view that CDP-deacyl-Y is identical with CDP-vinlose. It follows that CDP-Y is a derivative of CDP-vinlose bearing a carboxylic acid residue through an ester link.

Identification of Carboxylic Acid Residue—Information regarding the molecular weight of the carboxylic acid residue was obtained through comparison of the mass spectrum of sugar Y with that of vinlose.

The sample of sugar Y (Compound VI, Fig. 1) was prepared from CDP-Y (87 mg as the sodium salt or 110 μmoles as cytidine)
Fig. 2. The nuclear magnetic resonance spectrum of deacyl-Y-1-P (Compound III, Fig. 1) at 100 Meps. The measurement was performed in 0.4 ml of D2O with 49.4 µmoles of sample (ammonium salt). The spectrum of vinclose-1-P was essentially identical with this spectrum. TMS, tetramethylsilane (external standard).

### Table II
Summary of chemical shifts and coupling constants for deacyl-Y-1-P (or vinclose-1-P') in D2O

| Chemical shift, δ | Multiplicity of signal | Relative no. of protons | Coupling constant, J (Hz) | Assignment |
|------------------|------------------------|-------------------------|--------------------------|------------|
| 1.32             | Doublet                | 3                       | 7 (J6,6)                 | C3-CH3     |
| 1.36             | Singlet                | 3                       | 7 (J6,6)                 | C2-CH3     |
| 3.31             | Doublet                | 1                       | 2 (J1,1)                 | C1-H       |
| 3.39             | Doublet                | 1                       | 2 (J1,1)                 | C1-H       |
| 3.65             | Singlet                | 3                       | 7 (J1,1)                 | C2-OCH3    |
| 3.96             | Quartet of doublets    | 1                       | 7 (J1,1)                 | C1-H       |
| 5.29             | Doublet of doublets    | 1                       | 9 (J1,1)                 | C1-H       |

### Table III
Paper chromatography and paper electrophoresis of sugar Y and related sugars

| Compound | RF | Paper chromatography | Glucose: | Glucose: |
|----------|----|----------------------|----------|----------|
|          |    | Paper chromatography | glucose   | glucose: |
| Sample   |    | Paper chromatography | glucose   | glucose: |
| Y-sugar  | 0.70 | 0.78 0.68 0.80 0.82 | 0.00      | 0.02     |
| Deacyl-Y  | 0.71 | 0.74 0.66 0.75 0.76 | 0.02      | 0.02     |
| Standard | 0.71 | 0.74 0.66 0.75 0.76 | 0.02      | 0.02     |
| 6-Deoxy-3-C-methyl-2,4-Me-glucose | 0.68 | 0.71 0.68 0.81 0.77 | 0.18      | 0.18     |
| 6-Mycarose | 0.70 | 0.78 0.69 0.76 0.77 | 0.35      | 0.35     |
| 6-Mycarose | 0.69 | 0.78 0.66 0.76 0.77 | 0.35      | 0.35     |
| 6-3-Epi-mycarose | 0.58 | 0.76 0.58 0.71 0.73 | 0.18      | 0.18     |
| 6-4-Epi-mycarose | 0.63 | 0.79 0.67 0.74 0.75 | 0.57      | 0.57     |

* mobility of sample (in centimeters)/mobility of glucose standard (in centimeters).

By successive digestion with nucleotide pyrophosphatase and alkaline phosphatase in a similar manner as described for the preparation of vinclose from CDP-vinclose (3). The sugar was obtained from the chloroform solution as a colorless transparent liquid; [α]D -26.5° (c, 0.85, in CHCl3). The yield was 24 mg.

During these processes, the nucleoside monophosphate fragment was obtained from the pyrophosphatase digest as the crystalline barium salt and identified as 5'-CMP essentially as described above for the 5'-CMP fragment obtained from CDP-deacyl-Y. The sugar phosphate fragment (Y-1-P; Compound V, Fig. 1) was also obtained from the pyrophosphatase digest as the ammonium salt; [α]D +3500° (in H2O). The yield was 98 µmoles, as estimated from the phosphate content. This sample was used for the studies with nuclear magnetic resonance spectroscopy (see below).

The mass spectrum of sugar Y is presented in Fig. 4. As can be seen, the peak of maximum m/e value is situated at 246. Since, in most instances, "M - H2O" fragments, but not molecular ions, are recognizable in the mass spectra of free carbohydrates (18, 19), it is likely that the molecular weight of sugar Y is equal to 264 (i.e. 246 + 18). Assuming that sugar Y is a molecule made up of 1 mole each of carboxylic acid and vinclose with subtraction of 1 eq of water, the molecular weight of the carboxylic acid (as free acid) is given by 264 (molecular weight of sugar Y) + 18 (molecular weight of water) - 192 (molecular weight of vinclose) = 90. A conclusion can be drawn from this value that the carboxylic acid is O-methylglycolic acid, because the presence of an O-methyl group in the carboxylic acid moiety is indicated by the difference in methoxyl group contents in CDP-Y and CDP-deacyl-Y (see above) and the molecular weight, 90, is
sufficiently low to exclude from consideration all other methyl ether derivatives of hydroxycarboxylic acids.

In support of this conclusion the hydroxamate of the carboxylic acid (Compound VII, Fig. 1), prepared from CDP-Y by the treatment with hydroxylamine, was indistinguishable from the hydroxamate of O-methylglycolic acid in its behavior on paper chromatography (Table IV).

The nuclear magnetic resonance spectrum of Y-l-1' (Fig. 5; Table V), which gives rise to signals at 3.57 and 4.43 ppm corresponding to the 5 protons of O-methylglycolyl group (i.e. the protons of -OCH3 and -CH2-), is a further indication of the structure of the carboxylic acid.

Properties of Sugar Y—It may be appropriate here to make a few comments on the properties of the new sugar, Y. The \( R_p \) values of sugar Y in the solvents indicated in Table III are all higher than the respective values of vinose. Like vinose, sugar Y is revealed on the chromatograms by the aniline hydrogen phthalate reagent (20), silver nitrate reagent (21), and vanilline-perchloric acid reagent (22). It is not revealed, however, with the periodate-benzidine reagent (23) which is active with vinose. As expected from the proposed structure, reagents of broad specificity for carboxylic acid esters, such as hydroxylamine-FeCl3 (24), also reveal sugar Y on the chromatograms.

The infrared spectrum of sugar Y is shown Fig. 3, together with the spectra of vinose (or deacyl-Y) and 6-deoxy-3-C-methyl-2-O-methyl-\( \alpha \)-alloce, one of the 16 possible isomers (i.e eight diastereoisomers and their optical enantiomers) for vinose. The bands at 1740 and 1200 cm\(^{-1}\), which are not seen in the other two spectra, are a strong indication of the presence of an ester linkage in sugar Y. Also to be noted is the fact that the spectrum of vinose (or deacyl-Y) is distinct from that of 6-deoxy-3-C-methyl-2-O-methyl-\( \alpha \)-alloce.

Position of O-Methylglycolyl Group in Sugar Y—As noted above, sugar Y was not visualized on the chromatograms with the periodate-benzidine reagent. If sugar Y was treated with 0.0013 M periodate in 0.05 M acetate buffer, pH 5.0, at 20\(^\circ \)C, no periodate consumption was observed during 20 hours. Under the same condition, vinose (or deacyl-Y) consumed 2 moles of periodate in 20 hours. These results then suggest that the O-methylglycolyl group is located at position 4 of the vinose residue since with a C4-substituted vinose uptake of 1 mole of periodate would occur at C4.

Evidence that supports the above interpretation was also provided by the studies with nuclear magnetic resonance spectroscopy. When the spectrum of Y-1-P (Fig. 5; Table V) is compared with that of deacyl-Y-1-P (Fig. 2; Table II), the C6-H signal indicates a tendency toward shifting to a lower position (paramagnetic shift). Since it is known that signals from protons attached as in acyl-O-CH normally lie in a lower position than those attached as in HO-CH, the shift on the C6-H signal in the Y-1-P spectrum is consistent with the assignment of the position for O-methylglycolyl group.

Configuration of Vinose—It was reported in our previous paper (3) that the 3 carbon atoms at positions 4, 5, and 6 of vinose were converted, by successive oxidation of vinose-1-P with periodate and bromine and dephosphorylation with 5 N HCl at 100\(^\circ \)C for 6 hours, to a hydroxycarboxylic acid identified as

![Fig. 3. Infrared absorption spectra of A, deacyl-Y (Compound IV, Fig. 1); B, sugar Y (Compound VI, Fig. 1); and C, 6-deoxy-3-C-methyl-2-O-methyl-\( \alpha \)-alloce (the sample was contributed by Dr. G. B. Howarth, Queen's University, Kingston, Ontario, Canada). Samples were incorporated in KBr discs. The spectrum of vinose was essentially identical with that of deacyl-Y.](http://www.jbc.org/)

![Fig. 4. The mass spectrum of sugar Y (Compound VI, Fig. 1). The intensities of the peaks are expressed in relation to the m/e 45 peak, which is assigned an arbitrary value of 100%.](http://www.jbc.org/)

**TABLE IV**

| Compound                   | Solvent K | Solvent L | Solvent M | Solvent N | Solvent O |
|----------------------------|-----------|-----------|-----------|-----------|-----------|
| Sample                     | 0.35      | 0.19      | 0.24      | 0.55      | 0.55      |
| Standard (hydroxamate of)  |           |           |           |           |           |
| Acetic acid                | 0.34      | 0.21      | 0.26      | 0.49      | 0.67      |
| Propionic acid             | 0.55      | 0.36      | 0.43      | 0.62      | 0.72      |
| Lactic acid                | 0.26      | 0.42      | 0.45      | 0.40      | 0.55      |
| O-Methylglycolic acid      | 0.35      | 0.19      | 0.24      | 0.55      | 0.55      |
| O-Ethylglycolic acid       | 0.53      | 0.35      | 0.46      | 0.67      | 0.61      |
| O-Methylalaetic acid       | 0.48      | 0.22      | 0.43      | 0.62      | 0.67      |
lactic acid. Since this acid serves as a substrate for L-lactic acid dehydrogenase, an L configuration was suggested for the vinelose in CDP-vinelose. The use of 5 N HCl at 100°, however, resulted in significant breakdown of lactic acid, and it was difficult to increase the yield of lactic acid over 48%.

To overcome this difficulty, a modified method has been developed, in which the oxidation product is dephosphorylated by alkaline phosphatase and subjected to determination of D- and L-lactic acid with the use of Leuconostoc D-lactic acid dehydrogenase and bovine heart L-lactic acid dehydrogenase. The method has been applied to vinelose-1-P (and deacyl-Y-l-P) as follows: the sugar phosphate (1 pmole) was mixed with 0.04 M acetate buffer, pH 5.0 (500 µl), and 0.1 M sodium periodate (50 µl) and, after 8 hours at room temperature in the dark, excess of periodate was destroyed by the addition of 1 M glycerol (4 µl). The solution was kept for a further 1 hour at room temperature and then mixed, successively, with 1 M sodium acetate (30 µl) and bromine (25 µl). The tube was sealed and kept at 5° for 60 hours. Excess bromine was then removed by aeration and the pH of the solution was adjusted to 8.5 with ammonia. Alkaline phosphatase (1.5 units) was added to this solution and incubated at 37°. After 5 hours, the tube was placed in a boiling water bath for 3 min and then centrifuged to remove the denatured proteins. With the supernatant solution, enzymatic analyses of D- and L-lactic acid were carried out according to the method of Hayama, Koga, and Fukui (6) and the method of Hohorst (25), respectively. The lactic acid (Compound VIII, Fig. 1) formed in this way was shown to have the L configuration. The yields of L-lactic acid were 0.95 pmole from vinelose-1-P and 0.97 pmole from deacyl-Y-1-P, in close agreement with the amount of starting material. D-Lactic acid was not detected in the reaction mixtures more than trace amounts. D-Lactic acid, added to the test materials after bromine oxidation, gave about 98% of the theoretical recovery. It leaves little doubt, therefore, that the vinelose in the nucleotides has the L-configuration.

Information on the configuration of C3 and C4 has been obtained from the kinetic studies of periodate oxidation. As shown in Fig. 6, vinelose-1-P (or deacyl-Y-1-P) consumes periodate at almost the same rate as β-1-O-methyl-2,6-dideoxy-3-C-methyl-DL-ribo-hexoside (i.e. β-1-O-methyl-DL-glycuronoside with the cis-α-glycol group at C3 and C4). The rates of these glycosides are much higher than those of β-1-O-methyl-2,6-dideoxy-3-C-methyl-DL-arabo-hexoside (i.e. β-1-O-methyl-DL-3-epi-glycuronoside with the equatorial C3-hydroxyl group, being trans to the equatorial C4-hydroxyl group) and β-1-O-methyl-2,6-dideoxy-3-C-methyl-DL-zylo-hexoside (i.e. β-1-O-methyl-DL-4-epi-glycuronoside with the axial C3-hydroxyl group, being trans to the axial C4-hydroxyl group). Since it is known that the rate of periodate oxidation is dependent principally on the stereochemistry of the α-glycol group, the above data suggest the presence of a cis-α-glycol group in vinelose-1-P as well as in deacyl-Y-1-P. There are eight possibilities for the configurational structure of L-vinelose, and four of them are thus

![Diagram](image-url)
is ruled out because the diastereoisomers relating to \(\alpha\)-glucose, \(\alpha\)-mannose, \(\alpha\)-galactose, and \(\alpha\)-idose must have trans-\(\alpha\)-glycol groups at \(\alpha_2\) and \(\alpha_4\).

The availability of a synthetic sample of 6-deoxy-3-C-methyl-2-O-methyl-D-allose has shown that the sugar differs in position on paper chromatograms from vinelose (Table III).

Since pairs of optical enantiomorphs would not be readily distinguished by these means, it seems unlikely that vinelose is in a relation of optical enantiomorph with 6-deoxy-3-C-methyl-2-O-methyl-D-allose. Thus, it is tentatively concluded that vinelose has the configuration relating to one of the three diastereoisomers, L-altrose, L-galactose, and L-talose, although a definite identification must wait until all of the diastereoisomers are synthesized for comparison.

**DISCUSSION**

In view of the suggestion that vinelose has the configuration relating to L-altrose, L-galactose, or L-talose, the possible chair conformations of \(\beta\)-L-vinelose-1-P are illustrated in Fig. 7. Since nearly all, if not all, of the nucleotidyl sugars isolated from natural sources have been shown to have either \(\alpha\)-D or \(\beta\)-L configuration, it is reasonable to assume that the anomeric carbon of the L-vinelose residue has the \(\beta\) configuration. In \(\beta\)-6-deoxy-3-C-methyl 2-O-methyl D-allose 1-phosphate and its L-galactose isomer the bulky groups on \(\beta_1\), \(\beta_4\), and \(\beta_6\) must be axial in the C1 conformations (Conformations A and C, Fig. 7), whereas no such 1,3-diaxial interactions of bulky substituents are present in the 1C conformations (Conformations B and D, Fig. 7). It follows that 1C is the more likely possible form for these sugars. In Conformation B, \(\beta_4\)—H is in a 1,2-diaxial relation with \(\beta_5\)—H and, in Conformation D, \(\beta_5\)—H is in a 1,2-diaxial relation with \(\beta_4\)—H.

\(\beta\)-L-vinelose-1-P is indicated by the fact that, in the nuclear magnetic resonance spectrum of this compound, the signals for \(\beta_1\)—H, \(\beta_2\)—H, \(\beta_3\)—H, and \(\beta_4\)—H at \(\delta = 5.29, 3.39, 3.31,\) and \(3.96\), respectively, showed a small \(J_{1,2}\) or \(J_{1,4}\) constant (about 2 cps, see Fig. 2 and Table II). Accordingly, either of the chair conformations of the talose isomer (Conformations E or F, Fig. 7), in which a 1,2-diaxial arrangement of hydrogens is absent, is considered as being the most preferred conformation for \(\beta\)-L-vinelose-1-P (the isomers B and D would give larger values of \(J_{1,2}\) and \(J_{1,4}\), respectively).

Experiments on the biochemical significance of CDP-vinelose and its O-methylglycolic acid derivative are in progress, but preliminary results support our previous postulate (3) that CDP-vinelose is derived from CDP-glucose via a CDP-6-deoxy-4-keto-hexose. It was shown previously (2) that a pyrophosphorylase is present in \(A.\ vinelandii\) which synthesizes CDP-glucose from CTP and \(\alpha\)-D-glucose-1-P. We have found more recently that an enzyme preparation from \(A.\ vinelandii\) is able to convert CDP-glucose into a CDP-6-deoxy-4-keto-hexose. The product is similar in its chemical properties to CDP-6-deoxy-4-keto glucose, an intermediate in the synthesis of CDP-3,6-dideoxy-4-hexoses (27, 28), but it can be converted by another enzyme fraction from \(A.\ vinelandii\) into at least two methylated compounds in the presence of \(S\)-adenosylmethionine.\(^3\)

The mechanism whereby such transmethylation occurs has not yet been established experimentally, but it seems likely that the nucleotidyl keto sugar may undergo rearrangement to constitute the site (i.e., 3,4-enediol) to which the methyl group is transferred. Either the enediol nucleotide or its C-methylated product may

\(^1\) Dr. G. B. Howarth kindly informed us that he also compared vinelose with his synthetic sugar by paper chromatography and gas-liquid chromatography of their O-acetyl derivatives and found them to be different.

\(^3\) M. Takagi, K. Kimata, S. Okuda, and S. Suzuki, unpublished observations.
also serve as the acceptor for methylation at the C₂-hydroxyl group. Further, one may expect from the occurrence of an O-
methylglycolic acid derivative of CDP-vinelose that CDP-
vineose may undergo an acylation of the sugar residue with an
appropriate acyl donor (probably with an acyl-CoA compound).

Among the branched chain deoxysugars that have been re-
ported to occur in nature in the form of carboxylic acid ester are
4-O-isovaleryl-L-mycarose in magnamycin (29) and leucomycins
methylglycolic acid derivative of CDP-vinelose that CDP-
group. Further, one may expect from the occurrence of an O-
elucidated, the close similarity in their structures strongly sug-
rllthough the processes of their biosynthesis have not yet. been
these sugars have a C-methyl branch and an O-acyl group at
positions 3 and 4, respectively, of the G-deoxy-L-hexose skeletons.

Like 4-O-(4-methylglycolyl)-l-vinelose, all of
positions 3 and 4 occur in these sugars at a nucleotide level.

Apart from 4-O-(4-methylglycolyl)-vinelose, which has now
been identified, the only other glycolic acid derivative of sugar
isolated from nature is N-glycolylneuraminic acid from hog (35).
In the latter compound, however, glycolic acid is attached as
an acid amide to neuraminic acid. At any rate, the natural occur-
gence of glycolic acid derivatives requires the activated form of
acid amide to neuraminic acid. At any rate, the natural occur-
been from nature is N-glycolylneuraminic acid from hog (35).

Although the processes of their biosynthesis have not yet been
elucidated, the close similarity in their structures strongly sug-
gs that a comparable C-methylation and O-acylation at
positions 3 and 4 occur in these sugars at a nucleotide level.

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ussions.

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