Formation of 1-Deoxy-D-*threo*-pentulose and 1-Deoxy-L-*threo*-pentulose by Cell-free Extracts of Microorganisms

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A new enzymatic acyloin-type condensation between pyruvate (or acetoin or methylacetoin) and D-glyceraldehyde was found to be catalyzed by cell-free extracts of a transketolase mutant of *Bacillus pumilus* IFO 12089. The reaction product (1) was isolated and determined to be 1-deoxy-D-*threo*-pentulose (D-DTP), which is considered to be a precursor of the five-carbon unit of the thiazole ring thiamine. 1-Deoxy-L-*threo*-pentulose (L-DTP, 2) was synthesized similarly when L-glyceraldehyde was used instead of D-glyceraldehyde. The configurations of 1 and 2 were confirmed by reduction to the corresponding 1-deoxy-pentitols.

Similar enzyme activities were also detected in cell-free extracts of all the wild-type strains tested of bacteria, actinomycetes, yeasts, and molds. These results suggest that the D-DTP synthesizing enzyme plays an important role in the biosynthesis of the thiazole ring of thiamine *in vivo*.

While studying the mechanism of 1-deoxy-D-*altro*-heptulose formation in a transketolase mutant of *Bacillus pumilus*,^{1,2)} we found that a partially purified enzyme preparation of the mutant also contained D-DTP synthesizing activity, which catalyzes an acyloin-type condensation between D-glyceraldehyde and pyruvate, DL-acetoin, or methylacetoin (2-hydroxy-2-methyl-3-butanone). By a similar method using L-glyceraldehyde as a substrate instead of D-glyceraldehyde, L-DTP was synthesized.

We also found that D-DTP- and L-DTP-synthesizing activities existed in the cell-free extracts of many other microorganisms, including bacteria, actinomycetes, yeasts, and molds.

Recent evidence^{3~7)} has established that D-DTP (1) is a direct precursor in the biosynthesis of the thiazole ring of thiamine in *Escherichia coli*. D-DTP has been enzymatically prepared from 1-deoxy-D-xylitol by sheep liver L-iditol dehydrogenase,⁸⁾ isolated from

the culture broth of *Streptomyces hygroscopicus*,^{9,10)} and has also been chemically synthesized.⁵⁾ L-DTP has been enzymatically prepared from 1-deoxy-L-xylitol by pigeon liver L-*threo*-pentulose reductase.⁸⁾ However, enzymatic formation of D-DTP and L-DTP by acyloin-type condensation has not previously been reported.

The present paper deals with the enzymatic formation of D-DTP and L-DTP and with the distribution of D-DTP- and L-DTP-synthesizing enzyme activities in microorganisms.

Isolation and identification of D-DTP (1)

When D-glyceraldehyde was incubated with pyruvate, DL-acetoin or methylacetoin in the presence of a partially purified enzyme preparation of the mutant BG2532, a spot of a newly formed substance (*R_f* 0.59, solvent B) was detected by PC, as shown in Fig. 1. The product (compound 1) was purified and isolated as crystals from the reaction mixture.

FIG. 1. Paper Chromatography of the Product Formed by a Partially Purified Enzyme Preparation of the Mutant BG2532.

Reaction mixtures (1 ml) contained 40 μ mol Tris-HCl buffer (pH 8.0), 5 μ mol $MgCl_2$, 0.5 μ mol TPP, 2 μ mol EDTA, 100 μ mol each of substrates, and a partially purified enzyme preparation of the mutant BG2532, which were incubated at 37°C overnight. The reaction was stopped by heating at 80°C for 3 min. After centrifugation, the supernatants were subjected to PC with solvent A. The papers were sprayed with vanillin-perchloric acid reagent and heated at 120°C for 5 min. (1), D-glyceraldehyde + sodium pyruvate; (2), D-glyceraldehyde + DL-acetoin; (3), D-glyceraldehyde + methylacetoin; (4), D-glyceraldehyde; (5), sodium pyruvate; (6), DL-acetoin; (7), methylacetoin; (8), (1) minus enzyme; (9), (2) minus enzyme; (10), (3) minus enzyme; (11), purified D-DTP preparation.

Elemental analysis of **1** required a molecular formula of $C_5H_{10}O_4$. The PMR spectrum of **1** in deuterium oxide showed three methyl singlets (total 3H) at δ 1.64, 1.69 and 2.50 in the ratios 6 : 20 : 72, which suggests that **1** had one C-methyl group and existed as a mixture of the open chain ketone (δ 2.50) and the anomers of the cyclic form (δ 1.64 and 1.69). The PMR spectrum of the acetyl derivative (**2**) showed that **2** had four methyl groups at the CH_3CO -region (δ 2.03 ~ 2.20). The mass spectrum of **2** showed ions at m/z 260 and 261, which might correspond to the molecular ion M^+ and $M^+ + 1$, respectively.

From these data, **1** was assumed to be a 1-deoxy-pentulose.

Comparison of specific rotations of **1** and **2** with those of known 1-deoxy-pentuloses and

TABLE I. COMPARISON OF SPECIFIC ROTATIONS OF 1-DEOXY-PENTULOSES **1** AND **5**, AND THEIR TRIACETATE DERIVATIVES WITH THOSE OF KNOWN 1-DEOXY-PENTULOSES AND THEIR TRIACETATE DERIVATIVES

Compound	$[\alpha]_D$ (°)	Solvent
1-Deoxy-pentulose 1	+33.6	H ₂ O
triacetate 2	+44	CHCl ₃
1-Deoxy-pentulose 5	-44	H ₂ O
triacetate 6	-43	CHCl ₃
1-Deoxy-pentulose		
D-threo	+34, ^a +46 ^b	H ₂ O
D-threo, triacetate	+52 ^c	CHCl ₃
L-threo	—	
L-threo, triacetate	-53 ^d	CHCl ₃
D-erythro	-30, ^a -37 ^e	H ₂ O
D-erythro, triacetate	-8 ^e	CHCl ₃
L-erythro	—	
L-erythro, triacetate	—	

^a Data from ref. 5.

^b Data from ref. 9.

^c Data from ref. 10.

^d Data from ref. 11.

^e Data from ref. 12.

their acetyl derivatives,^{5,9~12)} as shown in Table I, suggests that the configuration of **1** and **2** is D-threo.

The configuration of **1** was further characterized by reduction and acetylation to the corresponding 1-deoxy-pentitol acetates. The products **3** and **4** were identified as tetra-O-acetyl-1-deoxy-D-lyxitol*¹ and tetra-O-acetyl-1-deoxy-D-xylitol, respectively, by comparing the physical data of these compounds with those of known 1-deoxy-pentitol tetraacetates^{10,13~16)} (Table II). These experiments settle the configuration of **1** as D-threo.

Therefore, the product (**1**) of the enzyme reaction was determined to be 1-deoxy-D-threo-pentulose (D-DTP) and **2** was to be tri-O-acetyl-1-deoxy-D-threo-pentulose (Fig. 2).

Isolation and identification of L-DTP (**5**)

When L-glyceraldehyde was incubated with pyruvate (or DL-acetoin or methylacetoin) by a similar method in D-DTP formation, a prod-

*1 Systematic name: Tetra-O-acetyl-5-deoxy-D-arabinitol.

TABLE II. COMPARISON OF PHYSICAL DATA OF COMPOUNDS 3 AND 4 WITH THOSE OF KNOWN 1-DEOXY-PENTITOL TETRAACETATES

Parent 1-deoxy-pentulose	1-Deoxy-pentitol tetraacetate	mp (°C)	[α] _D (°)
D-threo	Compound 3	Amorphous	+46.3
	Compound 4	Syrup	+9.2
	D-lyxo	52~54	+47 ^a
	D-xylo	58~59	+46.1 ^b
L-erythro		Syrup	+8 ^a
		62~63	+10.4 ^c
	L-arabino	115	-26.37 ^d
	L-ribo	113~114 ^e	—

^a Data from ref. 10.^b Data from ref. 13.^c Data from ref. 14.^d Data from ref. 15.^e Data from ref. 16.

TABLE III. COMPARISON OF PHYSICAL DATA OF COMPOUNDS 7 AND 8 WITH THOSE OF KNOWN 1-DEOXY-PENTITOL TETRAACETATES

Parent 1-deoxy-pentulose	1-Deoxy-pentitol tetraacetate	mp (°C)	[α] _D (°C)
Compound 5	Compound 7	Syrup	-41
	Compound 8	Syrup	-8.8
L-threo	L-lyxo	57~58	-47 ^a
	L-xylo	Syrup	-2 ^b
D-erythro	D-arabino	115~116	+27.30 ^c
	D-ribo	—	—

^a Data from ref. 17.^b Data from ref. 18.^c Data from ref. 15.

molecular ion M^+ and $M^+ + 1$, respectively. These data suggested that 5 was a 1-deoxy-pentulose.

The comparison of specific rotations of 5 and 6 with those of known 1-deoxy-pentuloses and their acetyl derivatives,^{5,9~12)} as shown in Table I, suggests that the configuration of 5 and 6 is L-threo.

The configuration of 5 was further characterized by reduction and acetylation by the same method as for the preparation of 3 and 4. From the results of elemental analyses and PMR spectra together with comparing the specific rotations of these compounds with those of known 1-deoxy-pentitol tetraacetates^{15,17,18)} (Table III), the reduction products 7 and 8 were identified as tetra-O-acetyl-1-deoxy-L-lyxitol*² and tetra-O-acetyl-1-deoxy-L-xylitol, respectively. These experiments settle the configuration of 5 as L-threo.

Therefore, the product (5) of the enzyme reaction was determined to be 1-deoxy-L-threo-pentulose (L-DTP), and the acetyl derivative 6 to be tri-O-acetyl-1-deoxy-L-threo-pentulose (Fig. 2).

Mechanism of the enzyme reaction

In the reaction mixture containing DL-acetoin and D-glyceraldehyde, acetaldehyde

FIG. 2. Structures of 1-Deoxy-D-threo-pentulose (1), 1-Deoxy-L-threo-pentulose (5) and Their Derivatives.

uct, which showed the same R_f values and color reactions as D-DTP on PC, was formed.

The reaction product (5) from L-glyceraldehyde and DL-acetoin by the enzyme preparation of the mutant BG2532 was purified and isolated as crystals from the reaction mixture. Elemental analysis of 5 required a molecular formula of $C_5H_{10}O_4$. The PMR spectra of 5 and its acetyl derivative (6) were identical with those of 1 and 2, respectively. The mass spectrum of 6 showed ions at m/z 260 and 261, which might correspond to the

*² Systematic name: Tetra-O-acetyl-5-deoxy-L-arabinitol.

FIG. 3. Effect of Incubation Time on D-DTP and Acetaldehyde Formation from D-glyceraldehyde and DL-Acetoin.

Reaction conditions are the same as those in Fig. 1. The reaction was terminated by cooling in an ice bath, and D-DTP and acetaldehyde formed were determined by the assay methods as described in EXPERIMENTAL.

FIG. 4. Gas Chromatograms of the Product of the Enzyme Reaction between D-Glyceraldehyde and Methylacetoin.

Experimental conditions are described in the text. Control, authentic acetone; A, D-glyceraldehyde and methylacetoin (0 hr); B, D-glyceraldehyde and methylacetoin (2 hr); C, methylacetoin (2 hr); D, D-glyceraldehyde (2 hr).

was detected as another reaction product associated with D-DTP (Fig. 3). DL-Acetoin could be replaced by pyruvate or methylacetoin for D-DTP and L-DTP formation. When methylacetoin was used, acetone was detected by gas chromatography as another reaction product associated with D-DTP (Fig. 4). These results, together with our previous results on 1-deoxy-D-*altro*-heptulose phosphate formation,²⁾ suggest that D-DTP- and L-DTP-synthesizing reactions by combinations of D- or L-glyceraldehyde with pyruvate, DL-acetoin, or methylacetoin proceed according to the following formulae:

The products of the condensation reaction are 1-deoxy-pentuloses with *trans* configuration of hydroxyls at carbons 3 and 4.

The apoenzyme preparation, obtained by gel filtration as described in EXPERIMENTAL, absolutely required Mg^{2+} and TPP, as shown in Table IV.

Distribution of D-DTP and L-DTP synthesizing enzyme activities in microorganisms

From the experiments of the incorporation of deuterated carbohydrates and related compounds into thiamine in *E. coli*, White^{3,4)} postulated that 1-deoxy-pentulose phosphate, probably produced by an acyloin condensation between D-glyceraldehyde 3-phosphate and pyruvate, might be a precursor of the thiazole moiety of thiamine. More recently, Therisod *et al.*⁵⁾ and David *et al.*^{6,7)} found that the carbon chain of D-DTP is the specific precursor of the five carbon chain of the thiazole moiety of the thiamine molecule. However, neither the presence of D-DTP (phosphate) nor the presence of its synthesizing enzyme in *E. coli* cells has yet been described.

On the other hand, White and Spenser^{19,20)} found that in *Saccharomyces cerevisiae* a five-carbon unit of thiazole might be derived from a phosphoketopentose (D-ribulose 5-phosphate or D-xylulose 5-phosphate) but not from

TABLE IV. COFACTORS REQUIRED FOR D-DTP FORMATION BY *B. pumilus* MUTANT BG2532 AND *E. coli* IFO 13168 APOENZYME PREPARATIONS

A complete reaction mixture contained 40 μ mol Tris-HCl buffer (pH 8.0), 5 μ mol $MgCl_2$, 0.5 μ mol TPP, 2 μ mol EDTA, 50 μ mol each of substrates, and 0.2 ml of apoenzyme solution in a total volume of 1 ml, which was incubated at 37°C for 16 hr. The reaction was stopped by heating at 80°C for 3 min. After centrifugation, the supernatant was subjected to TLC. Method for determination of D-DTP is described in EXPERIMENTAL. As substrate other than D-glyceraldehyde, sodium pyruvate was used in Expt. (1) and (2), DL-acetoin in Expt. (3) and methylacetoin in Expt. (4). Apoenzyme preparations from *B. pumilus* mutant BG2532 and *E. coli* IFO 13168 were used in Expt. (1)~(3) and in Expt. (4), respectively.

Expt.	Component omitted	D-DTP formed (mg/ml)
(1)	Complete	1.92
	– TPP	0
	– $MgCl_2$	0
	– enzyme	0
(2)	Complete	3.28
	– TPP	0
	– $MgCl_2$	0
	– enzyme	0
(3)	Complete	8.56
	– TPP	0.54
	– $MgCl_2$	0.46
	– enzyme	0
(4)	Complete	1.22
	– TPP	0.08
	– $MgCl_2$	0
	– enzyme	0

D-DTP (phosphate). The participation of the phosphoketopentose in thiamine biosynthesis was deduced from data on the mode of incorporation of ^{14}C -labelled hexoses.

D-DTP-synthesizing enzyme(s) having been detected in the transketolase mutant of *B. pumilus* as described above, we thought that it played an important role in the biosynthesis of thiamine. Therefore, we examined the distribution of this enzyme activity in various microorganisms.

As Table V shows, not only bacteria but also actinomycetes, yeasts, and molds had the ability to catalyze the acyloin-type condensation

between D-glyceraldehyde and pyruvate to form D-DTP. When L-glyceraldehyde was used as a substrate instead of D-glyceraldehyde, these cell-free extracts catalyzed the formation of L-DTP.

D-DTP and L-DTP-synthesizing enzyme activities were detected in the soluble fractions of cells of all strains of bacteria except for the acetic acid bacteria *Acetobacter* and *Gluconobacter*, in which the activities were detected in the insoluble fractions. Relatively weak activities were found only in the insoluble fractions of yeast strains, which suggests that these enzymes are also membrane-bound. The enzyme activities were detected in both the soluble and insoluble fractions in molds (Table V).

Reaction products from the cell-free extracts of *E. coli* IFO 13168 (strain B) and *B. subtilis* IFO 13719 (strain Marburg) were purified and isolated by the same method as that of the *B. pumilus* mutant. The specific rotations of those for *E. coli* and *B. subtilis* were $[\alpha]_D + 26.2^\circ$ ($c = 1$, H_2O) and $[\alpha]_D + 30.1^\circ$ ($c = 1$, H_2O), respectively. *Rf* values on PC and TLC were identical with those of D-DTP. Furthermore, the reaction products by a yeast, *Saccharomyces cerevisiae*, and a mold, *Aspergillus oryzae*, showed the same color reactions and *Rf* values on PC and TLC as those of D-DTP (data not shown).

The enzyme solution prepared from *E. coli* also showed absolute requirements for Mg^{2+} and TPP as cofactors, as shown in Table IV.

DISCUSSION

1-Deoxy-threo-pentulose synthesizing enzyme(s) requires TPP and Mg^{2+} as cofactors, suggesting that hydroxyethyl-TPP might participate in the enzyme reaction as an intermediate. The reaction mechanism was presumed to be analogous to that of 1-deoxy-D-altro-heptulose phosphate synthase, and therefore, D-DTP and L-DTP synthesizing enzyme(s) can also be regarded as a carboli-gase which is known to cause the condensation of acetaldehyde with another acetal-

TABLE V. D-DTP AND L-DTP SYNTHESIS BY CELL-FREE EXTRACTS OF VARIOUS MICROORGANISMS

Microorganism	IFO No.	Medium	Protein ^a (mg/ml)	D-DTP (mg/ml) ^c	Formation of L-DTP ^e
(Bacteria)					
<i>Acetobacter aceti</i>	3281	E	1.0	Trace	— ^f
<i>Acetobacter aceti</i>	3281	E	ppt ^b	1.24	+
<i>Acinetobacter calcoaceticus</i>	12552	B	1.4	0.32	+
<i>Aerococcus viridans</i>	12317	C	2.4	0.12	+
<i>Aeromonas hydrophila</i>	3820	B	1.6	0.65	+
<i>Alcaligenes faecalis</i>	13111	B	2.8	0.98	+
<i>Agrobacterium tumefaciens</i>	3058	B	2.5	0.43	+
<i>Arthrobacter globiformis</i>	12137	B	1.5	0.29	+
<i>Azotobacter chroococcum</i>	12994	D	1.2	1.28	+
<i>Bacillus pumilus</i>	12089	A	1.2	1.18	+
<i>Bacillus pumilus</i> BG2532		A	2.6	1.66	+
<i>Bacillus subtilis</i>	13719	A	1.2	0.68	+
<i>Brevibacterium lines</i>	12141	B	0.9	0.37	+
<i>Corynebacterium michiganense</i>	12471	B	1.2	0.47	+
<i>Cytophaga heparina</i>	12017	B	1.5	1.08	+
<i>Erwinia carotovora</i>	3380	B	0.7	0.51	+
<i>Escherichia coli</i>	13168	C	1.8	1.22	+
<i>Flavobacterium capsulatum</i>	12533	B	1.1	0.86	+
<i>Gluconobacter oxydans</i>	3189	E	0.4	— ^a	—
<i>Gluconobacter oxydans</i>	3189	E	ppt	0.09	+
<i>Klebsiella pneumoniae</i>	3317	B	1.6	2.45	+
<i>Microbacterium lacticum</i>	14135	B	1.5	0.59	+
<i>Micrococcus luteus</i>	3333	C	1.7	0.52	+
<i>Proteus vulgaris</i>	3851	B	2.1	0.65	+
<i>Pseudomonas aeruginosa</i>	12689	B	2.7	1.25	+
<i>Rhizobium leguminosarum</i>	14168	D	1.4	1.25	+
<i>Salmonella typhimurium</i>	13245	B	2.0	0.98	+
<i>Serratia marcescens</i>	3046	B	2.0	1.25	+
<i>Staphylococcus aureus</i>	3060	B	1.2	0.41	+
<i>Xanthomonas citri</i>	3835	B	2.1	0.58	+
(Actinomycetes)					
<i>Actinoplanes missouriensis</i>	13243	F	1.3	0.86	+
<i>Micromonospora coerulea</i>	13504	F	1.4	0.69	+
<i>Micropolyspora brevicatena</i>	12119	F	0.8	0.41	+
<i>Nocardia erythropolis</i>	12682	F	1.8	0.82	+
<i>Streptomyces griseus</i>	3430	F	0.8	1.54	+
(Yeasts)					
<i>Candida albicans</i>	1594	G	ppt	Trace	Trace
<i>Hansenula anomala</i>	0122	G	ppt	0.15	Trace
<i>Pichia farinosa</i>	1163	G	ppt	0.10	Trace
<i>Rhodotorula glutinis</i>	0667	G	ppt	0.09	Trace
<i>Saccharomyces cerevisiae</i>	2043	G	ppt	0.09	Trace
(Molds)					
<i>Aspergillus oryzae</i>	30104	H	0.8	0.10	Trace
<i>Aspergillus oryzae</i>	30104	H	ppt	0.16	Trace
<i>Monascus ruber</i>	9203	H	1.0	0.10	Trace
<i>Monascus ruber</i>	9203	H	ppt	0.09	Trace
<i>Mucor hiemalis</i> f. <i>hiemalis</i>	9404	H	0.8	0.09	Trace
<i>Mucor hiemalis</i> f. <i>hiemalis</i>	9404	H	ppt	0.19	Trace
<i>Penicillium funiculosum</i>	6345	H	1.0	0.10	Trace
<i>Penicillium funiculosum</i>	6345	H	ppt	0.11	Trace

^a Soluble fractions were added at the indicated concentration of protein to the reaction mixtures, which were

dehyde or other aldehydes to form acyloin compounds. Many acyloin-type condensation are known to be catalyzed by the enzymes α -acetolactate synthase,²²⁾ pyruvate oxidase,²³⁾ pyruvate dehydrogenase of the pyruvate dehydrogenase system,²⁴⁾ pyruvate decarboxylase,²⁵⁾ α -ketoglutarate dehydrogenase of the α -ketoglutarate dehydrogenase system,²⁶⁾ acetoin dehydrogenase,²⁷⁾ etc. However, 1-deoxy-ketose formation has not yet been reported by these enzymes.

When glycoaldehyde or D-erythrose or D-threose was used as a substrate instead of D- or L-glyceraldehyde, 1-deoxy-erythrulose, 1-deoxy-D-fructose or 1-deoxy-D-sorbose was also formed as a reaction product.²⁸⁾ 1-Deoxyketoses have been chemically synthesized by several routes from aldoses or their derivatives by reaction with one carbon compound or one carbon donor. The reaction sequences involve several steps and the overall yields are relatively low. Therefore, the enzyme reaction described here can be used as a convenient method for the preparation of 1-deoxyketoses, in a one-step synthesis by an enzymatic method with good yield.

In view of the catalytic properties of 1-deoxy-ketose-synthesizing enzyme(s), it appears appropriate to designate the enzyme acetaldehyde-transferase (EC 2.2.1) according to the enzyme nomenclature recommended by the Nomenclature Committee of the International Union of Biochemistry (1978).²⁹⁾ However, further investigation must be performed with purified enzyme preparations to characterize the enzyme(s).

The findings described in this report may provide some clues to elucidate the biosynthetic pathway of thiamine in microorganisms. White and Spenser^{19,20)} have proposed a biosynthetic pathway of thiamine in yeast including pentulose phosphate as the precursor. However, the present study suggests that D-DTP might be a precursor of thiamine in yeasts and molds as well as in bacteria. In eucaryotes thiamine might be synthesized partly through the same metabolic pathway as in procaryotes.

Very weak enzyme activity for D-DTP phosphate formation from D-glyceraldehyde 3-phosphate and pyruvate could be detected in cell-free extracts of only a few bacterial strains. Furthermore, the presence of D-DTP in the cells of *E. coli* was confirmed but not that of its phosphorylated derivative (data not shown). These preliminary experiments suggest that the precursor of the thiazole ring of thiamine may be D-DTP, not its phosphorylated derivative as suggested by White.³⁾

As L-glyceraldehyde and methylacetoin have not yet been isolated from biological material, L-DTP cannot be considered to be formed by this enzyme reaction *in vivo*, and methylacetoin cannot be considered an *in vivo* substrate for D-DTP formation.

EXPERIMENTAL

General methods.

Microorganisms and culture conditions. The mutant BG2532 of *B. pumilus* IFO 12089,¹⁾ which is leaky in transketolase activity, was mainly used. It was cultured in medium A, which contained 2% sodium maleate 1% yeast

incubated at 37°C for 16 hr.

^b Insoluble fractions of the disrupted cells which were suspended in the same volume of buffer A were used.

^c A reaction mixture containing 100 μ mol of L-glyceraldehyde, 100 μ mol of sodium pyruvate, 80 μ mol of Tris-HCl buffer (pH 8.0), 5 μ mol of MgCl₂, 0.5 μ mol of TPP, 2 μ mol of EDTA, and 0.2 ml of enzyme preparation in a total volume of 1.0 ml was incubated at 37°C for 20 hr. Method for determination of D-DTP is described in EXPERIMENTAL.

^d Not detected.

^e L-DTP formed was detected by PC as follows: A reaction mixture containing the same composition as in c) except for employment of L-glyceraldehyde as a substrate instead of D-glyceraldehyde, was incubated at 37°C for 20 hr. After incubation, the reaction mixture was heated at 80°C for 3 min and centrifuged at 3000 rpm for 10 min. An aliquot (10 ~ 20 μ l) of the resulting supernatant was applied on a filter paper. Vanillin-perchloric acid²¹⁾ was sprayed on the paper and heated at 120°C for 5 min.

^f The signs plus and minus represent positive and negative enzyme activities, respectively, which were examined by PC.

extract, and 0.5% ammonium sulfate, pH 7.0. Other wild-type strains of bacteria, actinomycetes, yeasts, and molds used were obtained from the IFO (Institute for Fermentation, Osaka) culture collection, and were cultured in the following media: medium B, 2% D-glucose, 0.05% peptone, 0.02% yeast extract, and 0.02% NaCl, pH 7.0; medium C, 2% D-glucose, 0.5% peptone, 0.2% yeast extract, and 0.2% NaCl, pH 7.0; medium D, 1.5% mannitol, 0.1% yeast extract, 0.02% K_2HPO_4 , 0.02% $MgSO_4 \cdot 7H_2O$, 0.02% NaCl, and 0.01% $CaSO_4 \cdot 2H_2O$, pH 7.2; medium E, 1% glycerol and 0.5% yeast extract, pH 6.0; medium F, 1% maltose, 0.1% yeast extract, 0.1% beef extract, and NZ Amine, pH 7.3; medium G, 2% D-glucose, 0.2% yeast extract, 0.1% ammonium sulfate, 0.05% KH_2PO_4 , 0.05% $MgSO_4 \cdot 7H_2O$, and 0.03% $CaCl_2 \cdot 2H_2O$, pH 5.0; medium H, 0.2% D-glucose, 2% malt extract, and 0.1% peptone, pH 6.0.

Cell-free enzyme preparations. Cells were harvested by centrifugation, washed with 0.01 M Tris-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM TPP, 2 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 5 mM 2-mercaptoethanol (buffer A), and suspended in the same buffer.

Cells of bacterial, actinomycete, and mold strains were then disrupted with an ultrasonic oscillator, while yeast cells were treated with Zymolyase 5000 (20 units/ml) at 30°C for 1 hr before disruption in the same way. After centrifugation at $13,000 \times g$ for 10 min, the supernatants were used as the soluble fractions and the precipitates as insoluble fractions.

Partial purification of D-DTP and L-DTP synthesizing enzyme(s) from mutant strain BG2532. The soluble fraction of the mutant BG2532 was obtained from cells cultured in medium A. To the soluble fraction was added 1% protamine sulfate solution (7:1). After 15 min, the precipitate was removed by centrifugation at $13,000 \times g$ for 20 min. Solid ammonium sulfate was added to the supernatant to 30% saturation. After 15 min, the precipitate formed was separated by centrifugation and discarded. Solid ammonium sulfate was added to the supernatant to 60% saturation. The precipitate formed was collected by centrifugation and dissolved in buffer A. The solution was dialyzed against the same buffer, and then used as the partially purified enzyme preparation.

Partial purification of D-DTP synthesizing enzyme from *B. subtilis* IFO 13719 and *E. coli* IFO 13168. Strains of *B. subtilis* and *E. coli* were cultured in the chemically defined medium of Tomlinson³¹ and medium C, respectively. Soluble fractions were obtained from the cells by the method described above. Solid ammonium sulfate was added to 80% saturation to the soluble fractions. The resulting precipitates were collected by centrifugation at $13,000 \times g$ for 20 min, and were dissolved in buffer A. The solutions were dialyzed against the same buffer, and then used as partially purified enzyme preparations.

Preparation of apoenzyme. Enzyme solution used for the examination of cofactor requirements was prepared as follows: the partially purified enzyme solution was applied

to a Sephadex G-25 column (1.0 $\times$ 27 cm) which had been equilibrated with 0.01 M Tris-HCl buffer (pH 7.5) and eluted with the same buffer. The active fractions were combined and used.

Assay of D-DTP. D-DTP formed by the enzyme reaction was assayed by the method of Iwakawa *et al.*³⁰ as follows. An aliquot (5–20 μ l) of the supernatant was applied on a TLC plate, which was developed with solvent B and air-dried. Ethylenediamine sulfate reagent was sprayed on the plate and the fluorescence intensity on the chromatogram was measured with a Shimadzu CS-910 TLC scanner.

Assay of acetaldehyde. A reaction mixture (100 μ l), was incubated in a microtube (7 $\times$ 50 mm) with a rubber bung, and the acetaldehyde formed was determined by the method described in a previous paper.²¹

Determination of protein concentration. Protein concentrations were determined by the method of Lowry *et al.*³² with bovine serum albumin as the standard.

PC, TLC and column chromatography. PC on Toyo Roshi No. 51 filter paper, TLC on silica gel plates (20 $\times$ 20 cm, 0.25 mm, E. Merck), and column chromatographies with CF-11 cellulose powder (Whatman) and with silica gel 60 (70–230 mesh, E. Merck) were performed at room temperature with one of the following solvent systems (by vol.): (A) isobutanol–pyridine–acetate–water (12:6:1:4), (B) ethyl acetate–pyridine–water (40:11:6), (C) acetone–ethyl acetate (1:1), (D) ether–petroleum ether (2:1), (E) ether–petroleum ether (1:1), (F) ethyl acetate–benzene (1:1) and (G) ethyl acetate–*n*-hexane (1:2). As spray reagents, alkaline silver nitrate, alkaline triphenyltetrazolium chloride, vanillin–perchloric acid,²¹ and orcinol–ferric chloride–hydrochloride were used for PC, and 50% sulfuric acid for TLC.

Gas chromatography. Acetone formed by the enzyme reaction was detected by gas chromatography. The reaction mixture was adjusted to pH 1–2 with 5 N HCl. After centrifugation, 2 μ l of the supernatant was injected into a Shimadzu GC-4BM gas chromatograph with a column (glass, 4 m) of 25% Flexol 8N8 on Chromosorb W. He gas was the carrier and a flame-ionization detector was the detector. The column temperature and the injector temperature were 80 and 100°C, respectively.

Physical and chemical analyses. Melting points were uncorrected. Optical rotations were measured with a Japan Spectroscopic DIP-181 polarimeter. PMR spectra were measured with a Varian EM-390 (90 MHz) spectrometer with tetramethylsilane as a reference. Mass spectra were measured with a JEOL JMS-01SG mass spectrometer. IR spectra were measured with a Shimadzu IR-420 spectrometer.

Chemicals. Alcohol dehydrogenase (yeast), D-glyceraldehyde, and L-glyceraldehyde were purchased from the Sigma Chemical Co. DL-Acetoin (monomer) was purchased from Wako Pure Chemical Industries, which was crystallized as the dimer before use. Methylacetoin was purchased from the Tokyo Kasei Industrial Co.

Isolation of D-DTP (1) from a reaction mixture containing enzyme preparation of *B. pumilus* mutant BG2532. A reaction mixture containing 1 g of D-glyceraldehyde, 1 g of DL-acetoin, 16 mmol of Tris-HCl buffer (pH 8.0), 1 mmol of $MgCl_2$, 0.1 mmol of TPP, 0.4 mmol of EDTA, and 20 ml of partially purified enzyme solution of the mutant BG2532 (460 mg of protein) in a total volume of 200 ml was incubated at 37°C for 20 hr. After incubation, methanol (3 vol.) was added to the reaction mixture, and the precipitate formed was removed by centrifugation. Methanol was removed by evaporation under reduced pressure. This methanol treatment was repeated two more times. Then the solution was concentrated to a syrup, and ethanol (3 vol.) was added to the syrup. After the precipitate had been removed by centrifugation, the supernatant was concentrated *in vacuo* to a syrup, which was chromatographed on a column (2.6 × 40 cm) of cellulose with solvent B. The fractions containing compound 1 were concentrated, and the residue was crystallized from ethyl acetate to give **1** (670 mg), mp 62~63°C, $[\alpha]_D +33.6^\circ$ ($c=1$, H_2O). IR ν_{max}^{Nujol} cm^{-1} : 1710 (C=O). PMR (D_2O) δ : 1.64, 1.69, 2.50 (total 3H, three singlets in the ratio 6:20:72, C-CH₃), 3.75~4.70 (4H, m). Anal. Found: C, 45.08; H, 7.52. Calcd. for $C_5H_{10}O_4$: C, 44.77; H, 7.51%. PC: *Rf* 0.60 (solvent A), 0.52 (solvent B). It reduced alkaline silver nitrate and alkaline triphenyltetrazolium chloride, and gave greenish orange with vanillin-perchloric acid²¹⁾ and yellow with orcinol-ferric chloride-hydrochloride. TLC: *Rf* 0.59 (solvent B), 0.43 (solvent C).

Tri-O-acetyl-1-deoxy-D-threo-pentulose (2). Compound **1** (20 mg) was dissolved in a mixture of dry pyridine (0.65 ml) and acetic anhydride (0.5 ml). The solution was heated at 80°C for 10 min and stood overnight at room temperature, and then methanol (10 ml) was added. The mixture was repeatedly evaporated with toluene. The residue was extracted with chloroform, and the chloroform extract was evaporated to give a syrup. The syrup was further purified by silica gel column chromatography (1.1 × 55 cm) with solvent E. Fractions (2 ml) were collected and analyzed by TLC with solvent E. Compound **2** was isolated by evaporation from fractions 48~66 as a syrup (14 mg), $[\alpha]_D +44^\circ$ ($c=1$, $CHCl_3$). PMR ($CDCl_3$) δ : 2.03 (3H, s, COCH₃), 2.06 (3H, s, COCH₃), 2.20 (6H, s, 2 × COCH₃), 4.10~4.40 (2H, m), 5.25 (1H, d, $J=3.5$ Hz), 5.50~5.70 (1H, m). MS *m/z*: 261 ($M^+ + 1$), 260 (M^+). TLC: *Rf* 0.52 (solvent D), 0.30 (solvent E), 0.80 (solvent F).

Preparation of tetra-O-acetyl-1-deoxy-D-lyxitol (3)*³ and tetra-O-acetyl-1-deoxy-D-xylitol (4) from 1. Compound **1** (160 mg) was dissolved in a 3% aqueous solution of sodium borohydride (10 ml). The solution was kept for 1 hr at room temperature, passed through a

column of ion exchange resin IR-120 (H^+), and concentrated by repeated addition and evaporation of methanol under reduced pressure to give a syrupy residue. The residue was then dissolved in a mixture of pyridine (1.5 ml) and acetic anhydride (1.0 ml), which was heated at 80°C for 30 min and stood for 4 hr at room temperature. Methanol (10 ml) was added to the solution, and the mixture was repeatedly evaporated with toluene. The residue was chromatographed on a silica gel column (1.2 × 50 cm) and eluted with solvent G. Fractions (5 ml) were collected and analyzed by TLC with solvent G.

Compound **3** was isolated by evaporation from fractions 72~90 as an amorphous material (98 mg), $[\alpha]_D +46.3^\circ$ ($c=1$, $CHCl_3$). PMR ($CDCl_3$) δ : 1.22 (3H, d, $J=6.0$ Hz, CH-CH₃), 2.05 (6H, s, 2 × OCOCH₃), 2.08 and 2.10 (each 3H, s, OCOCH₃), 3.93 (1H, dd, $J=10.0$ Hz), 4.35 (1H, dd, $J=10.0$ Hz), 5.00~5.40 (3H, m). Anal. Found: C, 51.40; H, 6.70. Calcd. for $C_{13}H_{20}O_8$: C, 51.31; H, 6.63%. TLC: *Rf* 0.46 (solvent D), 0.25 (solvent G).

Compound **4** was isolated similarly from fractions 96~124 as a syrup (64 mg), $[\alpha]_D +9.2^\circ$ ($c=1$, $CHCl_3$). PMR ($CDCl_3$) δ : 1.20 (3H, d, $J=6.0$ Hz, CH-CH₃), 2.02, 2.04, 2.07 and 2.12 (each 3H, s, OCOCH₃), 3.95 (1H, dd, $J=10.0$ Hz), 4.26 (1H, dd, $J=10.0$ Hz), 4.90~5.40 (3H, m). Anal. Found: C, 51.40; H, 6.79. Calcd. for $C_{13}H_{20}O_8$: C, 51.31; H, 6.63%. TLC: *Rf* 0.36 (solvent D), 0.20 (solvent G).

Isolation of L-DTP (5) from the reaction mixture containing an enzyme preparation of the mutant BG2532. A reaction mixture containing 1 g of L-glyceraldehyde and 1 g of DL-acetoin was treated similarly to compound **1**. A thick syrup obtained was applied to a column (2.6 × 45 cm) of cellulose with the same solvent used in the isolation of **1**. The fractions containing **5** were concentrated. The residue was crystallized from ethyl acetate to give **5** (417 mg), mp 63~65°C, $[\alpha]_D -33^\circ$ ($c=1$, H_2O). Anal. Found: C, 44.44; H, 7.66. Calcd. for $C_5H_{10}O_4$: C, 44.77; H, 7.51%. PMR and IR spectra, *Rf* values on PC and TLC and color reactions were identical with those of **1**.

Tri-O-acetyl-1-deoxy-L-threo-pentulose (6). The acetylation of 20 mg of compound **5** with acetic anhydride and pyridine and purification by silica gel column chromatography with solvent E yielded the syrupy acetyl derivative **6** (18 mg), $[\alpha]_D -43^\circ$ ($c=1$, $CHCl_3$). PMR spectrum, mass spectrum and *Rf* values on TLC were identical to those of **2**.

Preparation of tetra-O-acetyl-1-deoxy-L-lyxitol (7)*⁴ and tetra-O-acetyl-1-deoxy-L-xylitol (8) from 5. Compound **5** (200 mg) was reduced with sodium borohydride, acetylated, and purified by silica gel column chromatography (1.2 × 50 cm) with solvent G.

*³ Systematic name: Tetra-O-acetyl-5-deoxy-D-arabinitol.

*⁴ Systematic name: Tetra-O-acetyl-5-deoxy-L-arabinitol.

Compound **7** was isolated by evaporation from fractions 70~88 as a syrup (126 mg), $[\alpha]_D -41^\circ$ ($c=1$, CHCl_3). PMR spectrum and R_f values on TLC were identical with those of compound **3**. *Anal.* Found: C, 51.47; H, 6.77. Calcd. for $\text{C}_{13}\text{H}_{20}\text{O}_8$; C, 51.31; H, 6.63%.

Compound **8** was isolated similarly from fractions 92~110 as a syrup (75 mg), $[\alpha]_D -8.8^\circ$ ($c=2$, CHCl_3). PMR spectrum and R_f values on TLC were identical with those of compound **4**. *Anal.* Found: C, 51.33; H, 6.72. Calcd. for $\text{C}_{13}\text{H}_{20}\text{O}_8$; C, 51.31; H, 6.63%.

Isolation of 1 from a reaction mixture containing an enzyme preparation of E. coli IFO 13168. Reaction conditions were similar to those described in the isolation of **1** from the reaction mixture containing an enzyme preparation of the mutant BG2532 except that 1 g of sodium pyruvate was used instead of DL-acetoin. The method of isolation and purification of the reaction product of the *E. coli* enzyme preparation was also similar to that in the case of mutant BG2532. The product was obtained as a syrup (45 mg), $[\alpha]_D +26.2^\circ$ ($c=1$, H_2O). R_f values and color reactions on PC and R_f values on TLC were identical with those of the reaction product **1** formed by the mutant BG2532 enzyme preparation.

Isolation of 1 from reaction mixture containing enzyme preparation of B. subtilis IFO 13719. Reaction conditions and the methods of isolation and purification of the reaction product of the *B. subtilis* enzyme preparation were similar to those described in the case of *E. coli* except that 1 g of methylacetoin was used instead of sodium pyruvate. The product was obtained as a syrup (22 mg), $[\alpha]_D +30.1^\circ$ ($c=1$, H_2O). R_f values and color reactions on PC and R_f values on TLC were identical with those of the reaction product **1** formed by the mutant BG2532 enzyme preparation.

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