THYMIDINE DIPHOSPHATE N-ACETYLAMINO SUGAR COMPOUNDS FROM ESCHERICIA COLI STRAINS**

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Previously the isolation of four unidentified TDP-sugar compounds from E. coli strains has been reported, viz: TDP-X, TDP-Y, and TDP-Z from E. coli Y-10 (Strominger and Scott, 1959) and TDP-X_2 from E. coli B (Okazaki, Okazaki and Kuriki, 1960). Subsequent study (Okazaki, Okazaki and Strominger, 1961; Okazaki, Okazaki, Strominger and Michelson, 1962) has revealed that TDP-Y is TDP-4-keto-6-deoxy-D-glucose and is an intermediate in the enzymatic synthesis of TDP-L-rhamnose. In addition, TDP-Z has been identified as a mixture of TDP-D-fucose and TDP-6-deoxy-D-glucose (reduction products of TDP-Y). The present communication describes recent observations on TDP-X and TDP-X_2, which indicate that X and X_2 are unusual N-acetylamino sugars.

TDP-X and TDP-X_2 were isolated from cold perchloric acid extracts of E. coli, strains Y-10 and B respectively, by chromatography on Dowex-1 formate followed by paper chromatography in isobutyric acid-ammonia as previously described (Okazaki, 1960; Okazaki, Okazaki, Strominger and Michelson, 1962). The sugars (X and X_2) were obtained by mild acid hydrolysis of nucleotides or of sugar phosphates prepared from nucleotides by digestion

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with snake venom nucleotide pyrophosphatase. They were then isolated by paper chromatography in butanol-ethanol-water.

The ratio, thymidine: total P: reducing value was 1.00: 2.15: 0.90 for TDP-X and 1.00: 2.00: 1.00 for TDP-X_2. Reducing values, determined by ferricyanide reduction after acid hydrolysis, are expressed relative to a rhamnose standard. No reducing value was found before hydrolysis. Some other properties of the two compounds are shown in Table I.

Table I

Some Properties of Nucleotides and Sugars

Compound	R_{TMP} of nucleotide in solvent: A	B	$R_{rhamnose}$ of sugar in solvent: C	D	E	ε_{585} mμ in Morgan-Elson reaction
TDP-X or X	1.61	0.80	0.72	0.97	0.91	0
TDP-X_2 or X_2	2.02	0.76	0.90	1.03	0.99	0
N-acetylglucosamine			-	0.56	0.78	16,500
N-acetylgalactosamine			-	0.56	0.70	5,500
N-acetylfucosamine*			-	1.25	1.05	5,500

* Kindly given to us by Drs. P. O'Brien and V. Ginsburg
Solvent A: Ethanol-1 M ammonium acetate, pH 7-7.5 (7.5 : 3)
Solvent B: Isobutyric acid-1 N ammonia (10 : 6)
Solvent C: Pyridine-ethyl acetate-water (1.0 : 3.6 : 1.15)
Solvent D: Butanol-ethanol-water (52 : 32 : 16)
Solvent E: Butanol-pyridine-water (6 : 4 : 3)

Infrared spectra of X and X_2 (Fig. 1) were characteristic of N-acetylamino sugars; for comparison a spectrum of N-acetylgalactosamine is also shown. Absorption bands appear at about 1640 cm^{-1} (amide I), 1540 cm^{-1} (amide II), 1375 cm^{-1}, 1310 cm^{-1} (amide III) and 1050 cm^{-1} (-C-OH). These spectra differ in the fingerprint region (600-1000 cm^{-1}). The band at 3400 cm^{-1} is

probably due mainly to water in the samples, although this is also the region of an absorption band of secondary amides.

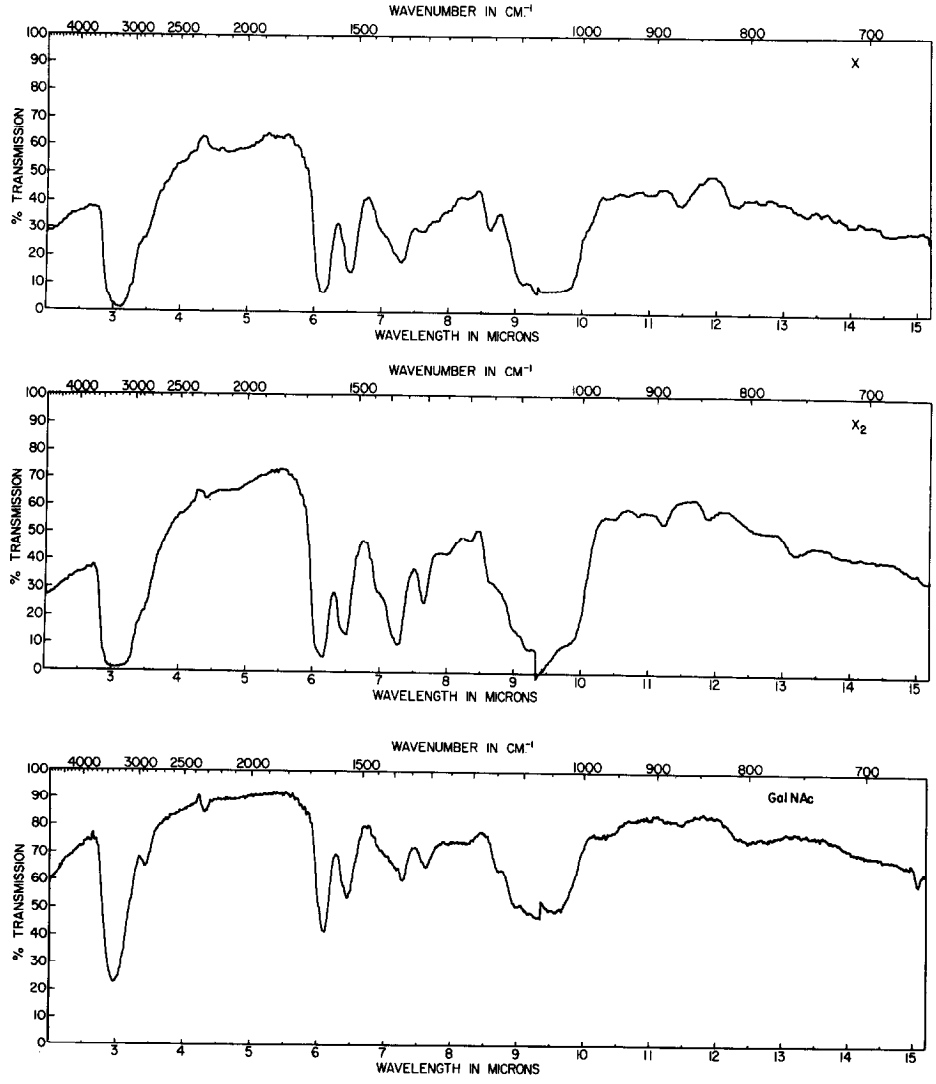

Figure 1. Infrared Spectra

About one mole of acetic acid (measured by gas chromatography) was formed per mole of X or X_2 on hydrolysis in 2N HCl, and one mole of methyl acetate (measured with alkaline hydroxylamine) was formed from each on hydrolysis in dry 2N methanolic HCl (Table II). Direct treatment of X and X_2 with alkaline hy-

droxylamine did not result in hydroxyamate formation, indicating the absence of O-acetyl groups. Several O-acetyl sugars used as standards formed hydroxamate directly under these conditions. The half-time for formation of methyl acetate from X in 2N methanolic HCl at 100° was less than 2 min, while from X_2 or from N-acetylglucosamine it was 10-20 min. The extreme lability of the N-acetyl group in X is unusual.

Table II

Acetyl and Amino Group Determinations

Data are expressed as μmoles per μmole of sugar. The amount of the sugars was determined by ferricyanide reduction using molar reducing values, relative to a rhamnose standard, of 0.90 for X and 1.00 for X_2.

	Acetyl*	Acetyl**	Amino Group+	Nitrogen++
X	1.17	1.13	1.04	1.04
X_2	1.24	0.85	0.53	0.97

* Identified and determined by gas chromatography following hydrolysis in 2N HCl for 4 hours. We are grateful to Dr. Gino Marco and Mr. Andrew Bybell, Monsanto Chemical Co., St. Louis, for assistance with these determinations.

** Measured by hydroxamate formation from methyl acetate after treatment with dry 2N methanolic HCl for 4 hours (Ludowieg and Dorfman, 1960).

\+ Measured with 2,4-dinitrofluorobenzene after hydrolysis in methanolic HCl. Data are expressed relative to an acetylglucosamine standard. The relative yields and extinction coefficients of the chromogens formed on treatment of X, X_2 and the standard are unknown.

++ Measured by an ultramicro method (Suzuki and Okuda, unpublished).

The appearance of an amino group from X or X_2 during hydrolysis in methanolic HCl could be followed with 2,4-dinitrofluorobenzene. The rates of their appearance paralleled the rates of formation of methyl acetate. Moreover, the amino sugars formed had the same electrophoretic mobility as glucosamine, migrating

toward the cathode in 0.05 M acetate, pH 5; they were detected with both ninhydrin and alkaline $AgNO_3$. X and X_2 themselves had no electrophoretic mobility. Analysis, furthermore, showed the presence of one nitrogen atom in both X and X_2 (Table II).

Neither X nor X_2 yielded any chromogen in the Morgan-Elson reaction for N-acetylamino sugars. Negative reactions are given by N-acetylamino sugars substituted at C-1 or C-4 (e.g. phenyl acetylglucosaminide, acetylneuraminic acid and 4-O-methyl-acetylglucosamine) or by those in which the N-acetylamino group is not at C-2 (e.g. the 4-acetylamino-2-amino-2,4,6-trideoxy-hexose from B. subtilis (Sharon and Jeanloz, 1960)). X or X_2 could not be identified with a known N-acetylamino sugar by paper chromatography (Table I).

Both TDP-X and TDP-X_2 were hydrolyzed to give TMP and a sugar phosphate by heating at 100° for 60 min in conc. NH_4OH. Under these conditions UDP-N-acetylglucosamine was not decomposed at all. This fact suggests the presence in TDP-X and TDP-X_2 of a hydroxyl group vicinal to the β-phosphate of TDP, i.e. at C-2 of X and X_2.

TDP-X_2 consumed one mole of periodate within 60 min, whereas X_2 consumed 3 moles of periodate in 1-2 hours. On the other hand, TDP-X and X each consumed 3 moles of periodate. No formation of formaldehyde, acetaldehyde or malonyl-dialdehyde was detected on periodate oxidation of X and X_2, excluding the structures: $-CHOH-CH_2OH$, $-CHOH-CHOH-CH_3$, $CHO-CH_2-CHOH-CHOH-$, $CHO-CHOH-CH_2-CHOH-CHOH-$ and $-CHOH-CHOH-CH_2-CHOH-CH_2OH$.*

* After oxidation of X_2 with periodate, the sample was treated with Br_2 and then deacetylated. Under these conditions threonine appeared to be formed as judged by paper chromatography of the amino acid and of its dinitrophenyl derivative and by oxidation to acetaldehyde with periodate. These data indicate the occurrence of the structure $-CHOH-CHOH-CHNHAc-CHOH-CH_3$ in this sugar. Both X and X_2 also gave weak reactions in the cysteine-H_2SO_4 reaction for 6-deoxyhexoses.

It is evident that these TDP-N-acetylamino sugar compounds contain unusual sugars, and much further work will be required to establish their structures.** To our knowledge the occurrence of sugars with these properties in bacterial polysaccharides or other products has not been reported. It may be noteworthy, however, that X is rapidly decomposed under conditions ordinarily employed for isolation of sugars by acid hydrolysis of polysaccharides.

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** While this paper was in preparation, the enzymatic synthesis of TDP-N-acetylglucosamine and TDP-N-acetylgalactosamine in extracts of Pseudomonas aeruginosa was reported (Kornfeld and Glaser, 1962). It is not clear at the present time whether or not these nucleotides could have some metabolic relation to the TDP-N-acetylamino sugar compounds described here.