The isolation of uridine pyrophosphogalacturonic acid from a Type I pneumococcus*

It has been demonstrated by SMITH, MILLS AND HARPER[1] that a rough pneumococcus, derived from a Type II organism, and a capsulated Type III pneumococcus contain the uridine nucleotides UPPG, UPPGA and UPPAG; certain enzymes involved in the formation of these nucleotides have also been described.

During the investigation of the nucleotides of a Type I capsulated pneumococcus, a uridine nucleotide has been found which is eluted from a Dowex-1 Cl′ column in the same position as UPPGA; this compound, when subjected to chromatography in the ethanol–ammonium acetate solvent of PALADINI AND LELOIR[2] behaves in a manner identical with UPPGA. On hydrolysis however, it was found to contain galacturonic acid instead of glucuronic acid.

Streptococcus pneumoniae, Type I, (National Collection of Type Cultures, London, Catalogue No. 7465) was maintained, grown, harvested and extracted as described for the Type III organism[1]. The extracts were applied to a Dowex-1 Cl′ column, eluted and concentrated by the method described by SMITH AND MILLS[3].

The material eluted in the 0.1 *N* HCl/0.05 *M* NaCl fraction, when chromatographed in the neutral ethanol–ammonium acetate solvent of PALADINI AND LELOIR[2], showed one main ultra-violet-absorbing spot with an R_F identical with that of UPPGA, and a smaller one in the position of UPP. The spot with the R_F of UPPGA was eluted with water after washing the paper with absolute alcohol, and the following analyses carried out.

(a) Hydrolysis with 0.01 *N* HCl (100° C, 10 min) followed by neutralisation and ionophoresis in borate buffer at pH 8.6 (CONSDEN AND STANIER[4]), 7 V/cm for 4 h. Uronic acids were located with the aniline hydrogen phthalate reagent of PARTRIDGE[5].

Hexuronic acid	*Migration (cm)*
Glucuronic acid	13.4
Galacturonic acid	12.1
Hydrolysed chromatogram extract	12.0

(b) Hydrolysis with 0.01 *N* HCl (100° C, 10 min) and 0.1 *N* HCl (100° C, 90 min) followed by neutralisation and chromatography in the neutral ethanol–ammonium acetate solvent[2].

Nucleotide	R_{AMP}
UMP	1.21
UPP	0.83
0.01 *N* HCl hydrolysis	0.82
0.1 *N* HCl hydrolysis	1.20

(c) Estimation of absorption at 262 mμ at pH 2, assuming a value of 10,000 for the molar extinction coefficient, estimation of total phosphorus and estimation of hexuronic acid by the method of HANSON, MILLS AND WILLIAMS[6] as modified by PAUL[7] (using galacturonic acid as standard), gave the ratios: uridine/phosphorus/galacturonic acid = 1/2.05/0.94.

These analyses would indicate that the compound isolated is, in fact, uridine pyrophospho-galacturonic acid with a structure identical with that of UPPGA (MILLS AND SMITH[8], STROMINGER, KALCKAR, AXELROD AND MAXWELL[9]).

This is the first recorded isolation of uridine pyrophosphogalacturonic acid and the presence of this nucleotide in the Type I capsulated pneumococcus is in accord with the concept[1] that the uridine pyrophosphoglycosyl compounds act as glycosyl donors in polysaccharide synthesis in the pneumococcus. In the Type I organism the capsular polysaccharide contains galacturonic acid and hexosamines, whereas the Type III capsular polysaccharide contains glucuronic acid and glucose.

* The following abbreviations have been used throughout: UPPG = uridine pyrophospho-glucose; UPPGA = uridine pyrophosphoglucuronic acid; UPPAG = uridine pyrophosphoacetyl-glucosamine; UPPGal = uridine pyrophosphogalactose; UMP = uridine-5′-monophosphate; UPP = uridine-5-pyrophosphate; AMP = adenosine-5′-monophosphate.

The absence of UPPGA in the Type I pneumococcus suggests that the uridine pyrophosphogalacturonic acid is derived from UPPGal by a mechanism similar to that recorded[9] for the conversion of UPPG to UPPGA, and not by a Waldenase type of enzyme acting on UPPGA.

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[1] E. E. B. SMITH, G. T. MILLS AND E. M. HARPER, *J. Gen. Microbiol.*, 16 (1957) 426.
[2] A. C. PALADINI AND L. F. LELOIR, *Biochem. J.*, 51 (1952) 426.
[3] E. E. B. SMITH AND G. T. MILLS, *Biochim. Biophys. Acta*, 13 (1954) 587.
[4] R. CONSDEN AND W. M. STANIER, *Nature*, 169 (1952) 783.
[5] S. M. PARTRIDGE, *Nature*, 164 (1949) 443.
[6] S. W. F. HANSON, G. T. MILLS AND R. T. WILLIAMS, *Biochem. J.*, 38 (1944) 274.
[7] J. PAUL, *Ph.D. Thesis*, University of Glasgow, 1952.
[8] E. E. B. SMITH AND G. T. MILLS, *Biochim. Biophys. Acta*, 13 (1954) 386.
[9] J. L. STROMINGER, H. M. KALCKAR, J. AXELROD AND E. S. MAXWELL, *J. Am. Chem. Soc.*, 76 (1954) 6411.

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A lipid present in yeast with certain properties similar to those of tocopherol

The unsaponifiable fractions of the lipids present in Fleischmann Yeast 50B and *Torula* yeasts (1 N and 3 N from Lake States Yeast Corporation, Rhinelander, Wisc.) have been shown to contain a substance which gives the Emmerie-Engel test. Moreover it behaves similarly to the tocopherols when chromatographed on $ZnCO_3$-coated filter paper according to the method of GREEN *et al.*[1], and has been shown to protect the erythrocytes of vitamin E-depleted rats from haemolysis *in vitro* in the test described by CHRISTENSEN *et al.*[2].

Two methods were used to isolate the unsaponifiable matter from the yeast:

(1) The yeast was heated under reflux (in presence of pyrogallol and in an atmosphere of N_2) with methanolic KOH until attack appeared complete (1–2 h). The mixture was then diluted with water and extracted with ether. Under these conditions, particularly with Fleischmann yeast, a lipid material sparingly soluble in benzene was produced.

(2) The yeast was extracted in the Soxhlet apparatus for 24 h with absolute ethanol containing 2% (w/v) dry HCl and 2% (w/v) ascorbic acid, the ethanol extract being then diluted with an equal vol. of water and extracted with petroleum ether. The lipid from an aliquot of the petroleum ether was saponified with ethanolic alkali in presence of pyrogallol and the unsaponifiable matter extracted with ether[3]. This method yielded 5–6% of total lipid, but in the case of Fleischmann yeast the unsaponifiable matter was considerably less than that obtained by the first method. No satisfactory method has been found for fractionating the unsaponifiable matter. Direct chromatography on $ZnCO_3$-coated filter paper gave a diffuse band of reducing material, and pretreatment with Filtrol earth resulted in some adsorption of this reducing material.

The method of GREEN *et al.* for chromatography of tocopherols was modified by the use of benzene as developing solvent, since cyclohexane was found to be unsatisfactory with the zinc carbonate obtainable here. Development with this solvent for one hour gave the following R_F values for the tocopherols: α 0.74; γ 0.58; δ 0.36. A reducing substance present in the yeasts had a mean R_F value of 0.50, although this varied somewhat depending on the purity of the spotting solution. The band was rather diffuse, but was always distinguishable from the clear-cut bands given by added β- or δ-tocopherols. Fleischmann yeast contained a substance of reducing power equivalent to 50 μg α-tocopherol/g yeast. *Torula* yeast contained more than six times this amount. Some reducing material also remained on the origin of the chromatogram.

The material obtained by alkaline hydrolysis of the yeast was purified by freezing out sterols and by passing the supernatant, in benzene solution, through a column of Filtrol earth purified by treatment with hydrochloric acid and stannous chloride[3]. Some of the reducing material was adsorbed to give a purple band. Chromatography on paper of the purified material resulted in the recovery of less than 50% of the total reducing matter present in the ethanolic spotting solution. When stored at 5° C this ethanolic solution showed no loss of reducing power, but when diluted with benzene A.R. quality (Merck) the total reducing power fell by more than