Short Communications

Structure of the paramylon from *Euglena gracilis*

Both photosynthetic and non-photosynthetic unicellular algae of the family *Euglenaceae* contain in their cytoplasm characteristic granules of diverse form and number. GOTTLEIB[1] in 1850 named them paramylon granules since they were morphologically similar to starch but did not show a blue colour with iodine. Similar granules have been described in the filamentous green alga *Cladophora rupestris*[2]. No chemical characterisation, beyond the demonstration that paramylon is a glucose-containing polysaccharide, has been recorded[3-6]. Further details of the chemistry of the paramylon from *Euglena gracilis* are now reported.

Cultures of *E. gracilis* were grown on an inorganic salts–vitamin medium[7] containing 15 % sucrose and the granules were separated as follows. Cells were collected by centrifugation, washed twice with water, once with ethanol and the pigments extracted with chloroform–ethanol (2:1, v/v). The depigmented cells were disrupted and most of the protein removed by incubation overnight at 40° with 1 % trypsin (B.D.H., Ltd.) in 0.1 *M* phosphate buffer at pH 7.6. The residue was extracted twice with 90 % urea and washed with water. The off-white material consisting mainly of paramylon granules was further deproteinised by SEVAG's method[8]. The white suspension of paramylon granules was collected by centrifugation, washed with water and dried *in vacuo* at 60°. The yield represented 25 % of the weight of the dried depigmented cells.

Microscopic examination of material prepared in this way showed no intact cells or cell debris. The granules were refractile, homogeneously anisotropic, somewhat lenticular in shape and measured 2.3–2.8 (mean 2.6) μ × 1.0–1.6 (mean 1.3) μ. Electron microscopy showed a membrane enclosing fibrils arranged lengthwise in the granule. Their staining behaviour corresponded to that reported in the literature[1,9] and in addition no yellow fluorescence was observed with ARNOLD's callose stain[10].

The granules were soluble in 5 % NaOH, 55 % H_2SO_4, formaldehyde and anhyd. formic acid, and were more or less swollen by lower concentrations of these reagents and by 5 % calcium thiocyanate, 25 % KI, satd. $ZnCl_2$, formamide and cuprammonium. The paramylon showed $[\alpha]_D^{16} = +26°$ in anhyd. formic acid (*c*, 2), $[\alpha]_D^{16} = +28°$ in 2.5 *N* NaOH (*c*, 2). Found: C, 42.4; O, 49.3; H, 6.49; N, 0.5; P, 0.05. $(C_6H_{10}O_5)_n$ requires C, 44.5; O, 49.4; H, 6.16 (Dr. K. W. ZIMMERMAN, C.S.I.R.O., Organic Microanalytical Laboratory, University of Melbourne).

Paramylon was completely hydrolysed by refluxing 20 mg with 2 ml 90 % formic acid on a boiling-water bath for 2 h followed by a further 3-h refluxing after the addition of 7 ml 3 *N* H_2SO_4. Paper chromatography of the neutralised hydrolysate showed a single reducing compound with the same mobility as glucose in four solvent systems. The identity of the hydrolysis product was confirmed by preparation of the 2,5-dichlorophenylhydrazone derivative (m.p., 160° (corr.); authentic glucose

2,5-dichlorophenylhydrazone, m.p. 160° (see ref. 11)). The polyglucose content of the paramylon was 98.8% after correction for the loss of glucose under the hydrolysis conditions.

A partial hydrolysate was prepared by refluxing paramylon with 90% formic acid (10 mg/ml) for 3 h on a boiling-water bath and after distillation of the formic acid the residue was dissolved in water. Chromatography in *n*-propanol–ethyl acetate–water (6:1:3, v/v) showed a series of six reducing compounds when sprayed with $AgNO_3$. The two compounds with the greatest R_F values corresponded in mobility to glucose and laminaribiose and a plot of the logarithm of the partition function against the expected number of hexose units/molecule was linear, suggesting that the reducing compounds were oligosaccharides forming a homologous series. Paper electrophoresis of the partial hydrolysate in borate buffer at pH 10 showed compounds with M_G's of 1.0, 0.69 and 0.59. The M_G of laminaribiose was 0.69. Preparative paper chromatography was used to separate the di- and tri-saccharides from a large-scale partial hydrolysate. The separated compounds were acetylated with acetic anhydride in pyridine. The disaccharide acetate had m.p. (corr.) 163–164° (β-laminaribiose-octa-O-acetate, m.p. 160–161° (see ref. 12)), the trisaccharide acetate m.p. (corr.) 122–123° (β-laminaritriose-hendeca-O-acetate, m.p. 120–121° (see ref. 12)). Both melting points were determined on a heated microscope stage.

On oxidation with sodium metaperiodate the paramylon granules as isolated and paramylon reprecipitated from NaOH solution showed a periodate consumption, measured spectrophotometrically[13], of less than 0.02 mole/mole anhydroglucose.

The infrared spectrum of the sample dispersed in KCl and examined as a pressed disc was well defined and a band of moderate intensity near 890 cm^{-1} provides evidence that the material is β-linked. The X-ray pattern of the paramylon showed relatively sharp rings with the following spacings and relative intensities: 13.6 Å (VS), 7.85 Å (mw), 6.78 Å (w), 5.35 Å (vw), 4.45 Å (m.dif.), 3.92 Å (m), 3.62 Å (mw), 3.45 Å (w), 3.00 Å (vw), 2.90 Å (w), 2.70 Å (vw), 2.65 Å (vw).

This pattern agrees closely with the pattern published by KREGER AND MEEUSE[14] although no spacing values were given. On the basis of the similarity of the X-ray patterns of paramylon from *E. viridis* and *E. geniculata*, and yeast glucan which had been boiled for 2 h with dil. HCl these authors suggested that these compounds are chemically identical. The main glycosidic linkage in yeast glucan has been shown to be β-1,3 (see ref. 15, 16). The absence of the reaction of paramylon with periodate-Schiff reagent observed by PRINGSHEIM[17], and also by SINGH[18] with the paramylon of *Trachelomonas grandis* suggests that few adjacent hydroxyls were present, which is consistent with a 1,3 glucan structure for paramylon. The identity of the partial hydrolysis products and the periodate consumption of the paramylon from *E. gracilis* together with the infrared spectrum and optical-rotation data confirms the 1,3-β-glucan nature of paramylon. Whether paramylon is the substance of the granules in all *Euglenaceae* remains to be demonstrated. Thus the birefringent granules from *Astasia sagittifera* (SKUJA)[19] are soluble in hydrocarbons and chloroform but are insoluble in ether and alcohol and not stained by osmic acid, Ruthenium red or Sudan III. Also the X-ray pattern of *Astasia ocellata* granules are quite different from those of *E. gracilis* and the *E. viridis*, *E. geniculata* mixture studied by KREGER AND MEEUSE[14].

Paramylon granules disappear from *Euglena* cells under certain physiological

conditions *e.g.* during cell division or during growth in the dark[20,21]; thus paramylon can probably be classified with those 1,3-β-glucans which act as reserve substances[22,23].

We wish to thank Miss J. GREY, Royal Melbourne Hospital for supplying the *Euglena* cells, Mr. H. HIGGINS and Dr. A. B. WARDROP, Division of Forest Products, C.S.I.R.O. for the infra-red and electron microscopical examinations respectively, Dr. A. MATHIESON and Mr. J. FRIDRICHSONS, Division of Chemical Physics, C.S.I.R.O. for the X-ray examination and Mr. E. MATTHAEI, Microscopy Laboratory, Melbourne University for advice and help with microscopy.

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Received May 31st, 1960

Biochim. Biophys. Acta, 44 (1960) 161–163

An improved synthesis of scopoletin

The occurrence of scopoletin (6-methoxy-7-hydroxy-coumarin) in cigarette smoke[1], and inhibition of root growth by external application of scopoletin to *Avena* and *Phleum* roots[2] have pointed out the need for a feasible synthesis to produce pure scopoletin in good yield. The present communication reports an improved synthesis of scopoletin based on modifications of the method reported by AGHORAMURTHY AND SESHADRI[3]. The pure scopoletin thus prepared has been used for preliminary studies of its metabolism in laboratory rats[4].

The new, modified procedure gives an overall yield of pure scopoletin of 55 %. In our hands, the procedure of AGHORAMURTHY AND SESHADRI gave a considerably