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The Soluble Carbohydrates of *Aspergillus clavatus*

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SUMMARY

A recent report that the major soluble carbohydrates of *Aspergillus clavatus* include ribitol and sorbose is wrong. The pentitol is arabitol and no sorbose could be detected, demonstrating that *A. clavatus* is not unlike many other moulds. Previous work on the metabolism of acyclic polyols in *A. clavatus* is re-evaluated.

INTRODUCTION

Corina & Munday (1971*b*) reported mannitol, ribitol and sorbose as major soluble carbohydrates of an unspecified strain of *Aspergillus clavatus*. To our knowledge, ribitol as a product of glucose catabolism has never been found in extracts of moulds whereas an isomer, D-arabitol, occurs in many higher fungi (Lewis & Smith, 1967*a*; Smith, Muscatine & Lewis, 1969). Also, sorbose has not been previously recorded as a major fungal product. We therefore decided to re-examine the soluble carbohydrates of this species.

METHODS

Growth of fungus and preparation of extracts for analysis of soluble sugars. Aspergillus clavatus (CMI 91910) was grown in submerged culture on a rotary shaker at 20 °C in the glucose (10 g/l)–salts medium described by Corina & Munday (1971*a*). Each 100 ml conical flask, containing 30 ml of medium, was inoculated with mycelium from a stock slope. Samples of fungal pellets (2 to 5 mm diam.) were harvested at intervals, washed in ice-cold distilled water, killed by immersion in boiling absolute ethanol and extracted under reflux with four changes of 80% ethanol. The extracts were combined, reduced in volume on a rotary evaporator at 40 °C and made up to a known volume with distilled water. For chromatography, samples of each extract were either cleared by shaking with an equal volume of 20% (w/v) $Al(OH)_3$ suspension (Harley & Jennings, 1958), or de-ionized by shaking with a mixture of Amberlite IR-120 (H) and IR-45 (OH) ion-exchange resins (Lewis & Harley, 1965).

Residual dry weight. After extraction, the residue was dried at 55 °C for 16 h and weighed.

Paper chromatography (see Lewis & Smith, 1967*b*). Samples of the cleared or de-ionized extracts were applied to Whatman no. 1 paper and developed in the following solvents: (i) ethyl acetate–acetic acid–water, 14:3:3; (ii) *n*-propanol–ethyl acetate–water, 7:1:2; (iii) methyl ethyl ketone–acetic acid–water saturated with boric acid, 9:1:1; and (iv) ethyl acetate–pyridine–water saturated with boric acid, 12:5:2. Carbohydrates were detected with silver nitrate–sodium ethoxide or by a modified *p*-anisidine technique (Lewis, Chen, Woods & Culpin, 1972) which is very sensitive and almost specific for free or combined

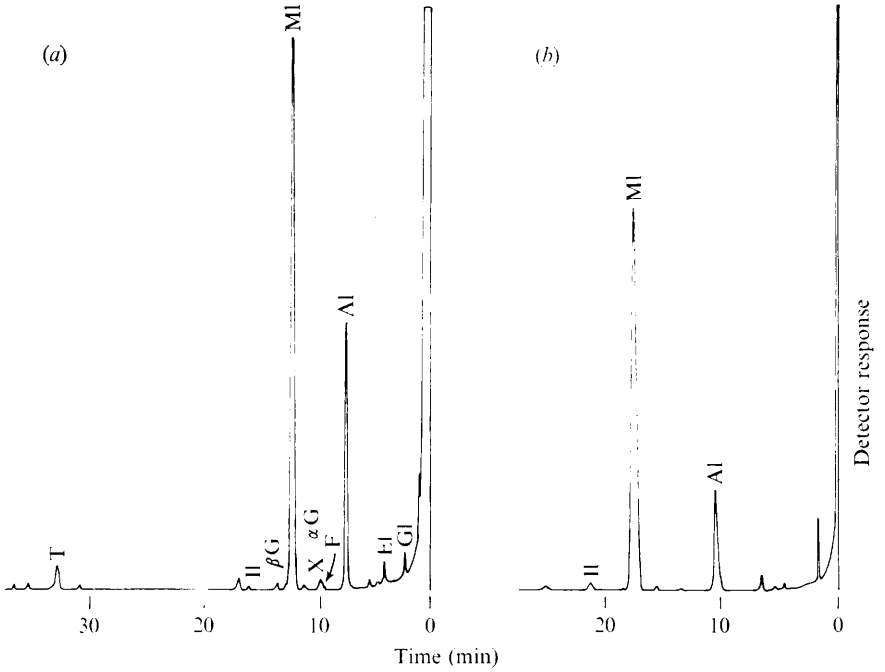

Fig. 1. Analyses by gas-liquid chromatography of the soluble carbohydrates of *Aspergillus clavatus* after 17 days of growth. (*a*) TMS derivatives resolved on a 2 % SE 52 column with a temperature programme of 140 °C+4 °C/min to 280 °C. (*b*) Acetate derivatives resolved on a 2 % ECNSS-M column with a temperature programme of 160 °C+2 °C/min to 210 °C. For abbreviations, see Table 2.

ketoses. Chromatograms developed in solvents containing boric acid were treated with hydrofluoric acid before application of silver nitrate (Britton, 1959).

Gas chromatography. The trimethylsilyl (TMS) and acetate derivatives of the soluble carbohydrates were prepared and quantitatively estimated (TMS only) as described by Holligan & Drew (1971). Analyses were carried out by using a Pye Model 64 gas chromatograph, fitted with flame ionization detectors and 5 ft × ¼ in glass columns containing 2 % SE 52 on Chromosorb W (80 to 100 mesh) for the TMS ethers and 2 % ECNSS-M on Universal B (85 to 100 mesh) for the acetates. Nitrogen was used as the carrier gas at a flow rate of 45 ml/min. Temperature programmes are given in the text.

Identity of compounds. In both gas and paper chromatography, the identity of compounds in each extract was checked by co-chromatography with authentic standards.

RESULTS

All the analytical techniques used showed mannitol and arabitol as the major soluble carbohydrates in *Aspergillus clavatus*, with glucose, *myo*-inositol and trehalose as minor components (Table 1, Fig. 1). Small amounts of glycerol, erythritol and fructose were detected by GLC of the TMS ethers. The two acyclic polyols could be clearly distinguished from isomers known to occur in other organisms by gas chromatography of their acetate derivatives. Also, arabitol and ribitol were well resolved on paper chromatograms developed in solvents containing boric acid (Table 1). There was no evidence for the presence of

Table 1. *Chromatographic mobilities* of compounds in Aspergillus clavatus, the identity of which is in doubt following the paper of Corina & Munday (1971b)*

Solvent or stationary phase	Paper chromatograms (i)†	(ii)	(iii)	(iv)	Gas chromatograms SE 52	ECNSS-M
Marker substances						
Arabitol	189	140	380	101	64	54
Ribitol	189	138	440	137	64	50
Sorbose	139	110	165	38	94	–
Fungal substances						
Pentitol	189	141	382	100	64	54
Unknown X (see Fig. 1)	+	+	+	+	87	–

* Relative to glucose on paper chromatograms, α-glucose on SE 52 and glucitol on ECNSS-M.
† See Methods for solvents corresponding to these numbers.
+, Not detected by silver nitrate or *p*-anisidine; –, not determined.

Table 2. *Soluble carbohydrates in the mycelium of a 17-day-old culture of Aspergillus clavatus*

	mg
Extracted dry wt of fungus	97·1
Mannitol (Ml)	4·57
Arabitol (Al)	1·72
Glucose (G)	0·09
Trehalose (T)	0·05
Inositol (Il)	0·34
Unknown (X)	0·11*
Glycerol (Gl), Erythritol (El), Fructose (F)	*t* (< 0·05 mg)

* Estimated by assuming a detector response factor of 1·00 (= glucose).

ribitol in any of the samples analysed. Gas chromatograms of the TMS derivatives (Fig. 1*a*) showed several minor unidentified peaks in both monosaccharide and disaccharide regions. Several of these are likely to represent non-carbohydrate substances or carbohydrate derivatives such as esters. One compound (X in Fig. 1) eluted before α-glucose (Mg = 0·87), though present in only small amounts, is possibly the substance identified as sorbose by Corina & Munday (1971*b*). However, it did not co-chromatograph with sorbose (Mg 0·94), and no ketose sugar could be detected on paper chromatograms by means of the very sensitive, modified *p*-anisidine reagent.

Quantitative data showed that the level of arabitol increased markedly between 13 and 17 days when glucose disappeared from the medium. Levels of mycelial carbohydrates after 17 days incubation are given in Table 2.

DISCUSSION

The major soluble carbohydrates of *Aspergillus clavatus* are mannitol and arabitol. This fungus, therefore, does not differ qualitatively from related fungi by possessing ribitol as suggested by Corina & Munday (1971*b*), but conforms to a predictable pattern (Lewis & Smith, 1967*a*). We would stress the importance of using more than one type of volatile derivative for identification of isomers by gas chromatography and more than one type

of paper chromatographic solvent. We not only failed to detect sorbose in *Aspergillus clavatus* but also any reducing sugar present in amounts approaching that of the putative sorbose identified by Corina & Munday (1971*b*).

It is not possible, from the experimental data of Corina & Munday (1971*b*), to determine the rate of absorption of glucose, the proportion of glucose absorbed that is converted to other carbohydrates, the distribution of carbohydrates between mycelium and medium, or yield of the fungus. Allaway & Jennings (1970) and Holligan & Jennings (1972*a*, *b*) have discussed the conditions under which fungal carbohydrates, especially mannitol and arabitol, leak from *Dendryphiella salina*. The latter authors describe the differences in time of synthesis and degradation of these two polyols during growth, and, from the data of Corina & Munday (1971*b*) and the present work, *Aspergillus clavatus* appears to behave similarly.

The rationale of the double-labelling experiments of Corina & Munday (1971*b*) is obscure and, in their interpretations, they did not consider that variation in the $^{3}H/^{14}C$ ratio in carbohydrates will be caused by relative changes in the specific activities of hydrogen and carbon (e.g. by addition of ^{3}H during polyol synthesis and loss of ^{14}C in the decarboxylation step of the pentose phosphate pathway), nor were the types of reaction that may have influenced the ratio or the probable degree of their effect indicated. Furthermore they appear to have ignored the fact that, as mannitol is a symmetrical molecule (i.e. C_6 and C_1 are equivalent), any synthesis of labelled mannitol from 1- or 6-labelled glucose will randomize the label between these positions. This seriously complicates interpretation of labelling patterns, especially in arabitol which is synthesized most rapidly, involving a decarboxylation, when endogenous mannitol is being re-utilized. This problem is discussed in more detail by Holligan & Jennings (1972*c*).

In Corina & Munday's (1971*b*) consideration of the roles of mannitol and pentitol, insufficient information was given to permit a meaningful discussion for the following reasons. An accumulation of any acyclic polyol represents a hydrogen-acceptor mechanism and a storage of carbon. Thus, mannitol cannot have a 'minor involvement in acceptor processes' since it is massively synthesized, utilizing reduced coenzyme. As no information was given concerning rates of turn-over of the polyol pools and no data on what factors influence polyol accumulation, few conclusions can yet be made about their metabolic role in *Aspergillus clavatus*.

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