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The Acid-soluble Nucleotides of *Polyporus squamosus* and *Amanita muscaria*

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The acid-soluble nucleotides of *Polyporus squamosus* and *Amanita muscaria* have been isolated and fractionated by ion exchange chromatography. These substances have been identified by ultraviolet absorption measurements, their chromatographic and ionophoretic behaviour both before and after hydrolysis, and by chemical and enzymic analyses.

In a previous publication from this laboratory [1] a method has been presented for the isolation and analysis of the acid-soluble nucleotides from plant tissues. Methods have also been described for the separation and quantitative analysis of varying amounts of nucleotides using paper chromatography and ionophoresis [2–4], and their application to the nucleotides in wheat, oats and barley [1,5]. It is the purpose of the present paper to extend the previous work on this subject to include the analysis of the acid-soluble nucleotides of two different mushrooms.

The acid-soluble nucleotides were isolated from *P. squamosus* and *A. muscaria* by homogenizing the fruiting bodies in cold perchloric acid. The nucleotides were then freed from interfering substances according to the procedure already described [1]. The separation and quantitative analysis of the nucleotide mixtures were accomplished by ion exchange chromatography on a Dowex 1 column (formate form) as described in the preceding papers. The elution of the nucleotides was followed by the changes in optical densities of the eluate. Figs. 1 and 2 show the effluent curves that were obtained. Since the nucleotide content of both mushrooms were quite similar, the analysis of the two materials are described together.

The eluate fractions from the Dowex column falling under the same peaks were pooled and concentrated by adsorption on charcoal and subsequently eluted with ethanol-ammonia. The isolated nucleotides were identified by their chromatographic and ionophoretic behaviour, both before and after hydrolysis, as compared with authentic nucleotides. The purified samples were then further characterized by the following criteria: (a) ultraviolet ab-

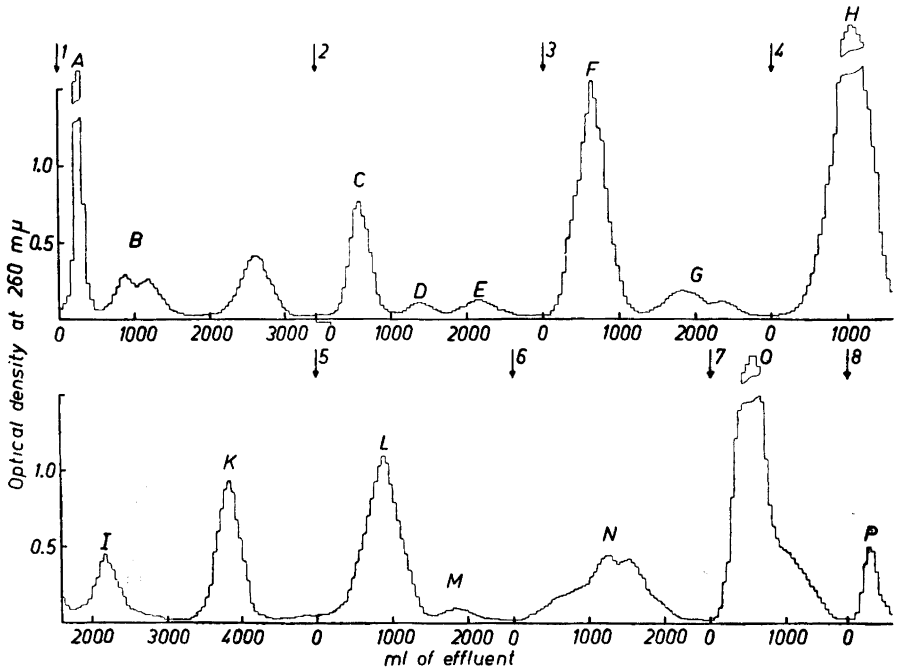

Fig. 1. Ion exchange chromatogram of acid-soluble nucleotides from 1 000 g of *P. squamosus*. The unmarked peaks contained no nucleotides. Exchanger: Dowex 1, X-10, 200 to 400 mesh, 2.4 × 70 cm, formate form; flow rate 2.0 ml/min.

Eluting agents:

1) 0.02 M formic acid
2) 0.1 M » »
3) 0.1 M » » + 0.05 M sodium formate
4) 0.1 M » » + 0.3 M » »
5) 0.1 M » » + 0.4 M » »
6) 0.1 M » » + 0.6 M » »
7) 0.2 M » » + 0.8 M » »
8) 0.5 M » » + 1.0 M » »

Identity of peaks:

A) Bases, uridine and DPN
B) 2′- and 3′-CMP
C) 5′-AMP
D) CMP-peptide
E) 2′-AMP
F) 5′-UMP
G) 2′- and 3′-UMP
2′- and 3′-GMP
TPN and CDP
H) UDPAG
I) UDPG
K) UDP
L) ADP
M) CTP
N) GDP, UTP and ATP derivative
O) ATP
P) GTP

sorption spectra in acid and alkali; (b) total and acid-labile phosphorus content; (c) sugar content; (d) paper chromatographic identification of the sugar components; and (e) ultraviolet absorption curves of the bases obtained after acid hydrolysis. The position of the phosphate groups was confirmed by the action of rattle snake venom and by oxidation with periodate.

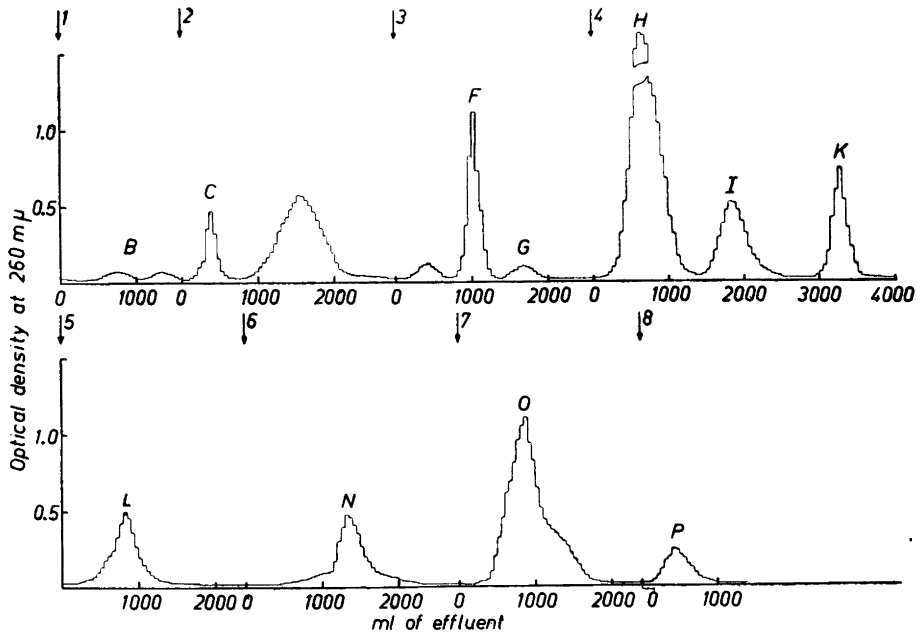

Fig. 2. Ion exchange chromatogram of acid-soluble nucleotides from 500 g of *A. muscaria*. The unmarked peaks contained no nucleotides. Exchanger: Dowex 1, X-10, 200 to 400 mesh, 1.6 × 50 cm, formate form; flow rate 1.5 ml/min. The eluting agents and identity of the peaks are the same as in Fig. 1.

The quantities of the nucleotides in the various column fractions were calculated by using the molecular extinction coefficients previously reported [1]. The results of the analysis are summarized in Table 1.

Peak A was obtained in the wash water or immediately after starting the elution with 0.02 M formic acid. This peak contained non-nucleotide material as the major component in addition to bases, uridine and DPN *.

Among the acid-soluble nucleotides of *P. squamosus*, a cytosine nucleotide is eluted from the resin after 5'-AMP. This compound has been identified as a cytidylic acid-peptide complex [6].

The different nucleotides were obtained as separate fractions with the exception of the peaks G and N. These fractions could be separated by either paper chromatography or ionophoresis. The separation could be made on a larger scale by applying the mixture to the paper as a streak instead of a spot, or by using continuous ionophoresis [5]. Fraction N from *P. squamosus* contained, in addition to the nucleotides, large amounts of a phosphorus-free sub-

* Abbreviations used: A, adenosine; G, guanosine; U, uridine; C, cytidine; MP, monophosphate; DP, diphosphate; TP, triphosphate; UDPG, uridine diphosphate glucose; UDPAG, uridine diphosphate acetylglucosamine; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

Table 1. The nucleotide content of *Polyporus squamosus* and *Amanita muscaria*. Concentration in μmoles per 1 000 g fresh weight.

Compound	Identity	*P. squamosus*	*A. muscaria*
B	2′- and 3′-CMP	25.3	8.1
C	5′-AMP	17.3	6.2
D	CMP-peptide	6.8	
E	2′-AMP	4.5	
F	5′-UMP	68.3	42.0
G	2′- and 3′-UMP	10.5	5.0
G	2′- and 3′-GMP	1.5	0.5
G	TPN	0.8	1.0
G	CDP	2.6	2.0
H	UDPAG	187.0	212.5
I	UDPG	13.5	48.5
K	UDP	32.5	37.0
L	ADP	37.5	30.9
M	CTP	4.0	
N	GDP	7.6	4.2
N	ATP derivative	1.5	13.5
N	UTP	2.6	1.8
O	ATP	122.0	114.5
P	GTP	13.0	6.0
Adenosine 5′-phosphates		178.3	165.1
Uridine 5′-phosphates		303.9	341.8
Guanosine 5′-phosphates		20.6	10.2
Cytidine 5′-phosphates		6.6	2.0

stance with an absorption maximum at 262 mμ. Peak N, from both materials, contained GDP, UTP and an adenosine derivative. The latter compound had an absorption spectrum that was typical for adenosine, but behaved both chromatographically and ionophoretically differently from known adenosine derivatives. The base liberated on acid hydrolysis was adenine, as determined by paper chromatography and spectrophotometry. Three phosphate groups per molecule of adenosine were found, only one of which was acid-labile and could be hydrolyzed in 10 min with 1 N hydrochloric acid at 100°C. Two pentose residues per adenosine were found. No periodate was consumed when the substance was tested by the method of Dixon and Lipkin [7]. The amount of material was not sufficient for a more detailed study of the structure of this adenosine triphosphate.

The chromatography of the nucleotides from these plant materials produced several non-nucleotide peaks. From *A. muscaria* two peaks appeared before the 5′-AMP peak, and from *P. squamosus* a single peak occurred after the 5′-AMP peak.

In addition to the 15 to 18 different nucleotides identified from the acid-soluble nucleotide fraction of these fungi, there are indications of many other nucleotides present in such small concentration that their identifications were not possible.

EXPERIMENTAL

Isolation and separation of acid-soluble nucleotides. Only the fruiting bodies of the mushrooms occurring out of doors under natural field conditions were investigated. 1 000 g of fresh material were homogenized in 1 000 ml of ice cold 10 % perchloric acid in a Waring Blendor and after reextraction with 1 000 ml of 5 % perchloric acid the nucleotides were isolated and freed from disturbing substances according to the method previously reported [1].

Fractionation of the isolated nucleotide mixture was carried out by ion exchange chromatography using Dowex 1, X-10, 200 to 400 mesh, in the formate form. Details about these separations are seen from Figs. 1 and 2. The nucleotides were recovered from the pooled fractions by norite adsorption and elution with 50 % aqueous ethanol containing 0.5 % ammonia.

Analytical methods. The analysis of the different nucleotides has been made using the same methods as described earlier and the same solvent systems have been used for the paper chromatographic identifications [1,5].

The one-dimensional paper ionophoretic separations were carried out with an acetate buffer of ionic strength 0.1 and pH 4.15. For continuous separations the ionic strength was decreased to 0.033. The details of these methods and the apparatus used have been reported in preceding papers [2,3].

The nucleotides used as standard substances were obtained from Sigma Chemical Company or isolated in our laboratory.

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