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The Acid-soluble Nucleotides of Different Mushrooms

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The acid-soluble nucleotides of eight different mushrooms have been separated and their concentrations determined by a combination of ionophoresis and chromatography on paper.

During the development of a scheme for the analysis of the acid-soluble nucleotides of plant materials, different methods have been developed for nucleotide separations. When large amounts of material are available, the use of ion exchange chromatography is most suitable for obtaining a complete resolution and a quantitative recovery of the various components of the nucleotide mixture [1]. Other less time-consuming methods have also been developed. Ionophoresis at pH 4.15 has been found to produce a sufficiently different separation from that obtained with paper chromatography for it to be used in combination with the latter. The nucleotides are fractionated into groups by paper chromatography and then completely separated by one-dimensional ionophoresis [2], or by continuous ionophoresis [3], depending on the quantities to be separated. Preliminary analyses of nucleotide mixtures were carried out by two-dimensional fractionation on a single sheet of filter paper using a combination of ionophoresis and chromatography [4]. This method is especially suitable when only small amounts of material are available, and these analyses can be completed in 24 h.

The latter method has been used for the analysis of the acid-soluble nucleotides of eight different species of mushrooms. The same types of apparatus which have been described earlier were used [4]. For the detailed description of the apparatus and the performance of the analyses the reader is referred to the preceding references.

An aliquot of a concentrated solution of the nucleotide mixture containing about 3 μmoles of nucleotides (calculated from the optical density at 260 mμ) was subjected to paper ionophoresis in a sodium acetate buffer of pH 4.15 and of ionic strength 0.1. The ionophoresis was carried out on a large sheet of Whatman No. 1 paper at 1 000 V for 10 h. The paper was dried at room temperature and then the separation in the second direction was achieved by paper chromatography. The previously described solvent system consisting

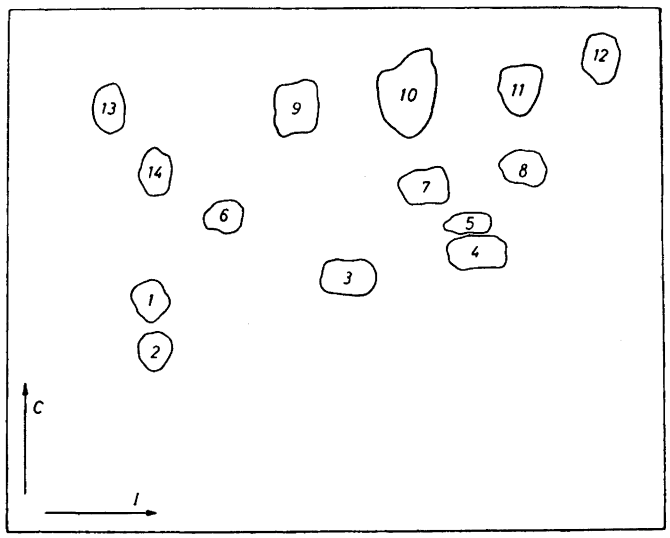

Fig. 1. Diagram of the locations of the nucleotides resolved by combined ionophoretic (I) and chromatographic (C) separations.

1) 5′-AMP
2) 2′-AMP
3) ADP
4) ATP
5) ATP derivative
6) GMP
7) GDP
8) GTP
9) UMP
10) UDPG and UDPAG
11) UDP
12) UTP
13) CMP
14) CMPX

of saturated ammonium sulphate solution-*n*-butanol-water (79:0.5:19) was used to get an almost complete separation of the different nucleotides. The various nucleotides were located on the paper photographically in ultraviolet light of 254 mμ, and prints were made with various exposure times to detect components of lower concentration. The spots were cut out from the paper and eluted with 0.1 N hydrochloric acid, and then the concentrations were calculated from the optical densities measured against the appropriate blanks. The uridine diphosphate derivatives, UDPG * and UDPAG, were obtained together as a single spot. To determine the concentrations of these two components an aliquot of the original nucleotide mixture was chromatographed in a solvent composed of 7.5 volumes of 95 % ethanol and 3 volumes of 1 M ammonium acetate of pH 7.5. This solvent separates these nucleotides from each other and from the main bulk of the nucleotide mixture.

To obtain a quantitative indication of the efficiency of this method, the acid-soluble nucleotides of *Polyporus squamosus* and of *Amanita muscaria* were analysed both by this method and by ion exchange chromatography.

* Abbreviations used: A, adenosine; G, guanosine; U, uridine; C, cytidine; MP, monophosphate; DP, diphosphate; TP, triphosphate; UDPG, uridine diphosphate glucose; UDPAG, uridine diphosphate acetylglucosamine; CMPX, cytidylic acid — peptide complex; TPN, triphosphopyridin nucleotide.

Table 1. Analysis of the acid-soluble nucleotides of *Polyporus squamosus* and *Amanita muscaria* by ion exchange chromatography (Ion ex.) and by combination of ionophoresis and paper chromatography (Combi.). Concentration in μmoles per 1 000 g fresh weight.

Compound	*P. squamosus*		*A. muscaria*	
	Ion ex.	Combi.	Ion ex.	Combi.
5′-AMP	17.3	17.0	6.2	6.5
2′-AMP	4.5	6.2		
ADP	37.5	37.9	30.9	30.8
ATP	122.0	122.0	114.5	113.0
ATP derivative	1.5		13.5	13.4
TPN	0.8		1.0	
2′- and 3′-GMP	1.5	2.0	0.5	
GDP	7.6	9.5	4.2	4.8
GTP	13.0	11.5	6.0	6.6
UMP	78.8	78.0	47.0	50.0
UDP	32.5	31.5	37.0	36.6
UTP	2.6		1.8	
UDPG and UDPAG	200.5	199.0	261.0	267.0
2′- and 3′-CMP	25.3	29.0	8.1	8.2
CDP	2.6		2.0	
CTP	4.0			
CMPX	6.8	5.2		

The separation and analysis of the nucleotides of these mushrooms by ion exchange chromatography are described in detail in the preceding paper [5]. Fig. 1 shows a map of the positions taken by the various nucleotides after a two-dimensional separation. The results of the analyses are summarized in Table 1.

The amount of each component that could be separated and detected on the filter paper was 0.01—0.50 μmoles. The great variations in the concentration of the different nucleotides in the mixture made the analyses more difficult. High recoveries were obtained for the substances present in high concentrations, while the recovery of nucleotides occurring in lower concentrations was less quantitative. The different monophosphates of the two pyrimidine nucleotides were isolated together, since they are not sufficiently separated by this method. The results of these analyses show that the method is useful for obtaining preliminary analyses of nucleotide mixtures. For more exact analyses of the components present in the lower concentrations, the other methods described must be used [1,2].

The acid-soluble nucleotides of eight different mushrooms were analysed with the method outlined above. The results of these analyses for the various

Table 2. Nucleotide content of eight species of mushroom. Concentration in μmoles per 1 000 g fresh weight.

Compound	*Polyporus squamosus*	*Amanita muscaria*	*Lycoperdon pratense*	*Hypholoma capnoides*	*Armillaria mellea*	*Pholiota squarrosa*	*Lactarius vellereus*	*Lactarius turpis*
5′AMP	17.0	6.5	10.5	13.0	3.0	3.5	11.8	8.3
2′-AMP	6.2							
ADP	37.9	30.8	50.2	76.7	27.3	86.3	54.8	15.5
ATP	122.0	113.0	113.5	105.5	138.5	115.0	158.0	95.0
ATP derivative		13.4						
TPN				6.5			1.9	6.7.
GMP	2.5		8.8					
GDP	9.5	4.8	15.5	19.5	4.0	8.6	10.9	
GTP	11.5	6.6	14.8	17.5	6.9	4.8	16.4	
UMP	78.0	50.0	72.0	54.7	22.5	48.4	43.7	26.5
UDP	31.5	36.6	46.0	62.9	48.5	11.3	31.6	6.2
UTP			11.0	5.0	14.5	4.2	15.4	2.0
UDPG + UDPAG	199.0	267.0	178.0	206.0	229.5	164.5	226.0	131.0
CMP	29.2	8.2	4.4	3.7				
CDP								
CTP				5.6			3.7	
CMPX	5.2							
UDPG	13.5	18.5	18.0	38.0	55.5	33.0	55.3	25.5
UDPAG	185.5	248.5	160.0	168.0	174.0	131.5	170.7	105.5
A derivatives	183.1	163.7	174.2	195.2	168.5	204.8	224.6	118.8
G derivatives	23.5	11.4	39.1	37.0	10.9	13.4	27.3	
U derivatives	308.5	353.6	307.0	328.6	315.0	228.4	316.7	165.7
C derivatives	34.4	8.2	4.4	9.3			3.7	

species are shown in Table 2. Since the uridine diphosphate sugar compounds were obtained as a single spot by the two-dimensional separation described above, they have been further separated by paper chromatography and the results of these analyses are also included in the same table.

There is a striking species variation of the relative nucleotide concentrations among the mushrooms. In all of the species studied the major nucleotide

components are the uridine and adenosine derivatives, as was also observed for wheat, oats and barley. In the mushrooms, however, UDPAG greatly predominates over UDPG while in the cereals UDPG is found in greater amounts than UDPAG.

EXPERIMENTAL

Isolation of acid-soluble nucleotides. 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].

The nucleotides were readsorbed on norite in order to free them from interfering salts and were eluted by repeated shaking with small volumes of 50 % aqueous ethanol containing 0.5 % ammonia. The combined eluents were neutralized and the volumes reduced to a small volume by blowing air through them.

Analytical methods. The nucleotides were analysed using the apparatus previously described [2,4]. The separations were carried out on Whatman No. 1 paper purified by washing with 1 N hydrochloric acid and water. The mixture to be separated was applied as a line to the dry paper and then the paper was impregnated with sodium acetate buffer of pH 4.15 and of ionic strength 0.1. After an ionophoretic separation at 1 000 V for 10 h, the paper was unfolded and dried at room temperature. The chromatographic separation was made after cutting away the extraneous parts of the paper sheet. Complete separation was obtained in 10 h by descending chromatography with the above mentioned solvent. Spectrophotometric determinations of the different substances were made with a Beckman DU spectrophotometer adapted for use with a volume of 0.6 ml in silica cells with a 10 mm light path.

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