INVESTIGATIONS ON CAROTENOIDS IN FUNGI
IV. MEMBERS OF THE BOLETUS GENUS

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ABSTRACT

The author investigated the presence of various carotenoids in the 7 members of the Boletus genus by means of column and thin-layer chromatography.

There are qualitative and quantitative differences in the carotenoid content of various species of the Boletus genus.

In the forests, apart from numerous berries, many types of fungi can be found which are a valuable source of food. Of the various species of fungi, those of the Boletus genus, are highly prized because their caps are very tasty. In addition to many mineral salts and specific proteins, edible fungi contain other biologically active compounds which play an essential role in human nutrition. To such groups of biologically active compounds belong the carotenoids which are a source of vitamin A. This is one of the reasons why we began a series of investigations on the carotenoid of fungi. A further factor of interest to us in this type of study was the application of carotenoid content analysis in taxonomic research.

The present paper is a continuation of work in this field. The first paper was devoted to a review of the literature on this subject (Czeczuga, 1974) and the second concerned the carotenoids found in Amanita species (Czeczuga, 1976). In our third paper of the series the presence and quantitative relations of different carotenoids in Suillus species from the forests of Białystok were discussed (Czeczuga, 1977).

MATERIAL AND METHODS

The investigations were carried out on the following species of the Boletus genus: B. aestivalis (Paulet) ex Fr., B. betulicola (Vasilk) Pilat & Dermek, B. edulis Bull. ex Fr., B. erythropus (Fr. ex Fr.) Kromb., B. luridus Schaeff. ex Fr., B. pinophilus Pilat & Dermek and B. satanas Lenz. With the exception of B. betulicola and B. erythropus, all these species were collected from the Knyszyn-Białystok forest. The specimens of the B. betuli-
were found in the Lublin Province near the town of Czerwony Staw and those of the *B. erythropus* species in the Stetin forests.

Column and thin-layer chromatography were used in the investigations, the method employed was as described in a previous paper (Czeczuga, 1972).

Separation of the carotenoids was begun, after preliminary analysis, by means of column chromatography: activated aluminium oxide (Al₂O₃) of Polish production (Gliwice), was used as absorbent.

For the experiments, a glass column, 1 cm in diameter and 15 to 20 cm in length, was used. The carotenoid extract was transferred to the column of Al₂O₃, which had been previously moistened with pure petroleum ether at boiling point (60° to 80° C).

Hydrolysis of the esters of the carotenoids was performed with 10% potassium hydroxide in methanol, under nitrogen in the dark, at room temperature (18° C) for 12 h.

Thin-layer chromatography on silica gel (6 plates) was also used to separate and identify the carotenoid pigments (Merc production) according to Stahl. The carotenoids were saponified with 10% KOH in methanol before separation. A Beckman spectrophotometer, model 2400 DU, and a Specol spectrophotometer were used for maximum absorption determinations.

The pigments were identified by the following methods: (1) behaviour on column chromatography; (2) absorption spectrum; (3) comparison of Rf values by means of thin-layer chromatography; (4) partition coefficient (Petracek & Zechmeister, 1956; Foppen, 1971).

Quantitative determinations of the various carotenoids were made by the method of Davies (1965).

**RESULTS**

1. *Boletus aestivalis*. The carotenoids isolated from the caps and pileus of this species of *Boletus* are shown in Table I from which it will be seen that they contained 9 carotenoids, chief of which was rhodoxanthin which comprised 35.6% of the total carotenoid content. A large amount of zeaxanthin was also found (18.3%).

2. *Boletus betulicus*. As is shown in Table II, these fungi contained, as did the above species, only xanthophylls of which the predominant one was found to be sarcinoxanthin; an average of 28.3% was found in these fungus species. Rubixanthin was also found in larger amounts, that is 12.1% of all the carotenoids.

3. *Boletus edulis*. The results of the analysis are shown in Table III. Here, in addition to numerous xanthophylls, two carotenes were found; γ-carotene and δ-carotene. The highest percentages of the carotenoid