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CAROTENOIDS OF SOUTHERN PINE RUST *CRONARTIUM FUSIFORME*

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Key Word Index—*Cronartium fusiforme*; Fungi; pine rust; aeciospores; carotenoids; β-carotene; γ-carotene; lycopene.

Large quantities of aeciospores of *Cronartium fusiforme* Hedg. and Hunt are found on loblolly pine (*Pinus taeda* L.) in the Athens area in April and the carotenoids of this fungus were investigated.

The following carotenoids were identified in the spore material before hydrolysis; β-carotene (34·8%), γ-carotene (63·1%) and lycopene (2·1%) in 161·7 μg/g dry wt total carotenoids. After harsh "hydrolysis" (see Experimental), the following carotenoids were observed: β-carotene (18·2%), β-zeacarotene (2·6%), ζ-carotene (1·9%), γ-carotene (64·6%), isomer-β-carotene? (8·4%), lycopene (2·3%), zeaxanthin (0·3%), flavoxanthin (1·1%) and auroxanthin (0·6%) out of a total of 485·5 μg/g dry wt.

Our results, expressed as a percentage of total carotenoids, before and after hydrolysis showed that the percentages of γ-carotene and lycopene were not altered but that of β-carotene was reduced by almost half, with the appearance of β-zea- and ζ-carotenes. The presence of small amounts of xanthophylls in the fraction after hydrolysis was unexpected.[1–4] The presence of 5,8-furanoid oxides, namely auroxanthin and flavoxanthin, is not known in organisms that lack chloroplasts. It is most likely, therefore, that xanthophylls were obtained from minute contaminants and not from the spores themselves.

EXPERIMENTAL

The aeciospores of *C. fusiforme* were collected from infected pines by brushing the spores from the galls into a polyethylene bag. The spores were then passed through a 100 mesh sieve to eliminate the larger debris, stored at −36°, and extracted within a week after collection. The extraction was carried out in a Virtis homogenizer 2 × MeOH, 2 × MeOH-Et_2O (1:1, v/v) and then 4 × with acetone at which time no more colour was extracted. This treatment is sufficient to extract all the carotenoids from most fungi; however, in this case, the spores were still brightly coloured. Irvine *et al.* found that if they digested their rust spores with N HCl for 1 hr at 100°, the residual pigments could be easily extracted.[1] This we carried out, followed by centrifugation and extraction of the spore material with Et_2O until no more colour came out. A large amount of carotenoids was released by this method, and the spore material was now dark brown. Two extracts were therefore obtained, one before and the other after hydrolysis.

Partition with Et_2O was performed and all the colour was transferred to the epiphasic layer after addition of H_2O. The upper layer which contained all the extracted pigments was concentrated under reduced pressure

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at about 35°. Procedures for the phase-partition of carotenoids between hexane and aq. 90% (v/v) MeOH and the separation and identification of carotenoids by column chromatography and TLC were as described.[5,6]

Concentrations of individual carotenoids were estimated[7] and results were calculated on a per g dry-wt basis. The dry wt of the spore material was 1·5 g.

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CHRYSOPHANOL AND EMODIN FROM *DRECHSLERA CATENARIA*

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Plant. Drechslera catenaria (Drechsler) S. Ito CBS 191·29 = *Helminthosporium catenarium. Previous work.* Isolation and characterization of catenarin and helminthosporin from *D. catenaria*;[1,2] chrysophanol and emodin from different organisms, including fungi.[3]

Present work. D. catenaria was grown at 24° on Czapek–Dox medium for 10 weeks. The dried mycelium (12·5 g) was finely ground and extracted successively with petrol and EtOAc. The residue (2·7 g) after the evaporation of EtOAc extract yielded 0·6 g helminthosporin and 1·7 g catenarin by means of fractional crystallization from EtOH. The mother liquor was concentrated *in vacuo* and the residue taken up in EtOAc. Neutral and acidic EtOAc fractions were prepared by means of the conventional method of 5% NA_2CO_3 separation. The neutral fraction (150 mg) was submitted to chromatography on silica gel G layers. The minor band was extracted with $CHCl_3$ leaving yellow leaflets (5 mg) m.p. 197–199° after concentration. (MS Found: 254·059235. Calc. for $C_{15}H_{10}O_4$: 254·057902). This led to a tentative identification of the compound as chrysophanol. Comparison with an authentic sample (m.m.p., UV, IR, MS, TLC) established its identity. The acidic fraction (270 mg) yielded a further small amount of catenarin (220 mg) after concentration. TLC of the residue revealed a yellow spot with nearly the same R_f value as catenarin. Good separation on thin layer plates only succeeded via the acetates. Hydrolysis of the individual acetates gave catenarin and a small amount of orange needles (1 mg) m.p. 259–261°. (MS Found: 270·053650. Calc. for $C_{15}H_{10}O_5$: 270·052817). The compound proved to be emodin by direct comparison with a commercial sample (Fluka) (m.m.p., UV, IR, TLC).

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