EXPERIMENTELLES

Leccinum aurantiacum (Kultur CBS 125 50, Nr 64) wurde auf Moser-b-Nahrmedium 6 Wochen bei 24 kultiviert Der Inhalt von 25 Kulturrohrchen (150 ml) wurde sorgfaltig mit Et_2O extrahiert, dem etwas HOAc zugesetzt worden war Man dampfte die getrockneten Extrakte ein und chromatographierte den Ruckstand an acetyliertem Polyamid 6-AC (Macherey, Nagel und Co, Duren) mit Aceton–MeOH (10 3) Nach wenig blaßgelbem Vorlauf wurde eine gelbe Zone eluiert, die nach Eindampfen und Zugabe von $CHCl_3$ uber Nacht kristallisierte 1 5 mg rote Kristalle nach MS identisch mit Atromentinsaure [4] UV (EtOH) λ_{max} = 370 258 230 nm Auftrennung mit Kieselgelfertigplatten Fa Merck Darmstadt (Laufmittel Benzol–HCOOEt HCOOH 10 5 3) ergab zwei Zonen **1**, R_f = 0 29 (leuchtend gelb) **2**, R_f = 0 24 (blaßgelb) von denen sich letztere mit konz H_2SO_4 tiefblau verfarbte

Anerkennung- Wir danken der Deutschen Forschungsgemeinschaft fur die großzugige Unterstutzung dieser Arbeit Frau D Ollig Munchen sei fur die technische Assistenz gedankt

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HYDROXYANTHRAQUINONE PIGMENTS FROM *ASCOCHYTA PISI*

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Key Word Index – *Ascochyta pisi*, Sphaeropsidaceae, fungi, chrysophanic acid hydroxyanthraquinones pachybasin

Plant *Ascochyta pisi* (Lib) *Source* ATCC 10954 *Importance* Pea-pod and leaf spot pathogenic fungus *Previous work* Isolation of the antibiotic, ascochitine, from the growth medium [1]

Present work We wish to report the isolation of two substances hitherto not found in this fungus The substances proved to be the closely related anthraquinone pigments, pachybasin and chrysophanic acid, the former occurring in the larger amount The substances were initially obtained from the culture medium, but the mycelium was subsequently found to contain much larger amounts The coexistence of these anthraquinones has also been reported in *Pachybasium candidum* Sacc,[2] *Phoma foveata* Foister,[3] *Trichoderma viride* Pers ex Fr,[4] and *Aspergillus crystallinus* Kwon and Fennell [5] but this is the first instance where they have been isolated from the culture medium

EXPERIMENTAL

M ps are uncorrected NMR spectra were recorded on a Perkin-Elmer Hitachi R-20 or a Varian HA-100 spectrometer, IR spectra on a Perkin-Elmer Model 257 Grating IR spectrometer UV spectra on a Coleman-Hitachi Recording Spectrophotometer Model EPS-3T and MS on a Perkin-Elmer RMU-6E low resolution spectrometer or on an Associated Electronics Industries MS-902 spectrometer equipped with a dual EI CI source [6]

[1] BERTINI, S (1956) *Annali Sperimentaz Agraria* (*Rome*) **11**, 545
[2] SHIBATA, S and TAKIDO, M (1955) *Pharm Bull* (*Tokyo*) **3**, 156
[3] BICK I R C and RHEE C (1966) *Biochem J* **98**, 112
[4] SLATER, G P HASKINS, R H HOGGI L R and NESBITT L R (1967) *Can J Chem* **45**, 92
[5] FARLEY T M (1965) *Diss Abstr* **25**, 6193
[6] BEGGS D VESTAL M L FALES H M and MILNE G W A (1971) *Rev Sci Instrum* **42**, 244

Culture medium Ascochita pisi, grown in 28 1-l flasks each containing 500 ml of modified Czapek-Dox medium,[7] was harvested after 49 days On acidifying the filtrates (pH 1) a ppt was obtained which was triturated with $CHCl_3$ Removal of the solvent left crude ascochitine which was recrystallized from EtOH (407 mg, m p 196 5–201°) Removal of the EtOH gave 155 mg of a dark brown residue which was chromatographed on silica gel (Woelm, activity III) using Et_2O, yielding 19 mg of an orange solid An additional 20 mg of the same material was obtained from $CHCl_3$ extractions of the aqueous medium These were combined and a portion was recrystallized (EtOH) yielding fine orange needles, m p 165–166 5° Although this material showed only one spot on TLC, combined high resolution and chemical ionization MS[8] indicated the presence of two parent ion peaks *m/e* 238 0619 (base peak, $C_{15}H_{10}O_3$ requires 238 0629) and *m/e* 254 0567 (25% of base peak, $C_{15}H_{10}O_4$ requires 254 0578). Using UV IR, MS and NMR spectra, the two components were identified as pachybasin and chrysophanic acid Acetylation of half the mixture and separation (TLC) gave one acetate as fine light yellow needles, m p 147 5–152 0° (EtOH), identified as pachybasin acetate by comparison with a synthetic sample[9] (co-TLC, MS, UV and m m p), and a second as light yellow needles, m p 194–199°, which was confirmed as chrysophanol acetate[10] (co-TLC, MS, UV and m m p)

The separation of pachybasin from the remaining mixture was accomplished by preparative TLC on a $MgCO_3$–5% $CaSO_4$ plate Development with C_6H_6 gave a high R_f orange band and a low R_f pink–violet band After separation, the orange band yielded pachybasin as orange needles, m p 175 8–176 8° (EtOH) [lit ,[4] m p 176–177°], identical (co-TLC, IR, MS and m m p) with an authentic sample [9] The pink band contained impure chrysophanic acid (MS) which was not further investigated

Mycelium The mycelium was dried (1 79 g) and blended with 200 ml H_2O The filtered aqueous phase was extracted with $CHCl_3$, which yielded 3 5 mg of the anthraquinone mixture on evaporation The residual mycelium was ground with fine sand and extracted with MeOH This yielded 123 mg of crude anthraquinone mixture which was chromatographed (silica gel, $CHCl_3$) to give 41 mg of the pachybasin–chrysophanic acid mixture (MS), m p 164–167 5° after one recrystallization from EtOH

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[7] RODIG, O R , ELLIS, L C and GLOVER, I T (1966) *Biochemistry* **5**, 2451
[8] MUNSON, B (1971) *Anal Chem* **43** (No 13), 28A , FIELD, F H (1968) *Accounts Chem Res* **1**, 42
[9] WALDMANN, H and SELLNER, P (1938) *J Prakt Chem* **150**, 145
[10] Aldrich Chemical Company, Milwaukee, 53233, U S A Commercial chrysophanic acid was found to contain a sizeable amount of physcion as an impurity, which was effectively removed before acetylation by dry-column chromatography on deactivated silica gel

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ANTHRAQUINONES OF *ASTROPLACA OPACA*

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Dedicated to Professor Dr OTTO STOCKER on his 85th birthday (17 December 1973)

Key Word Index—*Astroplaca opaca*, Lecideaceae, lichens, anthraquinones, anthrones

Plant Astroplaca opaca (Duf ap Fr) Bagl , syn *Lecidea opaca* Duf ap Fr , *Psora opaca* (Duf ap Fr) Massal Five specimens were collected (1) Greece, Kerkyra, NE of Piryi —leg