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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[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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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

et det J Poelt, (2) Tunisia, Djebel Zaghoran —leg et det H Hertel, (3) France, Vaucluse, NE of Joucas—leg et det G Clauzade, (4) France, Alpes de Haute Provence, Fourcalquier, Rochers de Mourres—leg G Clauzade, det C N Tavares, (5) France, Pays Basque francais Ossès—Irrisary—leg et det J Vivant, All samples with orange-coloured medulla, purple with KOH "var *crocea* (B de Lesd)" Voucher specimens in Herb Steiner, Pharmakognostisches Institut der Universitat Bonn *Previous work* None

Present work According to Santesson's[1] method of "lichen mass spectrometry" (LMS) thallus particles of the lichen were introduced directly into the inlet system Several peaks, characteristic of anthraquinones were found By combined use of LMS TLc and other methods[2] four constituents were identified

2-*Chloro*-1 8-*dihydroxy*-3-*methoxy*-6-*methylanthraquinone* (fragilinе) was identified by its two molecular ions at *m/e* 318/320 (high resolution $C_{18}H_{11}ClO_5$), its R_f value (0 84) in solvent system I, and cochromatography of authentic fragilinе from *Fulgensia subbracteata* (Nyl) Poelt [2]

2-*Chloro*-1-*hydroxy*-3,8-*dimethoxy*-6-*methylanthraquinone* has R_f 0 81 and molecular ions at *m/e* 332/334 ($C_{17}H_{13}ClO_5$) This yellow substance does not turn red in triethylamine vapour It was first detected in *Nephroma laevigatum* Ach [3] A compound with R_f 0 49 was isolated by sublimation and purified to m p 284–286 It gave peaks of molecular ions at *m/e* 304/306 (high resolution $C_{15}H_9ClO_4$) This is 2-*chloro*-1,3 8-*trihydroxy*-6-*methylanthraquinone* (7-chloro-emodine) [4 5]

A further substance with R_f 0 52 has molecular ions at *m/e* 290/292 (high resolution $C_{15}H_{11}ClO_4$) Its fragmentation pattern is consistent with an anthrone It was tentatively identified with 2-*chloro*-1,2,3-*trihydroxy*-6-*methyl*-10-*anthrone*, which has been reported from the lichen *Heterodermia obscurata* (Nyl) Trev [6]

A brown spot of R_f 0,61 is marked by a substance, which is extremely sensitive to oxidation With triethylamine vapour it yielded an intense brown violet colour Unfortunately it was impossible to isolate it, or to examine it in the mass spectrometer Two additional spots of R_f 0 27 and 0 16 respectively, both bright yellow and turning to intense violet with triethylamine, also underwent rapid oxidation All efforts to isolate these compounds failed, as they quickly change into blackish violet products, which remain on the start line of the TLC plates One might speculate on their possible relationship with the dark pigments present in the upper cortex of the *Astroplaca* thallus

EXPERIMENTAL

Extraction of the powdered thallus with C_6H_6–Me_2CO (1 1) Chromatography on silica gel H pretreated with EDTA,[2] in solvent system I first to 10 cm with C_6H_6–EtForm (1 1), then to 15 cm with $CHCl_3$ Results were checked by separation of compounds on thin and thick layer plates according to Hauschild *et al* [2]

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