cléiques et nécessite du magnésium. Les inhibiteurs classiques de la synthèse protéique ont également un action.

Nos résultats présentent une grande analogie avec ceux obtenus par RAMIREZ et al.[7] sur les membranes synaptosomales du cerveau de rat. Ces auteurs concluent à la présence de deux systèmes de biosynthèse protéique, l'un ergastoplasmique, l'autre mitochondrial. Dans les MGL, on observe un effet des inhibiteurs spécifiques des 2 systèmes, respectivement le cycloheximide et le chloramphénicol, auquel il faut joindre l'inhibition obtenue avec le 2,4-dinitrophénol. L'inhibition obtenue avec cet agent découplant montre qu'une source d'énergie mitochondriale est impliquée, au moins partiellement, dans l'incorporation. Il est intéressant de signaler que les MGL possèdent fréquemment une activité cytochrome oxydase[8], caractéristique de la membrane interne des mitochondries.

La présence, dans les MGL, d'éléments ergastoplasmiques et mitochondriaux est corroborée par des travaux de microscopie électronique[9,10]. Certains globules lipidiques présentent une excroissance contenant des éléments cytoplasmiques qui seraient entraînés par le globule au moment de sa formation et de son expulsion de la cellule épithéliale. Ces membranes granulaires ou mitochondriales ne se trouveraient, suivant les animaux, que dans 1 à 5% des globules, ce qui est en accord avec les variations individuelles que nous avons observées.

L'hétérogénéité des membranes des globules lipidiques du lait humain, déjà constatée par l'analyse des enzymes marqueurs[1], se trouve ainsi confirmée.

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Isolation of (-)-Cryptosporiopsin, a Chlorinated Cyclopentenone Fungitoxic Metabolite from *Phialophora asteris* f. sp. *helianthi*

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Summary. The (−)-enantiomer of cryptosporiopsin, a chlorinated cyclopentenone fungitoxic metabolite, was isolated from *Phialophora asteris* f. sp. *helianthi*. Next to a comparable fungitoxic activity as shown by crypstosporiopsin, the product particularly inhibits growth of *Sclerotinia sclerotiorum*, an important pathogen on sunflower. Two further metabolites were tentatively identified as a stereoisomer of cryptosporiopsin and its dehydrated derivative.

The vascular pathogen causing sunflower yellows was first isolated by HOES[3]. Further studies resulted in its determination as *Phialophora asteris* f. sp. *helianthi*[4]. We observed that in vitro cultures of the fungus possessed a fungitoxic activity and the present paper describes the isolation and identification of this antifungal metabolite.

Liquid stationary cultures (100 ml of 2% malt extract each) of *P. asteris* f. sp. *helianthi* produced the active substance in varying amounts. Addition of tryptone stimulated the growth, but at the same time strongly inhibited the formation of the toxic metabolite. The biological assay was carried out by measuring the development of *Pythium splendens* in liquid medium (5 ml) containing 1 µl of the test-solution (acetone). Sterilization of the medium was carried out before addition of the test-solution, because of the observed drastic 30-fold decrease of fungitoxic activity of preparations after being kept for 10 min at 120 °C.

Optimal toxin production by *P. asteris* f. sp. *helianthi* was observed after 12–15 days of incubation at 22 °C. The medium was filtered and the filtrate reduced to 20% of its original volume. The residue was acidified (in HCl) to pH 3, extracted with an equal volume of $CHCl_3$ and, after centrifugation, the organic layer was collected and rotavaporated. The acetone-soluble part of the viscuous residue was chromatographed twice (SiO_2, Merck precoated plates 20 × 20 cm; $CHCl_3$-MeOH 95:5). Variable amounts of the active compound were isolated with a maximal yield of 6–8 mg/l culture medium. The purified product was active towards *Pythium splendens* at a concentration of 5 µg/ml.

The colourless crystalline material (from ether m.p. 135–136 °C) thus isolated was pure by thin-layer (Merck Fertigplatten SiO_2; chloroform-methanol 5% and chloroform) and gas chromatography (3% OV-17 on Chromosorb G AW-DMCS, 80–100 mesh; 170° isothermally). GCMS measurements gave identical mass spectra at all positions of the gas chromatographically eluted peak, thus confirming its homogeneity. The most remarkable feature of the mass spectrum was the occurrence of isotope peaks of the molecular ion of *m/e* 264 at *m/e* $(M + 2)^+$ and $(M + 4)^+$ in the ratio of 9:6:1. Loss of water resulted in a strong fragment ion at *m/e* 246 still possessing the typical isotope peaks in a similar ratio as the molecular ion. Loss of 35 mass units gives a strong fragment ion at *m/e* 229 with an isotope peak at *m/e* 231 in the ratio 9:3. The base peak at *m/e* $(M-59)^+$ can be visualized by α-cleavage of a methyl ester group from the molecular ion. The observed isotope peaks and the fragmentation provide substantial evidence for the presence of 2 chloro atoms, a hydroxyl group and possibly a methyl ester group.

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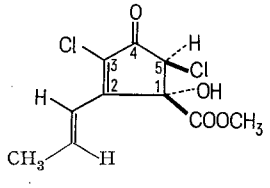
The relatively simple NMR-spectrum showed a doublet (3H, J = 5 Hz) at δ 1.9 ppm, a singlet at δ 3.8 ppm (3H), a broad singlet (exchangeable with D_2O) at 4.2 ppm (H), a sharp singlet (H) at 4.5 ppm and a complicated multiplet (2H) centered at 6.5 ppm. The possible presence of a methyl ester and hydroxyl group were thus supported by the NMR-spectrum. The methyl protons at 1.9 ppm must, through their chemical shift and coupling constant (J = 5 Hz), be attached to a double bond, and, as a consequence, the complicated multiplet centered at 6.5 ppm must be ascribed to olefinic protons attached to a methyl bearing double bond.

The IR-spectrum further confirmed these conclusions. The absorption at 3400 cm^{-1} is indicative of a hydrogen bonded hydroxyl group, while a non-conjugated carbonyl of an ester group was indicated by the strong absorption at 1755 cm^{-1}. The presence of a second carbonyl was revealed by the strong absorption at 1735 cm^{-1}, while the double bond, the ether linkage and carbon to chlorine bond were further confirmed in the appropriate regions.



The above findings accounted for the presence of 2 chlorine, 10 hydrogen and 4 oxygen atoms, and as a consequence of the molecular weight of 246, a molecular formula $C_{10}H_{10}Cl_2O_4$, was assigned to the compound.

A search in the present literature for fungal metabolites revealed the findings of McGAHREN et al.[5] and STRUNZ et al.[6] on chlorinated fungitoxic and antibiotic fungal metabolites isolated from *Sporormia affinis* Sacc. Bomm and Rous and a species of *Cryptosporiopsis*, respectively.

Comparison of our data with those given by these authors for their $C_{10}H_{10}Cl_2O_4$ compound and an authentic sample[1] revealed the identity of melting point and spectra including the UV-absorption. Singlecrystal X-ray analysis was used by McGAHREN et al.[5] to show the structure and absolute configuration of the dichlorinated metabolite to be (1S, 5S)-2-cyclopentene-1-carboxylic acid-2-*trans*-allyl-3, 5-dichloro-1-hydroxy-4-oxomethyl ester (I). This compound was named cryptosporiopsin by STRUNZ et al.[6].

For further comparison, the optical activity of the present compound was measured. Quite unexpectedly, the specific rotation was found to be of opposite sign (−96°) to the rotation measured by McGAHREN et al.[5] and STRUNZ et al.[6] indicating the antipodal nature of the product presently isolated from this *Phialophora*.

The observations of the production of optical antipodes by different living systems is not unusual: (+)-lactic acid by bacteria and its (−)-form in muscles and (+)- and (−)-isousnic and usnic acid in *Cladonia mitis* and *Cladonia pleurota*[7], respectively. Production of optical antipodes by related fungal species seems to be due to the ability either to produce or not to produce an isomerase which may then convert the originally formed product into its optical antipode[8,9].

It seems however, to the best of our knowledge, that the present findings are the second clear observation of the production of optical antipodes by different fungi. The production of (−)- and (+)-mellein by *Aspergillus melleus*[10] and an unidentified fungus[11], respectively seems unambigous, while strong doubts exist about the earlier observations on the production of dechlorogeodin[12]. We may point out here that the known sexual forms of some species of *Cryptosporiopsis* and *Phialophora* belong to the same family of Ascomycetes: the Dermateaceae. The isolation of cryptosporiopsin from both a *Cryptosporiopsis* sp. and a *P. asteris* f. sp. *helianthi* is equally in favour of the relationship between the two fungi.

Cryptosporiopsin[1,13] and the (−)-enantiomer showed comparable fungitoxic activity towards a variety of moulds. The activity of the (−)-enantiomer against *Sclerotinia sclerotiorum*, also an important pathogen on sunflower, seems noteworthy.

Finally, two further metabolites of *P. asteris* f. sp. *helianthi*, with weak to nil fungitoxic activity, were tentatively identified by GCMS measurements as a stereoisomer of cryptosporiopsin and its dehydrated derivative.

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Effect of Orotic Acid on Liver Glycogen of Different Animal Species

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Summary. The effect of orotic acid on the liver glycogen content in the mice, frogs and catfish was studied. It was observed that the orotic acid significantly increases the glycogen content in the liver of mice and catfish as it does in rats. On the other hand it causes a fall of the glycogen level in frogs in experiments made both in autumn and spring. This effect was modified by amino acids administrated together with orotic acid.

It is known that i.p. and oral administration of orotic acid elicits fatty changes in the liver. It has also been observed that on prolonged administration of orotic acid, besides fatty degeneration, a rise occurs in glycogen content of the liver associated with an increase of uridine nucleotides[1,2].

We have no data on the effect of orotic acid on the glycogen level of the organism in poikilothermal animals.

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