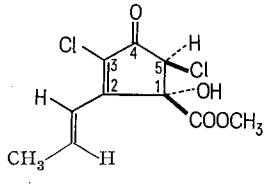
The relatively simple NMR-spectrum showed a doublet (3H, J = 5 Hz) at δ 1.9 ppm, a singlet at δ 3.8 ppm (3H), a broad singlet (exchangeable with D_2O) at 4.2 ppm (H), a sharp singlet (H) at 4.5 ppm and a complicated multiplet (2H) centered at 6.5 ppm. The possible presence of a methyl ester and hydroxyl 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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It therefore seemed justified to investigate the effect of orotic acid on the changes of glycogen content in lower animals.

Materials and methods. In the present work we report on our results obtained in experiments on mice and rats treated with orotic acid, and on the effect of orotic acid on liver glycogen in the catfish and edible frog. Parallel with the experiments, we have investigated the effect of DL-leucine on liver glycogen in the animals mentioned above. At the same time we have studied the effect of combined administration of orotic acid and DL-leucine. The effect of DL-leucine was compared with that of L-cysteine and DL-valine.

Male mice (CFLP strain) weighing 28–30 g, and rats (CFY strain) weighing 200–230 g were used. Treatment consisted of administration of daily i.p. doses of 30 mg orotic acid/kg body wt. Duration of the treatment was 10–14 days. 1 experimental group comprized 10 mice or rats. The frogs (*Rana esculenta*) weighed 80–100 g. 1 experimental group included 10 frogs (generally 5 males and 5 females). The animals received i.p. injections of 50 mg/kg/day orotic acid dissolved in physiological saline.

Catfish (*Ictalurus nebulosus*) weighing 120–140 g were treated with 60 mg/kg/day orotic acid. In one group 10 animals were included.

DL-leucine, DL-valine and L-cysteine, dissolved in distilled water, were given in daily i.p. doses of 250 mg/kg/mice or 500 mg/rats, frogs and catfish. The animals were treated twice a day, in the forenoon and afternoon. Orotic acid and aminoacid were injected together.

On the last day of the experiment (usually on the 10th–14th day) the animals were exsanguinated and the livers removed. From the liver 1 g was taken into 10 ml 10% trichloracetic acid. For the determination of glycogen in the liver samples, we used the combined method of GOOD, BLOOM et al.[3,4], the modified method of ROE et al.[5] respectively.

Results and discussion. The results on mice and rats have shown that under the effect of orotic acid the glycogen content of liver increases to about the double of the original amount (Figures A and B). A more moderate but still significant increase was noted in catfish, while a considerable decrease in glycogen content occurred in

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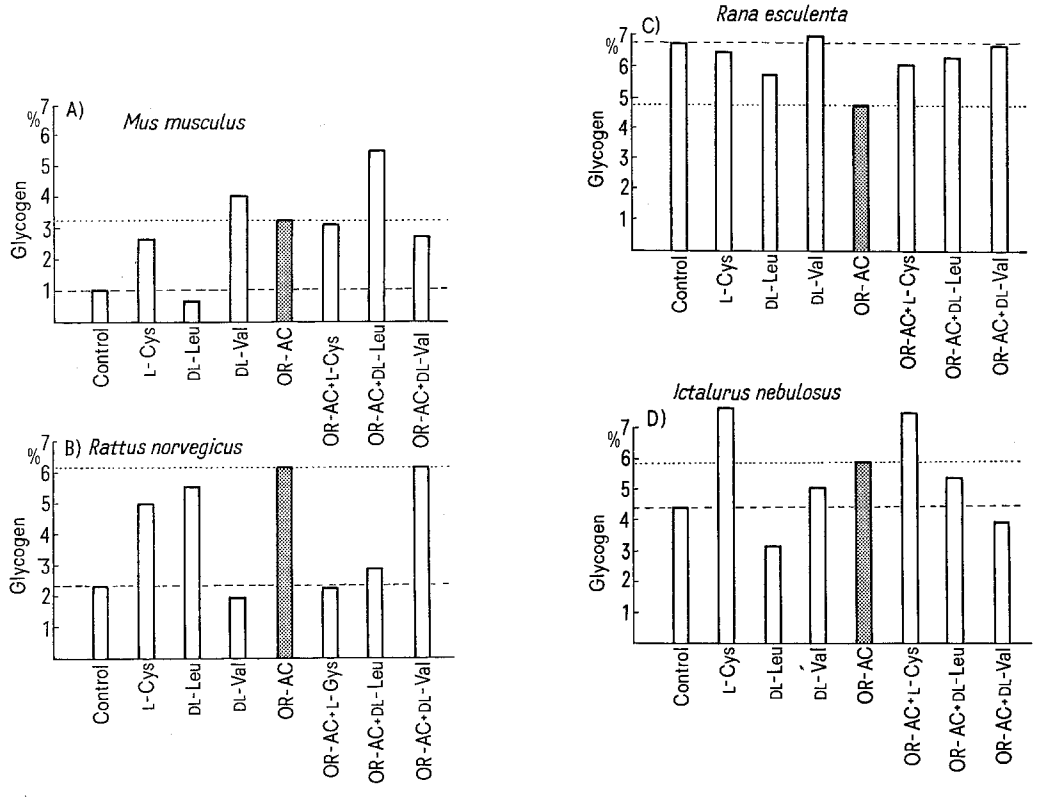

A. *Mus musculus.* L-Cys: 2.61 ± 0.36, $p < 0.01$; DL-Leu: 0.69 ± 0.05, $p < 0.05$; DL-Val: 4.06 ± 0.47, $p < 0.01$; OR-AC: 3.23 ± 0.36, $p < 0.01$; L-Cys + OR-AC: 3.15 ± 0.74, $p > 0.05$; DL-Leu + OR-AC: 5.42 ± 0.34, $p < 0.01$; DL-Val + OR-AC: 2.68 ± 0.29, $p < 0.02$; Control: 1.05 ± 0.12.

B. *Rattus norvegicus.* L-Cys: 4.97 ± 0.51, $p < 0.01$; DL-Leu: 5.55 ± 0.53, $p < 0.01$; DL-Val: 1.91 ± 0.20, $p > 0.05$; OR-AC: 5.38 ± 0.30, $p < 0.02$; L-Cys + OR-AC: 2.30 ± 0.54, $p < 0.01$; DL-Leu + OR-AC: 2.86 ± 0.83, $p < 0.02$; DL-Val + OR-AC: 6.13 ± 0.55, $p < 0.01$; Control: 2.39 ± 0.26.

C. *Rana esculenta.* L-Cys: 6.44 ± 0.26; DL-Leu: 5.67 ± 0.51; DL-Val: 6.99 ± 0.36; OR-AC: 4.70 ± 0.39, $p < 0.02$; L-Cys + OR-AC: 6.01 ± 55; DL-Leu + OR-AC: 6.28 ± 0.13; DL-Val + OR-AC: 6.70 ± 0.32; Control: 6.76 ± 0.61.

D. *Ictalurus nebulosus.* L-Cys: 7.61 ± 0.49, $p < 0.01$; DL-Leu: 3.15 ± 0.31, $p < 0.01$; DL-Val: 5.01 ± 0.37, $p > 0.05$; OR-AC: 5.89 ± 0.16, $p < 0.01$; L-Cys + OR-AC: 7.47 ± 0.80, $p > 0.05$; DL-Leu + OR-AC: 5.32 ± 0.61, $p < 0.01$; DL-Val + OR-AC: 3.87 ± 0.24, $p < 0.05$; Control: 4.46 ± 0.25.

L-Cys, L-cysteine; DL-Leu, DL-leucine; DL-Val, DL-valine; OR-AC, orotic acid.

frogs. Since this decrease of glycogen content in the frog liver was observed in autumn (November), at a time when – in agreement with other authors – we have found a considerable glycogen accumulation in several experiments[6], we have repeated the experiment in Spring (April). In the repeated experiment, after orotic acid treatment a slight but not significant decrease of glycogen content in the liver was obtained.

The observation of some authors that insulin enhances the activity of glycogen synthetase[7–9], and the finding that alloxan diabetes can be normalized by orotic acid[10], raises the possibility that orotic acid may stimulate glycogen accumulation through insulin secretion as well. Since an important role is attributed to leucine in the production of insulin, and it is known that leucine influences the blood sugar level, even if its role in glycogen production is still discussed[11–17], we have investigated the effect of DL-leucine on the glycogen level of the animal species mentioned above, by giving DL-leucine alone and together with orotic acid.

As can be seen from the Figure, in almost all animal species DL-leucine influenced, in one way or in other, the effect of orotic acid. With rats and catfish DL-leucine depressed the liver glycogen level increased by orotic acid. On the other hand, glycogen content in the liver of mice augments considerably on combined orotic acid and DL-leucine treatment as compared to the liver glycogen content of animals treated only with orotic acid. It is also remarkable that in frogs DL-leucine moderates the decrease of glycogen level due to orotic acid. Usually, combined administration of orotic acid and DL-leucine exerts a contrary effect on liver glycogen of the species studied, as compared to the values obtained in controls and orotic acid treated animals. Therefore, it may be supposed that both substances have an influence on glycogen synthetase enzyme as well. It is interesting that generally the simultaneous administration of orotic acid and DL-leucine favours the accumulation of glycogen, even if this accumulation is not always of high degree.

Although DL-valine differs in one CH_2-group from DL-leucine, still it exerts a fundamentally different effect on liver glycogen in certain species, both in combination with orotic acid or given alone, than does DL-leucine.

From the results obtained it may be concluded that amino acids containing an SH-group may play an important role in the production of glycogen, probably as enzyme components. In addition to the stimulatory effect of L-cysteine on glycogen production (even with frogs the decrease of glycogen due to cysteine treatment is not significant), it also modifies the effect of orotic acid in almost all species studied.

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Is Serotonin or are its Metabolites Responsible for Induction of Hypothermia?[1]

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Summary. Serotonin per se, rather than its metabolites, was shown to produce hypothermia in mice. This effect was mediated within the CNS and could be attenuated by methysergide.

The involvement of the central serotonergic system in neural regulation of body temperature in mammals has been the subject of numerous reports (for review see MYERS[3]). Several points of controversy are readily apparent within the literature. The major point has been the lack of agreement as to whether serotonin acts in the CNS to produce a hypothermic or hyperthermic response in core temperature. Many of the studies aimed at elucidating this point have used intracerebral administration of serotonin and monitored rectal temperature[4–7], and lack of agreement between studies has been attributed to species differences[4,5], and locale and route of administration[6,7]. Another possible explanation for the controversial results has been introduced by BAROFSKY and FELDSTEIN[8]. Their studies indicate that the serotonin metabolite 5-hydroxytryptophol produces hypothermia in mice. The possibility thus arizes that 5-HT may produce hyperthermia while its metabolites produce hypothermia. The conversion in brain of exogenously administered 5-HT to a metabolite which also modulates temperature may be responsible for some of the conflicting results. Our studies were, therefore, aimed at elucidating whether the effects of 5-HT, administered into the CNS, were a result of the action of the amine or primarily due to its metabolites.

Materials and methods. C57Bl/6J male mice, 60–80 days old, were used for all experiments. For at least 6 days prior to injections, mice were housed in a controlled

[1] This study was supported in part by grants No. NS-12759 and No. AA-2696 from the USPHS and a research grant from the Graduate College of the University of Illinois.
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