The germination is of epigeal type (Figures 2c–g). The seeds stored for 6 months were allowed to germinate at different temperatures. The result indicates that for germination the temperature range is 20–55 °C with an optimum of 30–36 °C (Figure 4). The seedlings appear in July when there is optimum temperature and moisture. This explains the cause of its distribution everywhere in the tropics during the rainy season.

Primary dormancy is of survival value for the species, as is indicated by its prevalence among wild plants[10–13]. Winter and summer are the dry part of the year and they are unfavourable for the growth of *Zornia diphylla*. Fresh seeds of the species are available in October–November, but they show marked dormancy as they do not germinate in any condition. The primary dormancy in freshly harvested seeds is due to rudiment embryo like Orchids, Ginkgo, Holly[5]. These incomplete seeds reach post-harvest maturation after passing through winter and summer seasons. Hence on the arrival of the rainy season, the seed gets released from its dormant phase, firstly due to the formation of complete embryo and secondly due to leaching of unknown chemical inhibitor/s in the rain water. Hence for survival over the unfavourable winter and summer seasons, *Zornia diphylla* possesses complex type of dormancy which does not permit its germination in fresh seeds. This behaviour is of great ecological significance[6,7].

The grassland and forest habitats of Gorakhpur have intense biotic activity of cattle and man. Grazing and scraping are special features. Monthly herbal removal by scraping is observed to stimulate shoot production in the species which is a character of non-palatable species of grassland[8]. The reason for good growth for this non-palatable species may be due to complete absence of palatable species[9].

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Structure of Furasterin, a Chlorinated Metabolite from the Fungus *Phialophora asteris* (Downson) Burge et Isaac[1]

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Summary. Furasterin, 4-chloro-7-hydroxy-5-methoxy-2-methyl-benzofuran, a new naturally occurring mono-chlorinated fungal metabolite was isolated from *Phialophora Asteris* (Downson) Burge et Isaac. Its possible relationship with the biosynthetical pathway of the fungitoxic dichlorinated fungal metabolite cryptosporiopsin is discussed.

In the scope of our work on different *Phialophora* vascular pathogens on plants[2], we have now studied *Phialophora asteris* (Downson) Burge et Isaac[3]. Mycological findings proved its close relationship with *P. asteris* f. sp. *helianthi*[4]. The latter species was earlier found to produce (–)cryptosporiopsin[5], the enantiomer of the fungitoxic and antibiotic product cryptosporiopsin[6,7] (Id, Scheme) Product Id was earlier isolated by MCGAHREN et al.[6], and STRUNZ et al.[7] from *Sporormia affinis* (Sacc.) Bomm and Rous and *Cryptosporiopsis* sp., respectively. Our aim was to prove a chemotaxonomic relationship between *P. asteris* f. sp. *helianthi* and *P. asteris* (Downson) Burge et Isaac. The present paper describes the isolation and identification of a chlorinated metabolite from the latter species. Its possible relationship to the biosynthesis of cryptosporiopsin is discussed. The fungus was similarly grown in liquid stationary cultures as was earlier described for *P. asteris* f. sp. *helianthi*[5]. Growth of the organism was somewhat slower (total period 24 days). The isolation of metabolites was identical to the method applied for (–)cryptosporiopsin[5].

Thin layer chromatography (using Merck Fertigplatten SiO_2; chloroform-methanol 5%) proved the presence of a substance with a similar Rf-value as (–)cryptosporiopsin (Rf 0.72). The unknown product (II b or c, Scheme) was purified by preparative thin layer chromatography in a final yield of 0.1 mg from each liter of culture medium. Gas chromatography (3% OV–17, isothermally at 160 °C) proved the product to be at least 95% pure, the remaining percentage being composed of several minor quantities of impurities.

Mass spectrometry revealed the molecular weight 212 with a typical intensity ratio between the ions M^+ and $(M + 2)^+$ of 3:1, indicative for the presence of a single isotopic chlorine atom. Loss of 36 mass units from the fragment at *m/e* 169 with simultaneous disappearance of the typical isotope fragment at *m/e* 133 + 2 further supported the mono-chlorinated nature of the unknown compound. At first instance, the above findings strongly suggested a close relationship between the unknown product and cryptosporiopsin (Id). The latter product with molecular weight 264 could yield – after replacement of one chlorine atom by hydrogen and additional dehydration – a product possessing the correct molecular weight 212 in agreement with the molecular formula obtained from exact mass measurements, $C_{10}H_9ClO_3$. However, the presence of the methylester group was not confirmed by the mass spectrum. Typical fragmentation by loss of 59 mass units and a strong fragment ion at *m/e* 59, as observed in the spectrum of Id, were lacking from the spectrum of the unknown.

The 100 MHz ^1H-NMR-spectrum measured in CCl_4 showed an OMe-resonance at δ 3.9 ppm in a very similar region as in the spectrum of Id, however, the typical resonances belonging to the allylic side-chain were not

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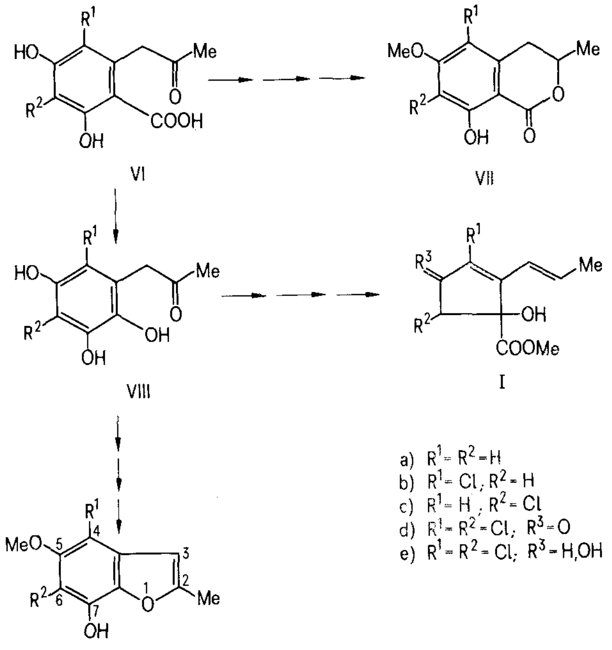
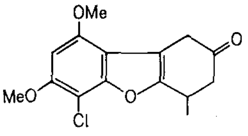
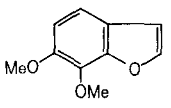
present and as a result the previously supposed close structural relationship between the unknown and Id could no longer be maintained. Further resonances in the ^{1}H-NMR-spectrum provided rather straightforward information with regard to the remaining structural features of the unknown. Next to the OMe-resonance, a methyl doublet ($J = 0.9$ Hz) was observed at δ 2.4 ppm. Decoupling experiments proved this resonance to be coupled to a single proton resonance in the olefinic region at δ 6.26 ppm. The position and small coupling constant of both resonances are in good agreement with a 2-methyl-furantype arrangement[8a, 9]. A 3-methyl-furantype arrangement can be excluded exclusively on the basis of the position of the olefinic proton, e.g. the C-2 proton in furan is found at δ 7.42 ppm[8b] or at δ 7.63 ppm in the spectrum of the benzofuran, Khellin[8c], whereas the position of the C-3 proton corresponds to the observed value of 6.26 ppm. 2 single proton resonances, one sharp and one very broad, were further observed at δ 6.34 and 5.1 ppm, respectively. These resonances were – in combination with UV-absorption observations – ascribed to an isolated proton of a penta-substituted aromatic nucleus and a phenolic hydroxyl. The UV-absorption spectrum (CH_3OH) showed maxima at 215, 235, 261 and 295 nm. Addition of a small amount of a weak alkaline solution caused a considerable bathochromic shift of primarily the longest wavelength band from 295 to 312 nm, thus indicating and confirming the presence of a phenolic hydroxyl group and as a consequence, an aromatic moiety.

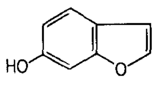




III



IV



V

Similar UV-absorption spectra were observed in the case of products III[10] and IV[11], whereas the longest wavelength band of compound V at 295 nm showed a bathochromic shift to 315 nm upon addition of NaOH.

The IR-spectrum confirmed the aromatic moiety by absorptions at 1610 and 1500 cm^{-1}, while the penta-substituted nature was supported by weak absorptions at 1730 and 1960 cm^{-1}. Further structural features were confirmed by absorptions in the appropriate regions.

The combined spectroscopical data suggested structure II b or c for the unknown product. The arrangement of the substituents is chosen as given in II b for a number of reasons.

Preceeding the isolation of cryptosporiopsin[6], McGahren and Mitscher[12] isolated several dihydroisocoumarins (VII, a, c and d, Scheme) and discussed later[6] their possible biosynthetic relationship with cryptosporiopsin. Recent findings by Holker and Young[13] on the biosynthesis of metabolites of *Periconia macrospinosa* similarly suggest that their metabolite Ie (Scheme) is biosynthesized from an aromatic precursor, structurally related to the β-ketide-(VI^a)-derived co-metabolite VII.

We now propose that similar oxidative decarboxylation of the β-ketide VIa to the intermediate VIII, instead of being followed by ring contraction to product I, may equally lead to the formation of product II[14]. The arrangement of the OH- and OMe-group is then assumed to be identical with that found for the dihydroisocoumarin metabolites from organisms which produce metabolites related to cryptosporiopsin. The formation of the furan ring seems unusual in being a polyketide-derived substance, instead of by the route where the furan carbon atoms are derived from mevalonic acid.

The remaining structural feature is the position of the chlorine atom at either position 4 or 6. The dihydroisocoumarins isolated by McGahren and Mitscher[12] and by Holker and Young[13] are equally in favor for both positions. Neither Lanthanide shift studies, nor a detailed study of the mass spectrometrical fragmentation in comparison with related compounds, allows unambiguous conclusions. Estimation of the aromatic proton chemical shift using additive increments provides support for a C-6 aromatic H (calculated δ 6.18 ppm, found δ 6.26) instead of a C-6 chloro-substitution (arom. H at C-4 calc. δ 6.76 ppm). An unequivocal structure assignment seems, however, at present best provided by synthesis. To the best of our knowledge product II, 4-chloro-7-hydroxy-5-methoxy-2-methyl-benzofuran, for which we propose the name furasterin, seems new among naturally occurring fungal metabolites.

In the scope of previous observations of the fungitoxic and -anti-bacterial activity of chlorinated metabolites, we similarly tested the activity of furasterin against *Pythium splendens*. The activity was considered of minor significance since a 20-fold amount (100 µg) of furasterin was required in order to produce an inhibitory effect equal to cryptosporiopsin (5 µg)[5].

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