Metabolites of the higher fungi. Part 29.[1] Maldoxin, maldoxone, dihydromaldoxin, isodihydromaldoxin and dechlorodihydromaldoxin. A spirocyclohexadienone, a depsidone and three diphenyl ethers: keys in the depsidone biosynthetic pathway from a member of the fungus genus *Xylaria*

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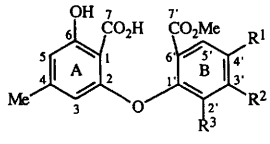
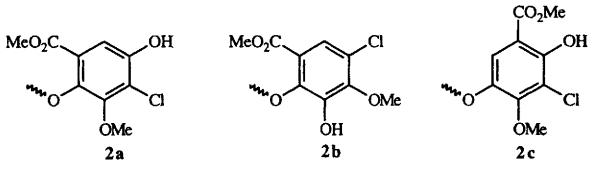
2-(3′-Chloro-2′-hydroxy-4′-methoxy-6′-methoxycarbonylphenoxy)-6-hydroxy-4-methylbenzoic acid (dihydromaldoxin 1), 2-(3′-chloro-4′-hydroxy-2′-methoxy-6′-methoxycarbonylphenoxy)-6-hydroxy-4-methylbenzoic acid (isodihydromaldoxin 2), dechlorodihydromaldoxin 3, a new chlorinated depsidone (maldoxone 4) and a new spirocyclohexadienone (maldoxin 5) have been isolated from the culture medium of an as yet unidentified *Xylaria* species. The structures have been determined by physical and chemical methods and the positions of the substituents in dihydromaldoxin 1 have been confirmed by a single crystal X-ray structure determination. Their role in the grisan–depsidone biosynthetic pathway is discussed.

The naturally occurring chlorinated spirocyclohexadienes such as griseofulvin, erdin, geodin and geodoxin have stimulated considerable experimentation designed to evaluate their biosynthetic pathways. At the same time the role of grisandienes as intermediaries in the formation of depsidones from benzophenones has been the subject of much synthetic work. However, to date no fungal species has been described which is able to produce all the possible intermediaries at the same time. We now describe the occurrence of five interrelated compounds, four of them chlorinated, from an as yet unidentified *Xylaria* species. From their common substitution pattern, these compounds show the relationship between diphenyl ethers, spirocyclohexadienones and depsidones. We name these compounds: dihydromaldoxin **1**, isodihydromaldoxin **2**, dechlorodihydromaldoxin **3**, maldoxone **4** and maldoxin **5**. Compound **1** was initially the main metabolite isolated from the medium as was **5** from the mycelium. On repeated subculturing, however, **1** was partially replaced by **2** and **3** in the medium and the new compound **4** appeared with **5** in the mycelium.

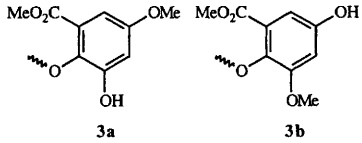
The *Xylaria* isolate used was collected in the Malaysian rain forest, unfortunately the original stromata bearing the spores decomposed before a formal identification could be made and we report here the interesting new chlorine containing metabolites produced in culture by this species. Attempts to induce it to sporulate continue. The fungus grows rapidly on malt extract medium forming an undulating, thin and almost colourless mycelium. No stromata are produced. Solvent extraction of the culture medium and evaporation of the solvent produced a bright orange gum which contained one main phenolic component; this was detected on SiO_2 as a bright orange colouration with diazotised *p*-nitroaniline. Column chromatography yielded the diphenyl ether **1**, $C_{17}H_{15}O_8Cl$, mp 195–196 °C, ν_{max}(KBr)/cm^{-1} 3550–3300, 1720 and 1688, which we name dihydromaldoxin. In the mass spectrum a strong M^+ ion at *m*/*z* 382 is accompanied by an M + 2 ion of approximately 1/3 intensity characteristic of chlorine-containing



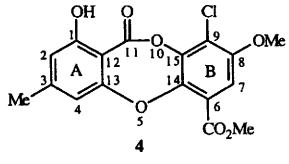
1 R^1 = OMe, R^2 = Cl, R^3 = OH
2 R^1 = OH, R^2 = Cl, R^3 = OMe
3 R^1 = OMe, R^2 = H, R^3 = OH



2a **2b** **2c**



3a **3b**



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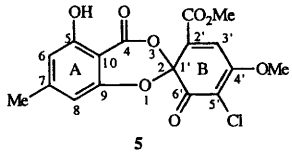

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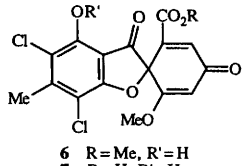
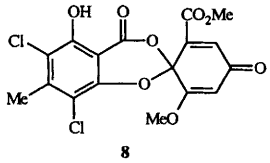


6 R = Me, R′ = H
7 R = H, R′ = H



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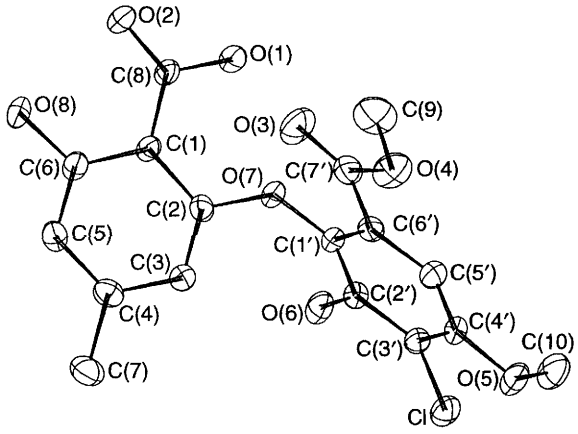


Fig. 1 The structure of dihydromaldoxin **1** showing the atom numbering scheme. Selected bond lengths (Å) and angles (°): C(2)–O(7) 1.383(4), C(1)–C(8) 1.467(5), C(6)–O(8) 1.368(4), C(4)–C(7) 1.517(5), C(8)–O(1) 1.303(4), C(8)–O(2) 1.233(4), C(9)–O(4) 1.415(5), C(10)–O(5) 1.450(5), C(1′)–O(7) 1.408(4), C(6′)–C(7′) 1.460(5), C(4′)–O(5) 1.375(4), C(3′)–Cl(1) 1.688(4), C(2′)–O(6) 1.379(4), C(7′)–O(3) 1.199(5), C(7′)–O(4) 1.361(5), O(1)–H(14) 1.144(3), O(6)–H(13) 1.051(2), O(8)–H(15) 1.184(3), O(7)–C(2)–C(1) 116.1(3), O(7)–C(2)–C(3) 121.2(3), O(8)–C(6)–C(1) 120.6(3); O(8)–C(6)–C(5) 116.9(3), O(1)–C(8)–C(2) 120.2(3), O(2)–C(8)–C(2) 121.7(3), O(2)–C(8)–O(1) 118.1(3), O(7)–C(1′)–C(6′) 125.0(3), O(7)–C(1′)–C(2′) 114.9(3), O(5)–C(4′)–C(5′) 122.1(4), O(5)–C(4′)–C(3′) 118.3(3), Cl(1)–C(3′)–C(4′) 119.7(3), Cl(1)–C(3′)–C(2′) 118.0(3), O(6)–C(2′)–C(1′) 121.3(3), O(6)–C(2′)–C(3′) 120.7(3), O(3)–C(7′)–C(6′) 124.3(4), O(4)–C(7′)–C(6′) 111.0(4), O(4)–C(7′)–O(3) 124.7(4), C(7′)–O(4)–C(9) 116.4(4), C(4′)–O(5)–C(10) 119.4(3), C(1′)–O(7)–C(2) 117.5(3).

compounds. The compound is soluble in aqueous sodium hydrogen carbonate and the presence of a chelated acid or ester carbonyl is indicated by a strong violet colouration with aqueous ferric chloride. The ^{1}H NMR spectrum shows only thirteen of the fifteen expected protons; the three between δ 6.5–7.25 are aromatic and two of them are *meta* or *para* coupled (J 1.1 Hz). The remaining ten protons give rise to a broad hydroxy signal, two methoxy signals and an aromatic methyl signal. The ^{13}C NMR data suggest the presence of two aromatic rings, five of whose carbons bear oxygen; these occur among the six resonances between δ 166–139. Two of these carbons bear the hydroxy groups which yield a diacetate with acetic anhydride and pyridine; in this derivative the two carbonyl absorptions of the parent now occur together as a strong band at 1735 cm^{-1}. The two rings share three aromatic methine resonances lying between δ 113–105; two of these carbons carry the *meta* or *para* coupled protons and the third the lone proton which is in a different ring. The ester carbonyl designation at δ 165.96 is confirmed in the ^{13}C–^{1}H NMR FLOCK spectrum in which the methoxy signal at δ 3.76 correlates only to this carbon resonance which in turn correlates to the lone aromatic proton at δ 7.24. The second methoxy group at δ 3.78 is identified as an aromatic ether group by its correlation with the carbon at δ 153.54 and these, together with the protons of the aromatic methyl resonance at δ 20.7, account for all the protons in the molecule.

The need to join the two remaining oxygen functionalised aromatic carbons by one oxygen atom characterises the compound as a monochlorinated multisubstituted diphenyl ether. The nuclear methyl at δ 2.07 is situated between *meta*-coupled protons because in the ^{13}C–^{1}H FLOCK spectrum it correlates to the methine carbons at δ 108.19 and 112.39 and to the unsaturated quaternary carbon at δ 144.37.

It would be a long and difficult synthetic task to unambiguously prove the structure of a multisubstituted diphenyl ether of this type. Fortunately the compound formed a good crystal and the structure of **1** was confirmed by a single crystal X-ray structure determination (Fig. 1). The acidic hydrogen atoms, H(13) [O(6)] H(14) [O(1)] and H(15) [O(8)], are responsible for intramolecular hydrogen bonding H(15) to O(2) and H(13) to O(7) and intermolecular crystal bonding of the inverse pairs of molecules centred on the unmethylated carboxylate groups in which H(14) is hydrogen bonded to O(2)(−1). The ether linkage atom O(7) is virtually sp^2 hybridised judged by the phenate-O(7)-phenate bond angle of 117.5°.

After this fungus had been repeatedly subcultured in malt medium, the yield of dihydromaldoxin **1** in the medium was much reduced and replaced by the metabolites **2** and **3**. Isodihydromaldoxin **2**, mp 205–206 °C, is isomeric with **1** and crystallises from alcohol as fine needles: like **1** it yields an intense orange colouration with diazotised *p*-nitroaniline, gives a violet colouration with aqueous ferric chloride and dissolves in aqueous sodium hydrogen carbonate. The mass and IR spectra of **1** and **2** are similar. In the ^{1}H NMR spectra there are small but noticeable differences in the chemical shift values of the three aromatic protons; these occur in **2** at δ 6.48, 6.81 and 7.35 compared with δ 6.58, 6.75 and 7.24 in **1**. The ^{13}C NMR resonance positions of the two also differ slightly. Calculations of the ^{13}C NMR chemical shifts of the nuclear carbons for the B ring arrangements **2a**, **2b** and **2c** clearly favour **2a** (Table 1) and the structure **2** for this molecule. If a choice between structures **1** and **2** had to be made without prior knowledge of structure **1**, the critical 2′-position would decide in favour of **2a**. This represents a diphenyl ether which could give rise to the as yet hypothetical *p*-quinonoid analogue of maldoxin **5** (see later). Similar ^{13}C–^{1}H FLOCK correlations justify assigning the same A ring substitution pattern as in dihydromaldoxin; similar arrangements of hydroxy and methyl substituents occur in geodin **6**, erdin **7** and geodoxin **8** (see later).

In addition to the two chlorinated diphenyl ethers **1** and **2** there is also a related unchlorinated analogue dechlorodihydromaldoxin **3**, $C_{17}H_{18}O_8$, mp 175–177 °C, which gives a salmon pink colouration on SiO_2 with diazotised *p*-nitroaniline, and like **1** and **2** dissolves in aqueous sodium hydrogen carbonate and yields the violet colouration with ferric chloride. The only difference in the ^{1}H NMR spectrum is the presence of an additional aromatic proton at δ 7.15 which replaces the chlorine atom; this proton is coupled (J 2.9 Hz) to what was the singlet proton and which has now become a *meta*-coupled doublet at δ 7.26 (J 2.9 Hz). In the ^{13}C NMR an additional methine carbon at δ 107.57 replaces the quaternary carbon which carried the chlorine. The calculated ^{13}C NMR shifts for the B aromatic ring in the alternative fragments **3a** and **3b** (Table 2) agree with the observed values at the 4′ and 6′ positions in structure **3a**. Dechlorodihydromaldoxin **3** is isomeric with asterric acid **12** obtained by Hassall *et al.*[2] from the mycelium of a strain of *Aspergillus terreus* Thom. The same compound is believed to occur in *Oospora sulfure ochrace*.[3] Asterric acid absorbs in the IR at 1686 (ester) and 1652 cm^{-1} (chelated carboxyl group); these values compare with the absorptions at 1685 and 1632 cm^{-1} respectively in **3**.

Maldoxone **4**, $C_{17}H_{13}O_7Cl$, mp 185–187 °C, ν_{max}(CH-Cl$_3$)/cm^{-1} 3680–2500, 1735 and 1697, appears in the mycelium after the fungus has been subcultured several times and is the most mobile TLC component in the solvent system used. The molecular formula suggested a loss of water from either dihydro- or isodihydro-maldoxin. The compound is insoluble in aqueous sodium hydrogen carbonate, yields the orange colouration with diazotised *p*-nitroaniline and gives the intense violet ferric chloride colouration; it forms a monoacetate, ν_{max}(KBr)/cm^{-1} 1756, 1733 and 1730. This identifies maldoxone **4** as a compound containing a chelated and unchelated ester grouping. The ^{1}H and ^{13}C NMR spectra resemble those of dihydro- and isodihydro-maldoxin and long range correlations in the FLOCK spectrum identify the presence of the same aromatic A ring and characterises maldoxone as the chlorinated depsidone **4**.

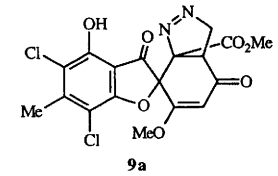
The fungus mycelium initially contained only **5** and this

Table 1 ^{13}C NMR chemical shift value (δ_C) calculations for isodihydromaldoxin **2**

Data set	Peak position (δ)					
	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
2a (calc.)	138.0	152.2	114.2	150.9	111.9	121.7
2b (calc.)	145.0	132.2	153.8	116.7	125.2	116.0
2c (calc.)	136.7	157.1	109.3	149.8	112.7	120.9
Observed	141.91	150.71	113.05	148.51	104.50	124.93

Table 2 ^{13}C NMR chemical shift value (δ_C) calculations for dechlorodihydromaldoxin **3**

Data set	Peak position (δ)					
	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
3a (calc.)	138.3	146.6	107.8	156.1	109.6	123.37
3b (calc.)	137.0	152.0	107.8	150.7	110.9	123.7
Observed	137.58	144.82	107.51	157.76	108.36	126.82



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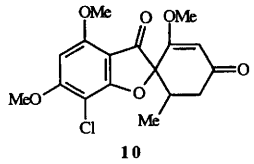

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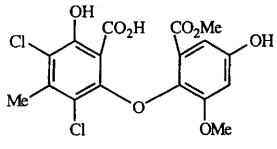

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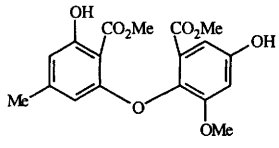

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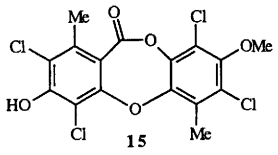

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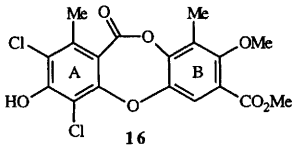

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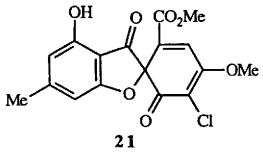

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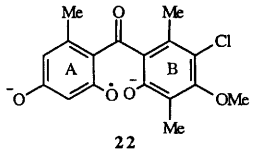

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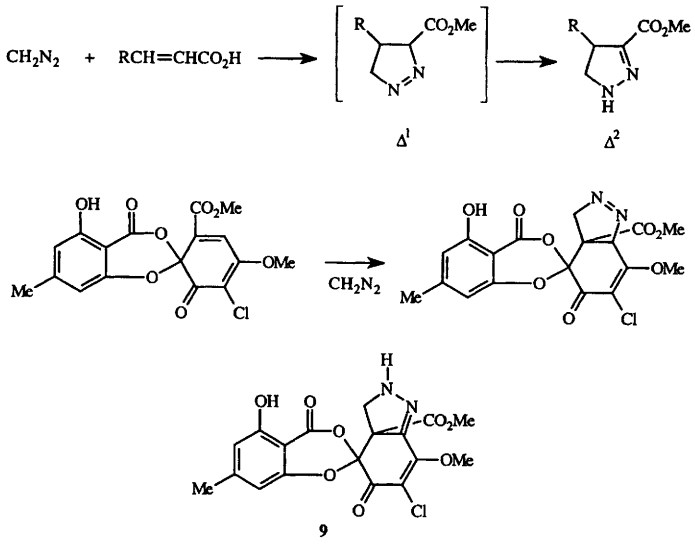
remained the major metabolite even after repeated subculturing. Maldoxin **5**, $C_{17}H_{13}O_8Cl$, ν_{max}(KBr)/cm^{-1} 3380–3220, 1728, 1709, 1680 and 1641, is bright yellow in colour and was isolated by chloroform extraction and purified by chromatography. The ^1H NMR spectrum is similar to that of dihydromaldoxin **1** but now one of the two methoxy resonances is at much lower field (δ 4.18). In the ^{13}C NMR spectra a new quaternary carbonyl carbon occurs at δ 183.09 and there is one less hydroxylated quaternary carbon between δ 163–150. Like dihydromaldoxin **1**, it stains orange with diazotised *p*-nitroaniline solution but unlike **1**, **2**, **3** and **4** it forms a 2,4-dinitrophenylhydrazone and it does not dissolve in aqueous sodium hydrogen carbonate nor give a colouration with ferric chloride. A monoacetate is formed with pyridine and acetic anhydride; in the IR spectra the hydroxy and 1709 cm^{-1} absorptions are missing, while those at 1728 and 1680 cm^{-1} are unaffected.

Proof that one of the rings is no longer aromatic was obtained by the action of diazomethane to produce a compound $C_{19}H_{18}O_8N_2Cl$, corresponding to the methylation of one phenolic hydroxy substituent and addition of diazomethane; such additions occur with α,β-unsaturated esters to yield initially Δ^1-pyrazolines which spontaneously rearrange to yield the Δ^2-isomer.[4,5] In cases where rearrangement is prevented by the presence of a substituent at the ester α-position as in a cyclic structure the product is the Δ^1-isomer. The formation of a pyrazoline is supported by the appearance of new signals at δ 6.77 (1 H, d), 4.43 (1 H, d) and 4.23 (1 H, dd) and the disappearance of the unsaturated methine proton at δ 7.63. The two new signals at δ 4.43 and 4.23 are due to non-equivalent methylene protons and their position results from their close proximity to nitrogen. Addition of D_2O caused the doublet at δ 6.77 to disappear and the methylene proton doublet of doublets at δ 4.23 to collapse to a doublet; this immediately identifies the low field signal as that of a secondary amine proton. These results mean that the compound is a Δ^2-pyrazoline **9** which could only be formed from a cyclic unsaturated ester if nitrogen has added at the ester β-position (Scheme 1). This is in contrast to the Δ^1 structure that has been given to a similar geodin diazomethane adduct[6] **9a**. Conclusions regarding the non-aromatic character of ring B can be drawn from the UV absorption which at 217, 270 and 381 nm (ε/dm^3 mol cm^{-1} 34 000, 11 000 and 6000) is quite different from that of any of the other compounds and resembles that expected from dienones.[7] The long wavelength band strongly suggests a 2,4-dienone rather than the cross conjugated 2,5 system. Unambiguous proof of the structure was obtained by catalytic hydrogenation over Pd/C which resulted in the absorption of one equivalent of hydrogen and the formation of dihydromaldoxin **1**. This could only result from a spiro cyclohexa-2,4-dienone system which attains aromaticity on hydrogenation. The alternative 2,5-enone system would have yielded iso-dihydromaldoxin **2**.

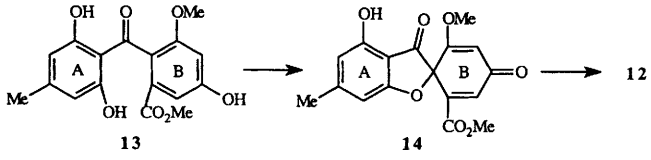
Naturally occurring chlorinated spirocyclohexadienones from fungi are uncommon. Geodin **6** and erdin **7** were first obtained from *Apergillus terreus* Thom by Raistrick and Smith[8] and their structures finally determined by Barton and Scott.[6] Geodoxin **8** was isolated from a strain of *A. terreus* which had ceased to produce geodin.[9] The antifungal antibiotic griseofulvin **10** although more saturated, is obviously related and is of widespread occurrence among *Xylaria* species.[10] The initial isolation of griseofulvin from *Penicillium patulum* and *P. griseofulvum*[11] and its structure determination[12] has resulted in much additional work and its biosynthetic origins are now firmly defined.[13]

Geodoxin **8** and maldoxin **5** although closely related, show



Scheme 1

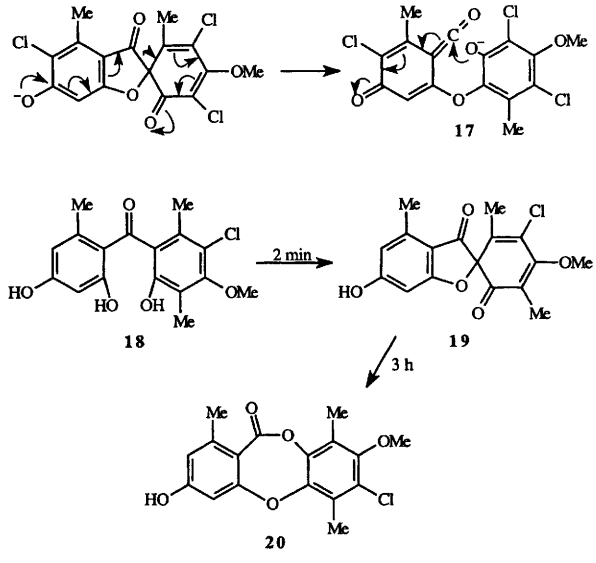
interesting structural differences. (i) The chlorine in geodoxin, geodin, erdin and griseofulvin occurs in the aromatic A ring; in maldoxin it occurs in the cyclohexadienone B ring. (ii) The hexadienone system in maldoxin is 2,4- but in the other compounds is 2,5-. The origin of geodoxin has been studied by using blocked mutant strains of *A. terreus*;[14] this work concluded that geodoxin arises *via* the diphenyl ether, dihydrogeodoxin (geodin hydrate) **11**, a compound which was unknown as a natural product at the time of the work but which was later isolated from another mutant strain of the fungus. Such a pathway is supported by the *in vitro* oxidative conversion of **11** into **8** by using PbO_2. The occurrence of the three diphenyl ethers **1**, **2** and **3** in the same metabolic broth supports such a route to maldoxin from dihydromaldoxin; chlorination and methylation having taken place at this pre-oxidative coupling stage. Dechlorodihydromaldoxin **3** and asterric acid **12** are the 4′ and 6′ monomethylated isomers of a common precursor and give rise to either the cyclohexa-2′,4′- or the -2′,5′-dienone systems. The major question then concerns the origin of the dechlorodihydromaldoxin. Does it arise by direct coupling of two aromatic phenols or does it arise *via* a proven route to such compounds, involving the formation of the acetate malonate derived benzophenone which by oxidative coupling forms the grisan which in turn forms the diphenyl ether, just as sulochrin **13** yields bisdechlorogeodin **14** and then asterric acid **12** (Scheme 2).[9] The chlorination step, whether it



Scheme 2

takes place at the benzophenone or diphenyl ether stage, is certainly an unusual one in that the chlorine is directed into the B ring and hence into the cyclohexadienone system; there is no precedent for this.

The presence of the depsidone **4** is significant. These compounds (like depsides and diphenyl ethers) are common lichen constituents but they are not common as fungus metabolites. The mollicellins[15,16] are a group of non-chlorinated compounds from *Chaetomium mollicellum* and nidulin, nornidulin and dechloronornidulin have been isolated from a strain of *A. nidulans*.[17,18] Nidulin and nornidulin are also co-metabolites with the compounds yasimin, haiderin, rubinin, strenin and nasrin from *A. unguis*.[19] A depsidone also occurs in *Sirrudesmium diversum*.[20] In the di-, tri- and tetra-chlorinated compounds both the A and B aromatic rings are chlorinated. Prior to 1972 it had been generally accepted that depsidones were biosynthesised from depsides with direct formation of the seven membered ring by oxidative coupling, and Ollis[21] synthesised diploicin **15** in this way by a direct oxidative coupling process which was considered to emulate the proposed biosynthetic pathway. Hendrickson,[22] in a re-examination of the structure of gangaleoidin **16**, sought to improve the synthesis of such compounds by making use of grisans as a source of diphenyl ethers bearing an *ortho*-carboxy group and capable of yielding a depsidone by ring closure. Sargent[7] has shown that oxidative coupling of benzophenones with potassium ferricyanide in aqueous potassium carbonate gives the depsidone directly and has proposed that the biosynthesis of such compounds follows the same course. *In vitro* the products obtained vary with structure, the length of time the reaction is allowed to remain basic and whether or not a ketene type intermediate **17** is possible. He found that brief oxidation (2 min) of the benzophenone **18** gave the grisadienone **19** in high yield, but if the compound was allowed to remain in contact with base for 3 h the depsidone **20** resulted, indicating that the grisadienone had rearranged to the depsidone (Scheme 3).



Scheme 3

This demonstration of the ready formation of depsidones from grisans could provide the explanation for the formation of maldoxone from an as yet hypothetical grisan **21**. The position of the chlorine atom in maldoxone is probably very significant. Hendrickson[22] observed during his synthetic work that chlorine (or another halogen) was necessary in the ultimate B ring in order to provide the necessary discrimination in the oxidative coupling of the benzophenone. Sargent reasoned that in such couplings of halogenated compounds the initially formed species has the phenoxyl radical *ortho* to the carboxy group in the unhalogenated A ring of the benzophenone **22** rather than on the halogenated B ring which would be expected to be of higher oxidising potential. If the biochemical reaction does proceed through a benzophenone then the same rules might be expected to apply and the positioning of the chlorine in the ultimate B ring ensures that formation and coupling of the diphenyl ether to yield the despsidone can take place. The arrangement of hydroxy and methyl groups on the A aromatic ring in geodin, erdin and trypacidin is the same as in the new metabolites and it is clear that their derivation follows a similar pathway. The absence of a depsidone among the metabolites found from *A. terreus* may be explained by the halogen having entered the ultimate A ring, thus making the necessary radical and phenate positions less conducive to the formation of a grisan able to produce a diphenyl ether capable of forming a depsidone. The presence of the 4-methoxycyclohexa-2,4-dienone also ensures the ready conversion to the depsidone. The presence of the *ortho* hydroxy acid chelated system in ring A of **1** has also produced an unusual depsidone possessing a seven membered chelated lactone carbonyl group; in most other depsidones the A ring 6-position is occupied by a methyl group and the presence of this unique chelation feature highlights its common biosynthetic origin with those of maldoxin and geodin, both of which have identical A ring hydroxy and methyl substitution patterns. We have found no benzophenones or spiro grisans as metabolites from this fungus.

Experimental

General

Mps were determined on a Kofler hot-stage apparatus and are uncorrected, IR spectra on either a Perkin-Elmer 681 or a Nicolet 205 spectrophotometer, mass spectra (FAB) using 3-nitrobenzyl alcohol as matrix on an AEI MS 902 spectrometer and optical rotations on a Perkin-Elmer 141 polarimeter. Extracts were dried over Na_2SO_4 and the metabolites were detected on TLC with diazotised *p*-nitroaniline solution. Column chromatography was carried out using Merck kieselgel GF_{254} or for flash chromatography, Fluka kieselgel 60 (230–400) mesh.

^1H and ^{13}C NMR spectra, using tetramethylsilane as internal standard, were determined at 270 or 67.8 MHz respectively with a JEOL GX270 spectrometer fitted with a dual 5 mm C/H probe. ^1H NMR spectra were acquired with 32 K data points over a spectrum width of 3001.2 or 6002.4 Hz; *J* values are given in Hz. Carbon atom types were established in the ^{13}C NMR spectrum by employing a combination of broad-band proton-decoupled and distortionless enhancement by polarisation transfer (DEPT) experiments with 32 K data points over a spectrum width of 17 605.6 Hz. Assignments were established by employing a combination of 1-D and 2-D NMR experiments. 2-Dimensional spectra were acquired and processed by standard JEOL software, ^1H–^1H correlations by double quantum-filtered COSY (VDQFN), resolution 2.93 Hz in the $f1$ and $f2$ domains, PW1 = PW2 = $\pi/2$; $[^1J_{C-H}]^{13}C$–^1H correlations (VCHSHF), resolution $f2$ 17.19 and $f1$ 5.9 Hz, pulse delay 1, 2 or 3 s, J_{C-H} 140 Hz; and $[^2J_{C-H}$ and $^3J_{C-H}]^{13}C$–^1H correlations were established by using the FLOCK pulse sequence of Reynolds *et al.*,[23] resolution $f2$ 17.19 and $f1$ 5.9 Hz, pulse delay 1, 2 or 3 s, Δ^1 86.5 and Δ^2 46.5 ms, or Δ^1 44 and Δ^2 24 ms.

Crystal structure determination. A four circle X-ray diffractometer (Stoe Stadi 4 Diffractometer, Stoe, Darmstadt, Germany) was used to acquire the reflection data on a single crystal. Cell constants and orientation matrix for intensity data collection were obtained from the least-squares refinement of the scattering angles of 25 selected reflections. Intensity data for the single crystal were measured using graphite monochromated Mo-Kα radiation; the ω–2θ scanning mode was used for data collection, and peak counts were corrected with background counts for mean linear backgrounds measured at both ends of each reflection. Intensities were additionally corrected for the Lorentz and polarization effects, but not for the extinction or absorption effect. The crystal examined was stable and did not deteriorate during analysis as monitored by three standard reflections measured at two hourly intervals during the data collection. The structure was solved by SHELX 86[24] and refined by CRYSTALS[25] from merged sets of Friedel Pair reflections of which 1514 reflections were in the unique set. A blocked full-matrix least-squares refinement of the non-hydrogen atoms was employed with the hydrogen atoms conventionally placed on the carbon atoms and the acidic hydrogen atoms found from a Fourier electron density difference map. The reliability index based on $F_o - F_c$ minimisation with unit weighting of the reflections (weighting did not yield a better result) converged to $R = 0.0357$, with parameter shift to esd ratios less than 0.02, and less than 0.2 e Å^{-3} in the residual electron density difference map.

Culture conditions. The unidentified *Xylaria* species (Mal 3) was collected in Malaysia and surface cultured at 23 °C for eight weeks on 3% malt extract in subdued daylight in Thompson bottles (2 dm^3) each containing 1 dm^3 of medium to yield a thin almost colourless mycelium whose black underside tended to curl upwards onto the sides of the bottles. No stromata were observed. The mycelium was separated from the culture medium by filtration through muslin and air dried.

Maldoxin (methyl 5′-chloro-5-hydroxy-4′-methoxy-7-methyl-4,6′-dioxospiro[4*H*-1,3-benzodioxine-2,1′-cyclohexa-2′,4′-diene]-2′-carboxylate) 5 from the mycelium

The air dried mycelium (30 g) from four bottles was extracted with chloroform (soxhlet 16 h). Evaporation of the solvent gave a dark brown gum (6.39 g) which was triturated with light petroleum (bp 60–80 °C) to yield a greenish yellow solid (0.64 g). The solid was dissolved in the minimum volume of the mixed solvent system toluene : ethyl acetate : acetic acid (75 : 25 : 1) and applied to a column of silica gel (40 × 2 cm) which was eluted with the same solvent system. Evaporation of the yellow eluate gave *maldoxin* **5** as a yellow solid which crystallised readily from alcohol as lustrous needles (0.36 g), mp 143 °C (Found: C, 53.9; H, 3.1; Cl, 9.3. $C_{17}H_{13}O_8Cl$ requires C, 53.6; H, 3.4; Cl, 9.3%); *m/z* 380 (M^+); ν_{max}(KBr)/cm^{-1} 3380–3220, 1728, 1709, 1680 and 1641; λ_{max}(EtOH)/nm 217 (ε/dm^3 mol^{-1} cm^{-1} 34 136), 270 (10 750) and 381 (6000); $\delta_H(CDCl_3)$ 2.28 (3 H, s, 7-H_3), 3.86 (3 H, s, 2′-CO_2Me), 4.18 (3 H, s, 4′-O*Me*), 6.17 (1 H, d, *J* 1.1, 8-H), 6.48 (1 H, d, *J* 1.1, 6-H), 7.63 (1 H, s, 3′-H) and 9.87 (1 H, s, 5′-OH); $\delta_C(CDCl_3)$ 183.09 (C-6′), 162.85 (C-4), 161.80 (2′-*C*O_2Me), 161.03 (C-4′), 160.69 (C-5), 153.09 (C-9), 150.21 (C-5′), 133.38 (C-2′), 128.55 (C-3′), 112.33 (C-7), 111.71 (C-6), 107.53 (C-8), 96.38 (C-10), 93.75 (C-1′), 58.34 (4′-O*Me*), 53.21 (2′-CO_2Me) and 22.53 (7-*Me*).

The procedure was repeated using the isolate that had been sub-cultured several times and kept viable on malt agar extract slopes. TLC of the mycelium extract indicated the presence of an additional metabolite (R_f = 0.82) which was identified by a salmon pink colouration with diazotised *p*-nitroaniline solution. The extract (0.81 g) was chromatographed as above and the eluted fractions (3 cm^3) collected.

Tubes 46–60. Evaporation of solvent yielded a solid which

crystallised from ethanol to yield *maldoxone* (*methyl 9-chloro-1-hydroxy-8-methoxy-3-methyl-11-oxo-1H-dibenzo-*[b,e][1,4]-*dioxepine-6-carboxylate*) **4** as needles (35 mg), mp 185–187 °C (Found: C, 55.8; H, 3.4; Cl, 9.4. $C_{17}H_{13}O_7Cl$ requires C, 56.0; H, 3.4; Cl, 9.3%); *m/z* 364 (M^+) and 329 ($M^+ - Cl$); $\nu_{max}(CHCl_3)/cm^{-1}$ 3650–2500, 1735 and 1697; $\delta_H(C_5D_5N)$ 2.19 (3 H, s, 3-H_3), 3.77 (3 H, s, 8′-O*Me*), 3.95 (3 H, s, 6-CO_2*Me*), 6.88 (1 H, s, 2-H), 7.18 (1 H, s, 4-H) and 7.37 (1 H, s, 7-H); $\delta_C(C_5D_5N)$ 21.68 (3-Me), 52.61 (6-CO_2*Me*), 56.73 (8-O*Me*), 105.90 (C-12), 109.68 (C-2), 113.13 (C-4), 116.10 (C-9′), 122.65 (C-6), 143.72 (C-15), 144.75 (C-3), 148.23 (C-14), 153.10 (C-8), 161.12 (C-13), 162.56 (C-1), 162.56 (6-*C*O_2Me) and 164.38 (C-11).

Tubes 65–77. Evaporation of the solvent and recrystallisation of the yellow residue from alcohol gave *maldoxin* **5** (120 mg) as lustrous yellow needles identical with the sample described above.

Dihydromaldoxin 1, isodihydromaldoxin 2 and dechlorodihydromaldoxin 3 from the culture medium

The dark brown culture medium (4 dm^3) was extracted with ethyl acetate (×3). The solvent was dried and evaporated to yield an orange gum (0.83 g) which was applied to a column of silica gel (40 × 2 cm) in the mixed solvent system (15 cm^3) described above. The column was eluted with the same solvent system; 3 cm^3 fractions were collected.

Tubes 65–77. The yellow eluent corresponded to the yellow band typical of maldoxin. Evaporation of the solvent gave only a trace of that compound (< 1 mg).

Tubes 78–105. On evaporation gave a white solid (7 mg) which yielded 2-(3′-chloro-4′-hydroxy-2′-methoxy-6′-methoxycarbonylphenoxy)-6-hydroxy-4-methylbenzoic acid (isodihydromaldoxin) **2** as needles from ethanol, mp 203–206 °C (Found: C, 53.4; H, 4.1; Cl, 9.1. $C_{17}H_{15}O_8Cl$ requires C, 53.3; H, 3.95; Cl, 9.3%); *m/z* 382 (M^+); ν_{max} and λ_{max} as for dihydromaldoxin; $\delta_H(C_5D_5N)$ 2.08 (3 H, s, 4-H_3), 3.75 (3 H, s, 6′-CO_2*Me*), 3.79 (3 H, s, 2′-O*Me*), 6.48 (1 H, d, *J* 0.7, 3-H), 6.81 (1 H, d, *J* 0.7, 5-H), 7.35 (1 H, s, 5′-H), 10.66 (1 H, s, 1-CO_2H) and 12.04 (1 H, s, 6-OH); $\delta_C(C_5D_5N)$ 21.71 (4-*Me*), 52.53 (6′-CO_2*Me*), 56.56 (2′-O*Me*), 104.50 (C-5′), 106.39 (C-1), 108.35 (C-5), 112.68 (C-3), 113.05 (C-3′), 124.93 (C-6′), 141.91 (C-1′), 145.41 (C-4), 148.51 (C-4′), 150.71 (C-2′), 158.95 (C-2), 164.47 (C-6), 169.17 (6′-*C*O_2Me) and 174.32 (1-*C*O_2H).

Tubes 108–124. Evaporation of the solvent and crystallisation of the solid from ethyl acetate gave 2-(3′-*chloro*-2′-*hydroxy*-4′-*methoxy*-6′-*methoxycarbonylphenoxy*)-6-*hydroxy*-4-*methylbenzoic acid* (*dihydromaldoxin*) **1** (2.4 mg) as rhombs, mp 198–199 °C (Found C, 53.4; H, 4.0; Cl, 9.1. $C_{17}H_{15}O_8Cl$ requires C, 53.3; H, 3.95; Cl, 9.3%); *m/z* 362 (M^+); ν_{max}(KBr)/cm^{-1} 3550–3300, 1720, 1688 and 1640; λ_{max}(EtOH)/nm 214 and 249 (ε/dm^3 mol^{-1} cm^{-1} 42 600 and 10 800); $\delta_H(C_5D_5N)$ 2.07 (3 H, s, 5-H_3), 3.76 (3 H, s, 6′-CO_2*Me*), 3.78 (3 H, s, 4-O*Me*), 6.58 (1 H, d, *J* 1.1, 5-H), 6.75 (1 H, d, *J* 1.1, 3-H), 7.24 (1 H, s, 5′-H) and 11.78 (1 H, s, 6-OH); $\delta_C(C_5H_5N)$ 21.71 (C-8), 52.34 (6′-CO_2*Me*), 56.31 (C 4′-O*Me*), 102.95 (C-5′), 106.86 (C-1), 108.19 (C-5), 112.39 (C-3), 115.85 (C-3′), 124.13 (C-6′), 139.16 (C-1′), 144.37 (C-4), 150.95 (C-2′), 153.58 (C-4′), 159.50 (C-2), 163.13 (C-6), 165.96 (6′-*C*O_2Me) and 174.58 (1-*C*O_2H).

Crystal data. M = 382.7, triclinic, space group $P\bar{1}$, a = 7.524(1), b = 8.834(5), c = 13.605(1) Å, α = 102.52(2), β = 108.36(7), γ = 94.30(4)°, Z = 2, V = 827.85 (Å^3), $F(000)$ = 396, D_c 1.535 g cm^{-3}, crystal size 0.3 × 0.05 × 0.05 mm, absorption coefficient 0.28 mm^{-1}, reflections collected 3028, unique reflections 1390 ($1 > 3\sigma I$), $(\sin\theta)/\lambda$ range 0 to 0.529 15, final R (unit weighting) 0.0357.

Tubes 135–145. Evaporation of the solvent and crystallisation of the solid from alcohol gave small cubes of 2-(2′-*hydroxy*-4′-*methoxy*-6′-*methoxycarbonylphenoxy*)-6-*hydroxy*-4-*methylbenzoic acid* (*dechlorodihydromaldoxin*) **3** (2.2 mg), mp 175–177 °C, subliming above 110 °C (Found C, 55.6; H, 4.9. $C_{17}H_{16}O_8 \cdot H_2O$ requires C, 55.7; H, 4.95%); *m/z* 348 (M^+); ν_{max}(KBr)/cm^{-1} 1685 and 1632; λ_{max}(EtOH)/nm 213 and 248 (ε/dm^3 mol^{-1} cm^{-1} 41 300 and 10 200); $\delta_H(C_5D_5N)$ 2.05 (3 H, s, 4-H_3), 3.76 (3 H, s, 6′-CO_2*Me*), 3.74 (3 H, s, 4′-O*Me*), 6.58 (1 H, s, 3-H), 6.73 (1 H, s, 5-H), 7.15 (1 H, d, *J* 2.6, 3′-H), 7.26 (1 H, d, *J* 2.6, 5′-H) and 8.62 (1 H, s, 6-OH); $\delta_C(C_5D_5N)$ 21.85 (4-*Me*), 52.24 (6′-CO_2*Me*), 55.64 (4′-O*Me*), 105.52 (C-3), 106.17 (C-1), 107.57 (C-3′), 108.36 (C-5), 111.95 (C-5), 126.82 (C-6′), 137.58 (C-1′), 144.82 (C-2′), 153.76 (C-4), 157.76 (C-4′), 160.31 (C-2), 163.58 (C-6), 166.34 (6′-*C*O_2Me) and 174.47 (1-*C*O_2H).

When the fresh isolate was initially used and the above procedure followed, compounds **2** and **3** were absent. The yield of dihydromaldoxin from 4 dm^3 of culture fluid was 14.3 mg.

Maldoxin acetate

A solution of maldoxin (50 mg) in acetic anhydride (3 cm^3) and pyridine (2 drops) was set aside 7 days at room temperature. The yellow solution was poured into water (25 cm^3) and the mixture set aside at 5 °C overnight. The yellow solid was filtered and recrystallised from aqueous ethanol to yield *maldoxin acetate* as yellow plates, mp 97 °C (Found: C, 53.7; H, 3.8; Cl, 8.2. $C_{19}H_{15}ClO_9$ requires C, 54.0; H, 3.6; Cl, 8.3%); *m/z* 422 (M^+); ν_{max}(KBr)/cm^{-1} 1760, 1728 and 1680; $\delta_H(CDCl_3)$ 7.62 (1 H, s, 3′-H), 6.65 (1 H, s, 6-H), 6.57 (1 H, s, 8 H), 4.17 (3 H, s, 4′-O*Me*), 3.84 (3 H, s, 2′-CO_2*Me*), 2.38 (3 H, s, 5-O*Me*) and 2.33 (3 H, s, 7-H_3). *Maldoxone acetate* was similarly prepared over 48 h and crystallised from toluene as needles, mp 210–213 °C; *m/z* 406 (M^+); ν_{max}(KBr)/cm^{-1}, 1756, 1733 and 1730; $\delta_H(C_5H_5N)$ 7.57 (1 H, d, *J* 0.7, 7-H), 7.29 (1 H, s, 4-H), 6.96 (1 H, dd, *J* 0.7 and 1.5, 2-H), 3.93 (3 H, s, 6′-CO_2*Me*), 3.76 (3 H, s, 8-O*Me*), 2.29 (3 H, s, OAc) and 2.23 (3 H, s, 3-H_3). Similarly *dihydromaldoxin* gave the *diacetate* as rhombs from toluene, mp 172–176 °C; *m/z* 464 (M^+); ν_{max}(KBr/cm^{-1}) 1776 and 1754; $\delta_H(C_5H_5N)$ 2.10 (3 H, s, 4-H_3), 2.33 (OAc), 3.69 (3 H, s, 6′-CO_2*Me*), 3.72 (3 H, s, 4′-O*Me*), 6.85 (1 H, s, 3-H), 6.85 (1 H, s, 5-H) and 7.58 (1 H, s, 5′-H); $\delta_C(C_5H_5N)$ 20.37 (OAc), 21.13 (4-H_3), 52.54 (6′-CO_2*Me*), 56.51 (4′-O*Me*), 110.80 (C-5′), 113.36 (C-5), 117.54 (C-1′), 118.38 (C-3), 124.80 (C-3′), 124.13 (C-6′), 141.36 (C-1′), 143.36 (C-4), 153.16 (C-2′), 157.07 (C-4′), 164.69 (C-2), 167.11 (C-6), 164.69 (6′-*C*O_2Me), 167.11 (OAc), 167.97 (OAc) and 169.42 (1-*C*O_2H).

Methylation of maldoxin

Maldoxin (30 mg) was treated with an ethereal solution of diazomethane. After 12 h the ether was evaporated to yield *methyl* 6-*chloro*-3′,3a′,4′,5′-*tetrahydro*-5-*hydroxy*-7′-*methoxy*-7-*methyl*-4,5′-*dioxospiro*[4H-1,3-*benzodioxine*-2,4′-2′H-*indazole*]-3a′-*carboxylate* **9** as pale yellow rhombs from ethanol (28 mg), mp 232–235 °C (Found: C, 52.3; H, 4.0; N, 6.3; Cl, 8.0. $C_{19}H_{17}N_2O_8Cl$ requires C, 52.1; H, 3.8; N, 6.4; Cl, 8.1%); *m/z* 437 (M^+); ν_{max}(KBr)/cm^{-1} 3371, 1760, 1734 and 1677; $\delta_H(CDCl_3)$ 6.77 (1 H, d, *J* 3.30, NH), 6.48 (1 H, s, 6-H), 6.43 (1 H, s, 8-H), 4.43 (1 H, d, *J* 10.3, 2′-CH_2), 4.28 (3 H, s, 5-O*Me*), 4.23 (1 H, dd, *J* 3.3 and 10.3, 2′-CH_2), 3.9 (3 H, s, 2′-CO_2*Me*), 3.82 (3 H, s, 4′-O*Me*) and 2.36 (3 H, s, 7-H_3); $\delta_C(CDCl_3)$ 188.4 (C-6′), 166.13 (C-4′), 161.32 (2′-*C*O_2Me), 158.68 (C-5), 158.52 (C-4), 154.92 (C-5), 149.59 (C-9), 135.37 (C-3), 112.76 (C-7), 108.76 (C-6), 107.08 (C-8), 99.47 (C-10), 98.75 (C-1′), 65.88 (C-2), 61.72 (5-O*Me*), 56.35 (2′-CO_2*Me*), 54.00 (4′-O*Me*), 50.74 (2-CH_2) and 22.36 (7-H_3).

Catalytic hydrogenation of maldoxin

A solution of maldoxin (105 mg) in absolute ethanol was hydrogenated at room temperature and pressure in the presence of pre-reduced palladium–charcoal catalyst (5%, 300 mg) until adsorption of hydrogen was complete. The catalyst was filtered off, the filtrate evaporated and the residue (82 mg) crystallised from ethanol to yield colourless rhombs of *dihydromaldoxin* (45 mg), mp 199 °C (Found: C, 53.4; H, 3.9; Cl, 9.3. $C_{17}H_{15}O_8Cl$ requires C, 53.3; H, 3.95; Cl, 9.4%). Identified as identical with **1** by comparison of their UV, IR and ^1H and ^{13}C NMR spectra.

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