Metabolites of the higher fungi. Part 31.[1] Longianone, a $C_7H_6O_4$ spiro bicyclic lactone from the fungus *Xylaria longiana* (Rehm.)

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Received (in Cambridge) 12th November 1998, Accepted 21st January 1999

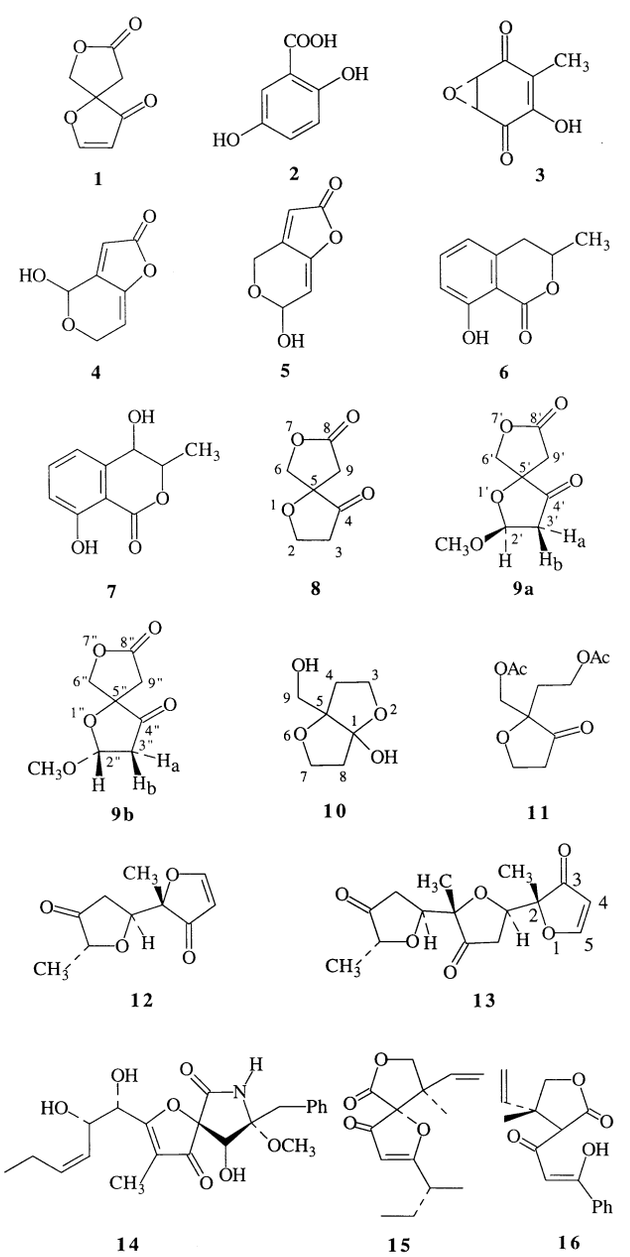
Longianone **1** from *Xylaria longiana* is identified as 1,7-dioxaspiro[4,4]non-2-ene-4,8-dione by chemical and physical methods. The possible biosynthetic origin is discussed.

Fungal metabolites of the simple formula $C_7H_6O_4$ are restricted to simple aromatic compounds like gentisic acid[2] **2**, quinones such as terreic acid[3] **3**, and the two lactones patulin[4] **4** and isopatulin[5] **5**. Patulin has been much studied because of its widespread occurrence, its toxicity and its intriguing chemistry and biosynthetic origin. Within the confines of the formula the scope for further structural variation would seem to be very limited, however, we now report a new $C_7H_6O_4$ metabolite **1** of unusual structure which is produced in static culture by the fungus *Xylaria longiana* and which we name longianone.

Xylaria longiana is a widely distributed but uncommon member of the fungus genus Xylaria, it occurs in tropical and temperate locations and has been reported on a number of hosts such as *Fagus silvaticus* (Switzerland)[6] and on wood in *Pinus-Quercus* forest (Mexico)[7] and leaves of *Nicotiana* (Georgia, USA).[6] Type material is from *Quercus* collected in Texas, USA[8] and for this work the fungus was collected from wood in Tennessee, USA. Static surface culture on 3% malt extract in Thompson bottles (2 dm^3), each containing 1 dm^3 of medium, for a period of 8 weeks at 24 °C in subdued daylight gave a hard grey–white mottled mycelium supporting copious unbranched stromata *ca*. 2 cm long. The mycelium was separated from the pale brown culture medium by filtration through muslin and air dried. A second isolate gave an off-white leathery mycelium with a light brown gelatinous underside; no stromata were observed in this case. Solvent extraction of the culture medium of both isolates gave longianone **1** and the known metabolites (*R*)-(−)-mellein **6** and 4-hydroxymellein **7**; these were separated by chromatography. The latter **7** was obtained as an optically inactive mixture of two diastereoisomers. Although the mixture was not separated, it is clear from the ^1H and ^{13}C NMR spectra that it consists of an unequal mixture of a *trans* and a *cis* isomer. Repeated subculturing caused a marked decrease in yield of **1,** but this was reversed by the addition of extra glucose (6%) to the culture medium.

Longianone **1** [$C_7H_6O_4$, mp 150 °C, *m*/*z* 154, $[\alpha]_D^{20}$ −85 (*c* 1.0 in EtOH); ν_{max}(KBr)/cm^{-1} 1787 and 1709; λ_{max}(EtOH)/nm 258 and 363 (ε/dm^3 mol^{-1} cm^{-1} 6016 and 678)], is soluble in polar solvents and insoluble in Et_2O and light petroleum. The compound is insoluble in aqueous sodium hydrogen carbonate but dissolves and yields a cherry red colouration in aqueous sodium hydroxide. The compound reduces Fehlings solution and yields an orange colouration on SiO_2 (TLC) with 2,4-dinitrophenylhydrazine solution.

The seven resonances in the ^{13}C NMR spectrum comprise



methylene carbons at δ 74.0 and 37.68, methine carbons at δ 177.67 and 106.88, and quaternary carbons at δ 199.32, 172.35 and 89.40. In the ^{1}H NMR spectrum doublets at δ 8.32 and 5.83 (J 2.6 Hz) are coupled olefinic methine protons, the former flanked by oxygen and the latter by a carbonyl group. The two single proton doublets at δ 3.04 and 2.72 (J 18.3 Hz) represent the non-equivalent geminal protons of the methylene group adjacent to the lactone carbonyl and the two-proton singlet at δ 4.41 represents the methylene group adjacent to the lactone oxygen. Significantly when the ^{1}H NMR spectrum is determined in acetone-d_6 the latter methylene occurs as two single proton doublets at δ 4.48 and 4.36 (J 10.6 Hz).

Catalytic reduction of **1** over Pd/C at room temperature and atmospheric pressure resulted in the absorption of one equivalent of hydrogen and the formation of **8**, $C_7H_8O_4$. In this compound the position of the lactone ν_{max} is unchanged, but the carbonyl absorption now at 1760 cm^{-1} is consistent with the formation of a saturated cyclic five-membered ring ketone. In the ^{1}H NMR spectrum of **8** the two olefinic methine protons at δ 8.32 and 5.83 in the spectrum of **1** are replaced by two new methylene signals at δ 4.22 and 2.62. In the ^{13}C–^{1}H COSY spectrum these correlate to signals at δ 63.30 and 35.94 respectively, resulting from the methine carbons originally present at δ 177.67 and 106.66. The reduction of the enone system is confirmed by the UV absorptions now at λ 206 and 301 nm. Since the two methylene groups of the lactone are isolated they must occupy the α and γ positions of the butyrolactone, leaving the β position quaternary and joined in a spiro arrangement with a 2*H*-furan-3-one system. The high ν_{max} at 1787 cm^{-1} in the parent and reduced compound indicates a strained lactone system rather than the β,γ unsaturated system seen in angelica lactone[9] at 1800 cm^{-1} or exocyclic system as in patulin[10] at 1774 cm^{-1}. The ^{1}H NMR olefinic proton resonance positions of several 2*H*-furan-3-ones[11] compare favourably with those of longianone. Significantly, 2*H*-furan-3-ones are also reported to reduce Fehlings solution.[12]

Although longianone can be crystallised from methanol, if the crystals remain in contact with the solvent, they slowly redissolve and addition of solvent occurs across the double bond to yield a mixture of **9a** and **9b**. The ^{13}C NMR spectrum of the gummy product shows 23 carbon signals, 16 of which appear in eight pairs. The remaining very low intensity signals correspond to unchanged **1**. Although **1** and (**9a** and **9b**) can be distinguished and separated by TLC, a dynamic equilibrium occurs between the two, and a small quantity of **1** is always reformed in the separated product. The eight pairs of signals indicate the presence of two isomers, which are present in unequal quantities. The loss of the original unsaturated methine carbon signals at δ 177.68 and 106.85 and the appearance of a methine carbon at δ 101.55/101.47, a methylene carbon at δ 42.85/42.79 and a methyl carbon adjacent to oxygen at δ 55.60/55.75 supports structures **9a** and **9b**. In the ^{1}H NMR spectrum the broad cluster of signals between δ 2.54 and 3.04 comprise five doublets of doublets, each doublet representing a single proton constituting the new 3′/3″-CH_2, the 9′/9″-CH_2 and the unchanged longianone 9-CH_2. The olefinic methine protons in longianone have been replaced by a methine proton adjacent to oxygen at δ 5.40/5.38 (2′-H and 2″-H) and two pairs of methylene protons adjacent to carbonyl at δ 2.88 (3′-H_a) and 2.87 (3″-H_a) and 2.55 (3′-H_b) and 2.54 (3″-H_b). The lactone methylene protons adjacent to oxygen in the residual longianone in the mixture appear as doublets in chloroform compared with singlets in the spectrum of longianone. Surprisingly the UV long wavelength absorption of the methanol addition product is in a similar position to that of longianone (363 nm, ε/dm^3 mol^{-1} cm^{-1} 678), but with much increased intensity (365 nm, ε/dm^3 mol^{-1} cm^{-1} 2980). This is to be compared with the reduced product which shows an absorption at 301 nm (ε/dm^3 mol^{-1} cm^{-1} 586). When **9a** and **9b** are placed under vacuum at 40 °C sublimation occurs and longianone is reformed. A similar

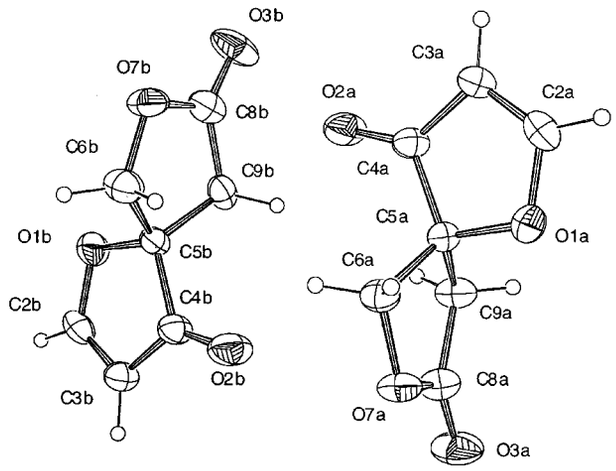


Fig. 1 The structure of longianone **1** showing the two molecules of the crystallographic asymmetric unit.

Michael addition reaction takes place with ethanol, two new methylene proton signals appear at δ 3.81 and 3.60 and two new methyl signals appear at δ 2.24 and 2.20 corresponding to the added ethoxy group in the two new isomers.

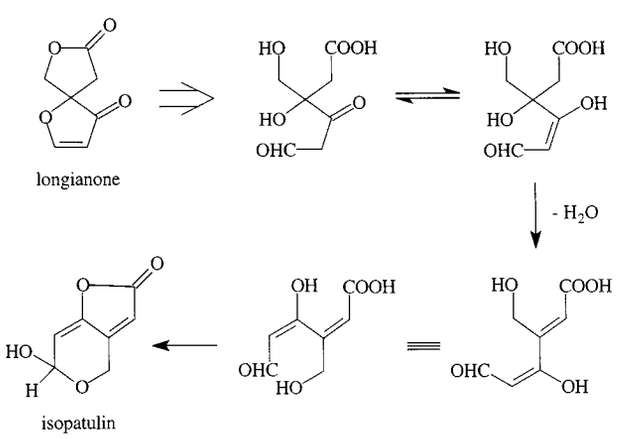
Reduction of **1** with $LiAlH_4$ gave a gummy product **10**, $C_7H_{12}O_4$, resulting from reductive fission of the lactone ring, conjugate addition of hydrogen at the double bond and hemiketal formation between the intermediate ketone and the propan-1-ol lactone derived residue. The protons on the methylene carbons adjacent to oxygen occur in the ^{1}H NMR spectrum as a six-proton cluster between δ 4.1 and 3.6. Within the cluster it is possible to identify a pair of doublets at δ 3.77 and 3.69 (J 11.9 Hz) which represents a pair of non-equivalent geminal methylene protons. A multiplet centred at δ 3.85 represents one proton of a methylene group adjacent to oxygen, the other proton signal occurs in a three-proton cluster of signals centred at δ 4.02. The splitting pattern of these two methylene groups adjacent to oxygen confirms that they are each adjacent to other methylene protons, which appear as a four-proton cluster between δ 2.2 and 2.0. The ^{13}C–^{1}H FLOCK spectrum exhibits a two bond correlation between the methylene group at δ 2.02 (δ_C 34.72) and the methylene group at δ_C 67.69 and confirms these two are adjacent and by deduction that those at δ_C 39.21 and 66.18 are also adjacent. The assignments for C-3 and C-4 are interchangeable with those for C-7 and C-8 respectively. There is also a link between the methylene group at δ 2.20 (δ_C 39.21) and the quaternary carbon at δ_C 113.79, however, this latter correlation provides no additional information regarding the structural position of the adjacent methylene carbon assignments. The exact structural positions were finally determined by reduction with $LiAlD_4$. The methylene protons at C-3 become CD_2 and the methylene protons at C-7 become CHD. In the ^{13}C NMR there is only methylene adjacent to oxygen at δ 64.24 which represents the primary alcohol substituent at C-9. Treatment of **10** with acetic anhydride and pyridine yields the diacetate **11**; this results in the appearance of a quaternary ketone carbon at δ 214.65 and two quaternary ester carbons at δ 170.44 and 170.17. This decomposition of the hemiacetal occurs spontaneously overnight in pyridine and can be followed by the appearance of the ketone carbon at δ 214.65.

The structure of longianone was finally confirmed by a single crystal X-ray determination (Fig. 1).

Significantly, although the 2*H*-furan-3-one nucleus is quite a common component of a number of natural product structures, only in chilenone A and B, **12** and **13** respectively, from the red marine alga *Laurencia chilensis*[13,14] do the 4,5 positions occur unsubstituted. These compounds are dimers and trimers of 2-methyl-2*H*-furan-3-one, which has not been detected naturally. Spirocyclics involving the nucleus are also uncommon

and can be found only in pseurotin A **14** and its analogues B, C, D and E from cultures of *Pseudeurotium Ovalis*[15–18] and, more pertinently, in hyperolactone A **15** and analogues B and C from the stems and leaves of *Hypericum chinensis*.[19] In the latter compounds there is a structural similarity to longianone but they have a substituent on the 5-position of the furanone ring, a substituent on the lactone ring and the spiro fusion position of the lactone ring is different. Surprisingly, catalytic hydrogenation is reported to result in the saturation of the exocyclic double bond, leaving the *endo* double bond unchanged. $LiAlH_4$ reduction results in the formation of a hemiacetal, similar apart from the substituents to that from longianone, however, acetylation of this is reported to yield a diacetate of the hemiketal; this must be a mistake since the reported ^{13}C NMR spectrum includes a ketone carbonyl carbon. The hyperolactones were isolated by methanol extraction of the plant stems and there is no report of adduct formation with the solvent.

It has been suggested that the hyperolactones might be biosynthesised by condensation of a triketide with isopentenyl pyrophosphate, the latter contributing a carbon of the lactone ring. This suggestion is supported by the occurrence of the non-spiro hyperolactone D **16** in *Hypericum chinensis*.[20] Such a mechanism is unlikely in the case of longianone and simple inspection of the system provides no clue to the positioning of the polyketide starter unit, the point of entry of a carbon into a possible triketide chain, or the point of loss of a carbon from a tetraketide system. However a hypothetically possible non-cyclic precursor to longianone is very similar to the hypothetical precursors postulated in the biosynthesis of patulin and isopatulin, two structures that have been shown to originate from *m*-cresol and 6-methylsalicyclic acid *via* gentisic alcohol.[21] (Scheme 1).



Scheme 1

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, and mass spectra (EI) and (FAB using 3-nitrobenzyl alcohol as matrix) on an AEI MS 902 spectrometer equipped with a MSS Data System for Windows (Data Version 2.03, Software Version 10.0). Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Extracts were dried over Na_2SO_4. All chromatography columns, thick layer (PLC) and thin layer (TLC) glass plates were made up using Merck Kieselgel GF_{254}. Column sizes and solvent systems used are specified in each case.

^1H and ^{13}C NMR spectra, using tetramethylsilane as internal standard, were determined at 270 and 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 32K 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 Hz 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 using the FLOCK pulse sequence of Reynolds *et al.*,[22] resolution $f2$ 17.19 Hz and $f1$ 5.9 Hz, pulse delay 1, 2 or 3 s, Δ^1 86.5 ms and Δ^2 46.5 ms, or Δ^1 44.0 ms and Δ^2 24.0 ms.

X-Ray structure determination of 1

Crystal data. $C_7H_6O_4$, $M = 154.12$, monoclinic, $a = 6.3316(13)$, $b = 17.483(4)$, $c = 6.3606(13)$ Å, $\beta = 97.70(3)°$, $V = 697.7(2)$ Å^3 (from least-squares refinement of 24 centred reflections with $7.6 \leq \theta \leq 13.0°$, $\lambda = 0.71073$ Å, $T = 293(2)$ K, space group $P2_1$, $Z = 4$, $D_x = 1.467$ g cm^{-3}, colourless block $0.12 \times 0.08 \times 0.06$ mm^3, μ(Mo-Kα) = 0.123 mm^{-1}.

Data collection and processing. Stoe STADI-4 diffractometer, graphite-monochromated Mo-Kα radiation, ω–2θ scans; 2009 reflections collected ($2.33 \leq \theta \leq 22.46°$, $-6 \leq h \leq 6$, $-18 \leq k \leq 18$, $-6 \leq l \leq 6$), all 1801 unique reflections ($R_{int} = 0.0304$) used in calculations. Correction for linear isotropic crystal decay (6.2%) applied. No absorption correction applied.

Structure solution and refinement. Structure solved by direct methods[23] and remaining atoms [excluding H atoms attached to C(9a)] located from ΔF^2 synthesis. Full-matrix refinement[24] on F^2 with all non-H atoms anisotropic; H atoms positionally refined with C–H distances for methyl and methine hydrogens restrained to be equal; hydrogens on C(9a) were included in idealised positions with $U_{iso}(H) = 1.2U_{iso}(C)$. Weighting scheme $w^{-1} = [\sigma^2(F_o^2) + (0.0461P)^2]$, $P = (F_o^2 + 2F_c^2)/3$. Refinement converged at $R_1[F \geq 4\sigma(F)] = 0.0381$, wR_2[all data] = 0.0870, $S[F^2] = 0.987$ for 239 parameters and 13 restraints. The final ΔF^2 map showed maximum features of +0.129 and −0.143 e Å^{-3}.

Full crystallographic details, excluding structure factor tables, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 1*, available *via* the RSC web page (http://www.rsc.org/authors). Any request to the CCDC for this material should quote the full literature citation and the reference number 207/299.

Metabolites from the culture medium

The culture medium (4 dm^3) was extracted with ethyl acetate (1 dm^3 × 3). Evaporation of the dried solvent yielded a light brown gum (1.3 g) containing three components which were detected by the orange–yellow colourations (TLC) produced with diazotised *p*-nitroaniline spray reagent. A solution of the gum (1.3 g) in ethyl acetate (10 ml) was pre-absorbed on silica gel and applied to a column of silica gel (40 × 2 cm). The column was eluted with the solvent system toluene–ethyl acetate–acetic acid (50 : 49 : 1) and the eluent collected in 5 cm^3 fractions.

Tubes 1–10. Evaporation of the solvent gave a pale yellow oil, which crystallised from light petroleum (bp 80–100 °C) to yield (*R*)(−)-mellein **6** as colourless plates (22 mg), mp 57 °C (lit.,[25] 56 °C); *m/z* 178 (M^+); $[\alpha]_D^{20}$ −96 (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 1676; λ_{max}(EtOH)/nm 246 and 314 (ε/dm^3 mol^{-1} cm^{-1} 6000

Table 1 Crystal data and structure refinement for longianone **1**

Identification code	ray 3a
Empirical formula	$C_7H_6O_4$
Formula weight	154.12
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	*P*2(1)
Unit cell dimensions	
a	6.3316(13) Å
b	17.483(4) Å
c	6.3606(13) Å
α	90°
β	97.70(3)°
γ	90°
Volume	697.7(2) Å^3
Z	4
Density (calculated)	1.467 Mg m^{-3}
Absorption coefficient	0.123 mm^{-1}
F(000)	320
Crystal size	0.1200 × 0.0800 × 0.0600 mm^3
Theta range for data collection	2.33 to 22.46°
Index ranges	$-6 \leqslant h \leqslant 6$, $-18 \leqslant k \leqslant 18$, $-6 \leqslant l \leqslant 6$
Reflections collected	2009
Independent reflections	1801 [*R*(int) = 0.0304]
Completeness to $\theta = 22.46°$	100.0%
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1801/13/239
Goodness-of-fit on F^2	0.987
Final *R* indices [$I > 2\sigma(I)$]	*R*1 = 0.0381, *wR*2 = 0.0773
R indices (all data)	*R*1 = 0.0789, *wR*2 = 0.0870
Largest diff. peak and hole	0.129 and −0.143 e Å^{-3}

and 4000); $\delta_H(CDCl_3)$ 11.03 (1 H, s, 12-H), 7.41 (1 H, t, *J* 7.7, 6-H), 6.89 (1 H, d, *J* 7.9, 7-H), 6.60 (1 H, d, *J* 7.9, 5-H), 4.75 (1 H, m, 3-H), 2.93 (2 H, d, *J* 7.3, 4-H_2) and 1.53 (3 H, d, *J* 6.2, 11-H_3); $\delta_C(CDCl_3)$ 169.96 (C-1), 162.15 (C-8), 139.39 (C-10), 136.15 (C-6), 117.91 (C-5), 116.20 (C-7), 108.26 (C-9), 76.10 (C-3), 34.58 (C-4) and 20.75 (C-11).

Tubes 14–24. Evaporation of the solvent afforded a pale yellow oil, which crystallised from light petroleum (bp 80–100 °C) to yield 4-hydroxymellein **7** as colourless needles (10 mg), occurring as a mixture of two diastereoisomers, mp 121–123 °C (lit.,[26] 131–132 °C); *m*/*z* 194 (M^+); $[\alpha]_D^{20}$ 0 (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 1680; λ_{max}(EtOH)/nm 248 and 317 (ε/dm^3 mol^{-1} cm^{-1} 5300 and 4200); $\delta_H(CDCl_3)$ (* identifies one isomer) 11.01* and 10.99 (2 H, s, 2 × 13-H), 7.55 and 7.53* (2 H, t, *J* 7.7, 2 × 6-H), 7.01 and 6.95* (2 H, m, 2 × 5-H), 7.01 and 6.95* (2 H, m, 2 × 7-H), 4.70* and 4.61 (2 H, m, 2 × 3-H), 4.70* and 4.61 (2 H, m, 2 × 4-H), 1.60* and 1.52 (6 H, d, *J* 6.0, 2 × 11-H_3); $\delta_C(CDCl_3)$ 169.32 and 169.24 (C-1), 162.02 and 161.94 (C-8), 141.27 and 140.54 (C-10), 136.90 and 136.83 (C-6), 118.48 and 118.38 (C-5), 117.75 and 116.36 (C-7), 106.85 and 106.66 (C-9), 80.02 and 78.30 (C-3), 69.06 and 67.19 (C-4), 17.92 and 16.02 (C-11).

Tubes 39–59. Evaporation of the solvent afforded *longianone* (*1,7-dioxaspiro[4,4]non-2-ene-4,8-dione*) **1** as a yellow powdery solid, which was crystallised from ethanol to yield colourless needles (52 mg), mp 150 °C (Found: C, 54.6; H, 3.7. $C_7H_6O_4$ requires C, 54.6; H, 3.9%); *m*/*z* 154, $[\alpha]_D^{20}$ −85 (*c* 1.0 in EtOH); ν_{max}(KBr)/cm^{-1} 1787 and 1709; λ_{max}(EtOH)/nm 258 and 363 (ε/dm^3 mol^{-1} cm^{-1} 6016 and 678); $\delta_H(CDCl_3)$ 8.32 (1 H, d, *J* 2.6, 2-H), 5.83 (1 H, d, *J* 2.6, 3-H), 4.41 (2 H, s, 6-H_2), 3.04 (1 H, d, *J* 18.3, 9-H_a) and 2.72 (1 H, d, *J* 18.3, 9-H_b); $\delta_C(CDCl_3)$ 199.32 (C-4), 177.67 (C-2), 172.35 (C-8), 106.88 (C-3), 89.40 (C-5), 74.00 (C-6) and 37.68 (C-9).

A second strain of *Xylaria longiana*, when cultured as above, produced an off-white leathery mycelium with a light brown gelatinous underside, no fruiting bodies were observed. The medium (32 dm^3) was extracted in 3 dm^3 fractions with ethyl acetate (1 dm^3 × 3). Evaporation of the dried solvent yielded a dark brown semi-crystalline gummy solid (12.8 g), which was triturated with ethyl acetate and set aside overnight. Filtration afforded longianone **1** as a yellow powdery solid (4.6 g), which was crystallised as described above. Repeated sub-culturing of both these strains resulted in a substantial reduction in the yield of all the metabolites.

Addition of glucose (6%) to the growth medium resulted in restoration and increased yield of longianone (320 mg dm^{-3}).

Catalytic hydrogenation of longianone

A solution of longianone **1** (50 mg) in ethyl acetate (15 cm^3) was hydrogenated at room temperature and pressure (20 °C, 697 mmHg) in the presence of a pre-reduced Pd/C (10%) catalyst (25 mg) until absorption of hydrogen was complete. The catalyst was filtered off, the filtrate evaporated and the residue (39 mg) crystallised from ethyl acetate to yield *dihydrolongianone* (*1,7-dioxaspiro[4,4]nonane-4,8-dione*) **8** as colourless cubes (30 mg), mp 98 °C (Found: C, 51.1; H, 5.2. $C_7H_8O_4$ requires C, 50.8; H, 5.2%); *m*/*z* 156 (M^+); $[\alpha]_D^{20}$ −63 (*c* 1.0 in $CHCl_3$); ν_{max}(KBr)/cm^{-1} 1787 and 1760; λ_{max}(EtOH)/nm 206 and 301 (ε/dm^3 mol^{-1} cm^{-1} 5888 and 586); $\delta_H(CDCl_3)$ 4.33 (2 H, dd, *J* 10.0 and 2.2, 6-H_2), 4.22 (2 H, m, *J* 7.3, 2-H_2), 2.80 (1 H, d, *J* 17.0, 9-H_a), 2.63 (1 H, d, *J* 17.0, 9-H_b) and 2.62 (2 H, m, *J* 7.3, 3-H_2); $\delta_C(CDCl_3)$ 211.98 (C-4), 173.14 (C-8), 84.36 (C-5), 74.25 (C-6), 63.30 (C-2), 37.71 (C-9) and 35.94 (C-3).

Methanol addition product

A warm concentrated solution of longianone **1** in methanol was set aside at room temperature. After 1 h fine colourless needles of longianone were deposited. The mixture was set aside until the crystals had redissolved (4 days) and the solvent evaporated. The resulting gum was separated into its components by PLC in the solvent system toluene–ethyl acetate–glacial acetic acid (50 : 49 : 1). The least mobile component yielded unchanged longianone. The more mobile component comprised a semi-crystalline gummy solid which TLC indicated was still a mixture of a new product and unchanged longianone. This separation procedure was repeated several times but the more mobile component could not be isolated alone, the product always existing as a mixture of longianone and an inseparable mixture of the two stereoisomers of *2-methoxy-1,7-dioxaspiro[4,4]-nonane-4,8-dione* **9a** and **9b**, $C_8H_{10}O_5$; *m*/*z* 154 (M^+) and 186.05310 (M^+), $C_8H_{10}O_5$ requires 186.05283, (FAB) *m*/*z* $(M + Na)^+$ 177 and 209; $\nu_{max}(CHCl_3)$/cm^{-1} 1790, 1760 and 1715; λ_{max}(EtOH)/nm 237 and 365 (ε/dm^3 mol^{-1} cm^{-1} 4120 and 2980); $\delta_H(CDCl_3)$ 8.41 (1 H, d, *J* 2.6, 2-H), 5.83 (1 H, d, *J* 2.6, 3-H), 5.40 and 5.38 (2 H, d, *J* 3.0, 2′-H and 2″-H), 4.42 and 4.34 (4 H, s, 6′-H_2 and 6″-H_2), 4.38 (1 H, d, *J* 10.3, 6-H_a), 4.27 (1 H, d, *J* 10.3, 6-H_b), 3.49 and 3.47 (6 H, s, 10′-H_3 and 10″-H_3), 3.04 (1 H, d, *J* 18.0, 9-H_a), 2.91 and 2.86 (2 H, d, *J* 18.0, 9′-H_a and 9″-H_a), 2.88 and 2.87 (2 H, d, *J* 18.7, 3′-H_a and 3″-H_a), 2.72 (1 H, d, *J* 18.0, 9-H_b), 2.67 and 2.65 (2 H, d, *J* 18.0, 9′-H_b and 9″-H_b), 2.55 and 2.54 (2 H, d, *J* 18.7, 3′-H_b and 3″-H_b); $\delta_C(CDCl_3)$ 210.41 and 210.38 (C-4′ and C-4″), 199.31 (C-4), 177.68 (C-2), 173.40 and 173.14 (C-8′ and C-8″), 172.37 (C-8), 106.85 (C-3), 101.55 and 101.47 (C-2′ and C-2″), 89.37 (C-5), 83.61 and 83.42 (C-5′ and C-5″), 77.22 and 74.93 (C-6′ and C-6″), 73.98 (C-6), 55.60 and 55.57 (C-10′ and C-10″), 42.85 and 42.79 (C-3′ and C-3″), 40.37 and 38.33 (C-9′ and C-9″) and 37.66 (C-9). On vacuum drying overnight (0.2 mm 40 °C) this mixture gave a sublimate of longianone **1** as fine colourless needles. There was no residue.

1-Hydroxy-5-hydroxymethyl-2,6-dioxabicyclo[3.3.0]octane 10

A solution of longianone (100 mg) in dry THF (7.5 cm^3) was added over 5 min to a mixture of $LiAlH_4$ (104 mg) in dry THF

(15 cm^3). The mixture was stirred for 1 h at 0 °C and then ethyl acetate (5 cm^3) was added followed by sulfuric acid (5 cm^3, 1 M) at 0 °C. The colourless solution was extracted with ethyl acetate (×3) and the extract washed, dried and evaporated to yield *1-hydroxy-5-hydroxymethyl-2,6-dioxabicyclo[3.3.0]octane* **10** as a pale yellow gum (48 mg), $C_7H_{12}O_4$; *m/z* 142.06255 ($M^+ - 18$), $C_7H_{10}O_3$ requires 142.06299; $\nu_{max}(CDCl_3)/cm^{-1}$ 3600–3200 (br); $\delta_H(CDCl_3)$ 4.02 (2 H, m, 3-H_2), 4.02 (1 H, m, 7-H_a), 3.85 (1 H, m, 7-H_b), 3.77 (1 H, d, *J* 11.9, 9-H_a), 3.69 (1 H, d, *J* 11.9, 9-H_b), 2.20 (2 H, m, 8-H_2) and 2.02 (2 H, m, 4-H_2); $\delta_C(CDCl_3)$ 113.79 (C-1), 90.46 (C-5), 67.69 (C-8), 66.18 (C-7), 64.24 (C-9), 39.21 (C-8) and 34.72 (C-4).

2-Acetoxymethyl-2-(2-acetoxyethyl)tetrahydrofuran-3-one 11

A solution of 1-hydroxy-5-hydroxymethyl-2,6-dioxabicyclo-[3.3.0]octane (50 mg) in acetic anhydride (2 cm^3) and pyridine (2 drops) was set aside overnight at room temperature. The pale orange solution was poured into water (25 cm^3) and the mixture left at 5 °C overnight. The solution was extracted with ethyl acetate (×3) and the extract washed and dried to yield the *diacetate* **11** as a pale yellow gummy solid (30 mg), $C_{11}H_{16}O_6$; *m/z* 245 ($M^+ + 1$); $\nu_{max}(CDCl_3)/cm^{-1}$ 1740; $\delta_H(CDCl_3)$ 4.29 (2 H, m, 5-H_2), 4.29 (1 H, m, 10-H_a), 4.11 (2 H, s, 6-H_2), 4.11 (1 H, m, 10-H_b), 2.63 (2 H, m, 4-H_2), 2.07 (1 H, m, 9-H_a), 2.06 (3 H, s, 8-H_3 or 12-H_3), 2.00 (3 H, s, 8-H_3 or 12-H_3) and 1.86 (1 H, m, 9-H_b); $\delta_C(CDCl_3)$ 214.65 (C-3), 170.44 (C-7 or 11), 170.17 (C-7 or 11), 80.62 (C-2), 67.04 (C-6), 64.08 (C-5), 59.08 (C-10), 36.38 (C-4), 32.10 (C-9), 20.77 (C-8 or 12) and 20.52 (C-8 or 12).

Acknowledgements

We thank the EPSRC for a research studentship to C. L. Oliver and Pfizer Central Research PLC for providing additional financial support, Dr I. J. Scowen (University of Bradford) for helpful assistance with the X-ray diffraction data, and the Chemical Database Service[27] for access to SpecInfo and Beilstein-on-line. A culture of *Xylaria longiana* is retained at Bradford.

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