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Biosynthesis of longianone from *Xylaria longiana*: a metabolite with a biosynthetic relationship to patulin

Rebecca J. M. Goss, Jens Fuchser and David O'Hagan*

Department of Chemistry, Science Laboratories, South Road, Durham, UK DH1 3LE.
E-mail: david.o'hagan@durham.ac.uk

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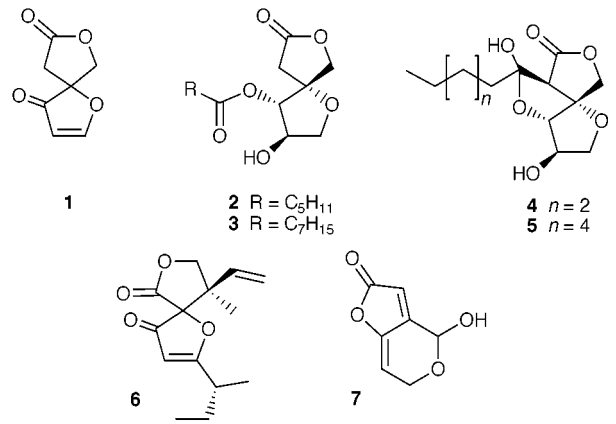
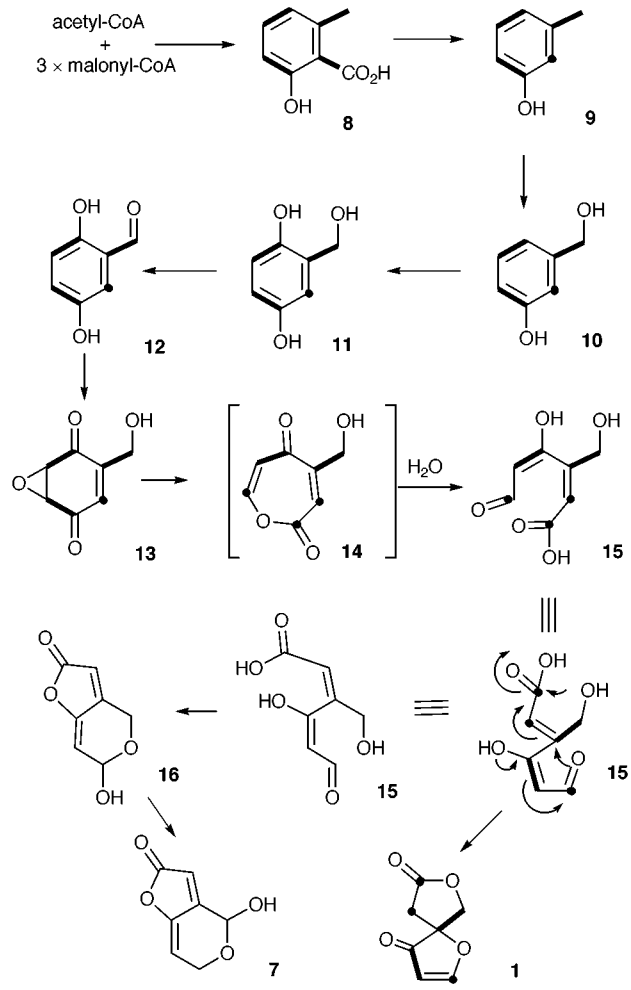
Longianone, a metabolite of *Xylaria longiana*, is an isomer of the well known fungal toxin, patulin; it is demonstrated that longianone is biosynthesised from 6-methylsalicylic acid in a pathway closely related to that found in patulin biosynthesis.

The isolation and structure of the metabolite longianone **1** was recently reported from *Xylaria longiana*.[1] Longianone **1** posesses an intriguing spiro-bicyclic ring structure (1,7-dioxaspiro[4,4]non-2-ene-4,8-dione). It is optically active, however the absolute configuration of the natural product is unknown at present. Structurally, longianone **1** represents a rare parent ring system. The only related bicyclic ring systems are the secosyrins **2** and **3** and their more elaborated co-metabolites, the syringolides **4** and **5**, metabolites of the bacterium *Pseudomonas syringia* pv. *tomato*.[2,3] A biosynthesis has been proposed[3] for the secoserins **4** and **5** which involves the combination of a polyketide chain with a pentose sugar moiety, however this is not based on experimental evidence. Another related ring system is found in the hyperlactones A–C **6** isolated from *Hypericum chinensis*.[4] However, in that case the ester functionality occupies a different site in the ring system and the hyperlactones are clearly terpenoid in origin and have no biosynthetic relationship to longianone **1** or the secosyrins. It is noteworthy that longianone **1** has a structural formula ($C_7H_6O_4$) which is isomeric with the fungal mycotoxin, patulin **7**. Patulin **7** has an intriguing biosynthetic origin derived from oxidative ring opening of the aromatic polyketide metabolite, 6-methylsalicylic acid (6-MSA) **8**[5–8] and the pathway to patulin biosynthesis is illustrated in Scheme 1. The interesting structure of longianone **1** and its constitutional relationship to patulin **7** stimulated us to initiate a biosynthetic investigation of **1** from *X. longiana*.



An initial experiment involved feeding [1,2-$^{13}C_2$]acetate to static cultures of *X. longiana*.[1] This resulted in a low (0.4%) but detectable incorporation of isotope into the resultant longianone **1**. ^{13}C NMR analysis revealed reciprocal couplings between C-3/C-4 ($J_{13C\text{-}13C}$ = 55 Hz) and C-5/C-6 ($J_{13C\text{-}13C}$ = 34 Hz) of **1**, indicating the incorporation of two intact acetate units. This was the first indication of a polyketide origin, and the labelling pattern was consistent with the intermediacy of 6-MSA **8** and a similarity to patulin biosynthesis, as illustrated in Scheme 1. In order to reinforce this hypothesis an experiment with [3,5-$^2H_2$]-6-MSA **8a** was conducted. A sample of ethyl 2-hydroxy-6-methylbenzoate was synthesised following an established route,[9] then deuterium was exchanged into the aromatic nucleus by refluxing with DCl, D_2O and MeOD for 24 h and the ester was hydrolysed to [3,5-$^2H_2$]-6-MSA **8a** by refluxing with NaOH. The subsequent feeding experiment resulted in a sample of longianone which was enriched with deuterium (^2H NMR analysis) at C-2 [Fig. 1(*b*)]. There was a minor enrichment at C-3 arising from some exchange into the aromatic precursor **8a** at C-4 during isotope exchange [Fig. 1(*b*)]. *m*-Cresol **9** and *m*-hydroxybenzyl alcohol **10** are established intermediates in patulin biosynthesis[7] and in order to assess if they have a role in longianone biosynthesis they were each prepared enriched with deuterium, and fed in separate experiments to *X. longiana* cultures. An experiment with [2,4,6-$^2H_3$]-*m*-cresol **9a**, prepared



Scheme 1

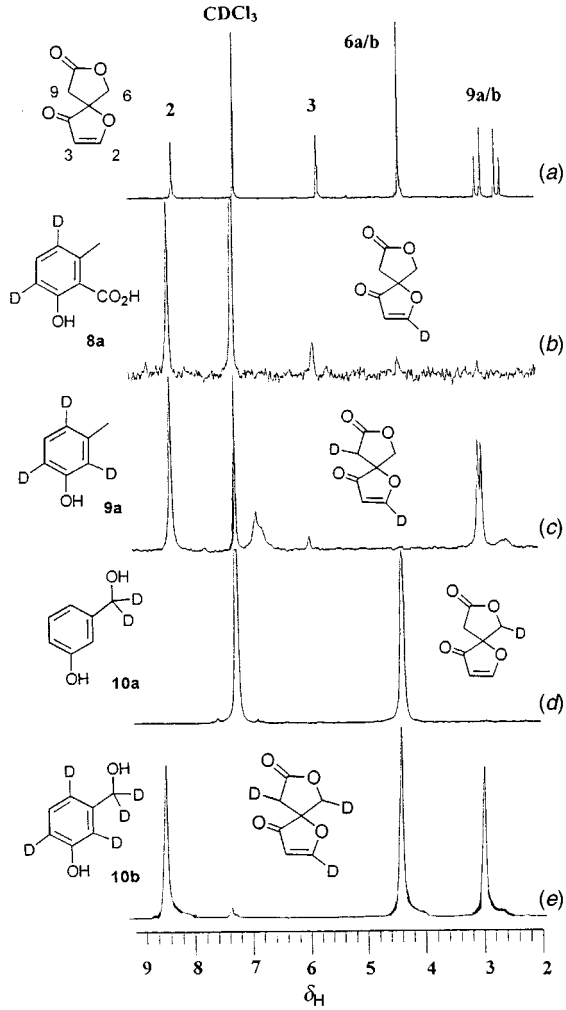
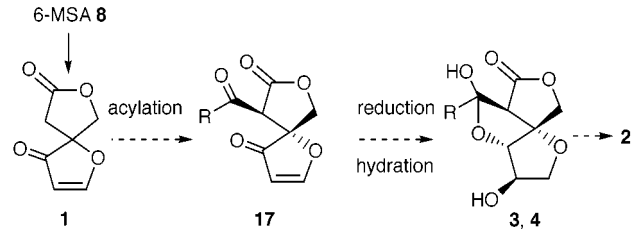


Fig. 1 (*a*) ^{1}H NMR ($CDCl_3$) spectrum and assignment of longianone **1**; (*b*) ^{2}H NMR ($CHCl_3$) spectrum of **1** after feeding **8a**; (*c*) ^{2}H NMR ($CHCl_3$) spectrum of **1** after feeding **9a**; (*d*) ^{2}H NMR ($CHCl_3$) spectrum of **1** after feeding **10a**; (*e*) ^{2}H NMR ($CHCl_3$) spectrum of **1** after feeding **10b**.

by reaction with PBr_3 and D_2O,[10] resulted in deuterium incorporation into **1** at C-2 and C-9 as determined by ^{2}H NMR [Fig. 1(*c*)]. The methylene hydrogens at C-9 are diastereotopic and well resolved by NMR, and the resulting ^{2}H NMR spectrum after feeding **9a** indicated a stereospecific enrichment into C-9, however upon storage (3 months) a slow racemisation was witnessed. Incorporation of 3-hydroxy[α,α-$^2H_2$]benzyl alcohol **10a**, prepared by reducing methyl 3-hydroxybenzoate with $LiAlD_4$, resulted in labelling at C-6 in **1** [Fig. 1(*d*)]. It was not clear from this latter experiment whether both deuterium atoms were processed through to the C-6 methylene group of longianone **1** as these deuterium atoms, which are formally diastereotopic, have nonetheless similar chemical shifts in the resultant ^{2}H and ^{1}H NMR spectra. In order to gain information on this issue a second experiment was carried out using **10b**, which was prepared with deuterium atoms located both at the hydroxymethyl group and at C-2, C-4 and C-6 of the aromatic ring. The resultant ^{2}H NMR spectrum [Fig. 1(*e*)] of longianone **1** showed three clear enrichments at C-2, C-6 and C-9, with *similar intensities*. We have drawn the conclusion therefore that only one deuterium atom from the methylene group of 3-hydroxybenzyl alcohol **10b** became incorporated into the C-6 methylene group of longianone and therefore the hydroxymethyl group is oxidised up to the aldehyde level during the biosynthesis.

The isotopic labelling experiments reveal a common biosynthetic pathway to both longianone **1** and patulin **2** which



Scheme 2

diverges at a late stage. Both metabolites are derived from 6-MSA **8** and have **9** and **10** as common biosynthetic intermediates. Also oxidation of the hydroxymethyl group to the aldehyde level is revealed as common to both cases and the aromatic ring is cleaved across the same bond. The conversion of gentisaldehyde **12** to phyllostine **13** is a biochemically characterised step[11] in patulin biosynthesis and the intermediacy of neopatulin (also termed isopatulin) has been established from mutant studies,[8] so reasonably the pathway progresses through **14**[12] with hydrolysis to **15**. Different *E/Z* isomers of **15** emerge as the likely branchpoint of the two pathways, with straightforward ester and intramolecular Michael-type cyclisations to generate both rings of longianone. An alternative cyclisation of **15** (ester and hemiacetal formations) generates neopatulin **16** which is a precursor to patulin **7** requiring an oxidation and a reduction. Perhaps longianone **1** is a shunt metabolite of an organism which has lost the capacity to complete patulin **7** biosynthesis.

In view of the above experimental evidence an alternative hypothesis to that recently proposed[2,3] emerges for the biosynthesis of the secosyrins **2** and **3** and the syringolides **4** and **5**. This would involve an origin from a longianone skeleton derived from 6-MSA, followed by acylation α to the lactone to generate intermediate **17**, as illustrated in Scheme 2. Elaborations such as reduction and hydration would generate **4** and **5** and a retro-aldol reaction would generate **2** and **3**. It remains to be determined whether this or the previous proposal[3] is the more valid for the biosynthesis of those metabolites.

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