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# Biomimetic Synthesis of the Microbial Elicitor Syringolide 2

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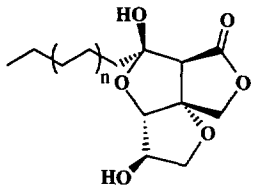
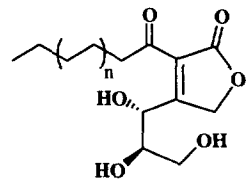
***Abstract***: Syringolide 2 (**1**; n=2), an elicitor metabolite of the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato*, has been synthesised in four steps from D-xylulose. The key stage involves triple cyclisation of the putative biosynthetic intermediate 1-(3'-oxodecanoyl)-D-xylulose (**9**), and provides evidence for the biosynthesis of the syringolides.


Syringolides 1 (**1**; n=1) and 2 (**1**; n=2)[1,2] are microbial elicitors, specific signal molecules produced by the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato*, which trigger a hypersensitive defense response in resistant cultivars of soybean plants. Their production by the bacterium reflects the expression of an avirulence gene, *avrD*, and their recognition by the plant requires the presence of a complementary resistance gene *Rpg4*.[3] Reported in 1993, they are the first non-proteinaceous specific elicitors of a plant hypersensitive response.




**1**; n=1,2

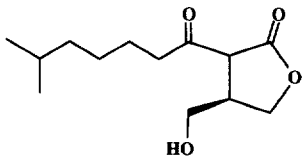



**2**; n=1,2

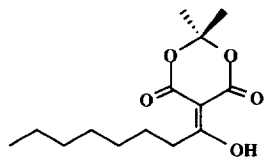
Midland *et al.*[2] proposed that the syringolides (**1**) were formed biosynthetically from the appropriate β-keto fatty acids and D-xylulose. Linkage of these two units was envisaged to occur either by esterification followed by intramolecular Knoevenagel condensation, or alternatively by intermolecular Knoevenagel condensation followed by lactonisation, to yield the butenolides (**2**). Intramolecular Michael addition of the primary hydroxyl group to the electron-deficient olefinic bond and hemiketal formation at the ketonic centre would then be thermodynamically favoured, affording the syringolides (**1**).

Of these two proposed processes leading to the butenolide intermediates (**2**), we favoured that involving esterification to the respective β-ketoacyl xylulose esters (*e.g.* **9**) followed by Knoevenagel condensation. This chemistry is directly parallel to that which we ourselves[4] and others[5] proposed for the biosynthesis of the

microbial autoregulator A-factor (**3**), and which has been established by biomimetic studies as extremely facile[4,6] and by labelling studies[5] as biogenetically plausible. Accordingly we have synthesised and examined the cyclisation behaviour of the 3'-oxodecanoate ester (**9**) of D-xylulose, and now report a biomimetic synthesis of syringolide 2 (**1**; n=2) (Scheme 1).[7]

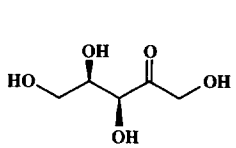



**3**



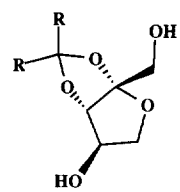

**4**

D-Xylulose (**5**) is available commercially, or it can be prepared from the cheaper sugar D-xylose by isomerisation with pyridine.[8] The xylulose can be conveniently isolated from the resultant mixture of pentoses as its crystalline acetonide (**6**),[9] but this seemingly attractive acetal protecting group subsequently proved too difficult to remove selectively in the presence of sensitive β-ketoester functionality. We therefore reverted to the more labile anisylidene ketal (**7**). A 1:1 mixture of epimers of this derivative (**7**) could be prepared directly from the same pentose mixture as the acetonide in 64% yield (based on the D-xylulose content of the mixture[9]) by sonnication with anisaldehyde in the presence of anhydrous zinc chloride catalyst. Alternatively, it could be prepared from the acetonide itself (**6**), either by boron trifluoride-catalysed exchange with anisaldehyde (45% yield), or by hydrolysis with aqueous oxalic acid to free D-xylulose (95%) and re-ketalisation.



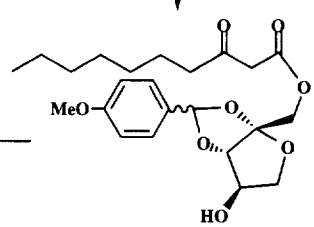

**5**

a)



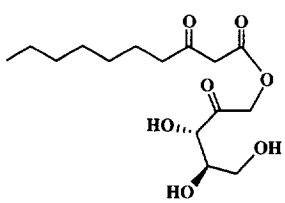

**6**; R=Me, Me
**7**; R=*p*-MeOC$_6$H$_4$, H

b)




**8**

c)




**9**

**Scheme 1.** a) **5** -> **7**: *p*-MeOC$_6$H$_4$CHO, $ZnCl_2$, sonnication, 6 h; b) **4**, THF, reflux 6 h; c) AcOH, $H_2$ / $Pd(OH)_2$, 8 h.

Coupling a fivefold excess of the ketal (**7**) with 3-oxodecanoic acid, mediated by dicyclohexylcarbodiimide, afforded the two epimers (1:1) of the desired 1-monoester (**8**), the 4-monoester and the 1,4-diester in 47, 21 and 13% yields respectively The addition of 4-dimethylaminopyridine to the reaction mixture increased decarboxylation of the β-ketoacid, and was not helpful. Selectivity of the acylation for the primary hydroxy group was improved by the use of the C-octanoyl Meldrum's acid derivative (**4**)[10] under thermal coupling conditions, the monoester (**8**), the 4-monoester and the 1,4-diester now being obtained in yields of 67, 22 and 8% respectively.

Removal of the anisylidene protecting group from the ester (**8**) could be accomplished with trifluoroacetic acid in aqueous tetrahydrofuran, but was accompanied by considerable hydrolysis of the β-ketoester functionality. Accordingly, the ketal was removed by hydrogenolysis, preferably with Pearlman's palladium hydroxide on carbon catalyst[11] in acetic acid, to furnish 1-(3'-oxodecanoyl)-D-xylulose (**9**) in 83% yield after radial chromatography. $^{1}H$ and $^{13}C$ NMR spectroscopy[12] showed that this product existed as an equilibrium mixture of three primary components in an approximately 9:4:1 ratio, representing probably the β- and α-furanose anomers and the acyclic keto form, respectively, of the xylulose moiety.[13] This mixture elicited no hypersensitive response when applied to resistant soybean cultivars.[14]

Exposure of 1-(3'-oxodecanoyl)-D-xylulose (**9**) to a variety of mild reaction conditions at room temperature gave syringolide 2 (**1**; n=2), together with considerable unavoidable decomposition of the unstable product.[2] The preferred but not yet optimised procedure involved exposure to basic alumina (THF, $Al_2O_3$ : **9** 10 : 1, 3.5 h, r.t., 6%) and gave syringolide 2 as a white solid after radial chromatography. This product showed identical TLC behaviour and $^{1}H$ and $^{13}C$ NMR spectra (in $CDCl_3$) to authentic syringolide 2, and had $[\alpha]_D^{22}$ -82° (*c* 0.001, $CHCl_3$) {lit.[2] $[\alpha]_D^{24}$ -75.91° (*c* 0.22, $CHCl_3$)}.

Recent publications[15,16] have described two syntheses of the syringolides by Knoevenagel condensation of *multiply protected* β-ketoacyl xylulose esters to the corresponding *protected* butenolides, followed by deprotection and further cyclisation to the syringolides (**1**). The D-xylulose segment, and consequent chirality in the products, was in each case derived from D-tartaric acid. The present biomimetic synthesis proceeds in only four steps from D-xylulose itself (**5**), and uses a single protecting group. The observed facile triple cyclisation of the presumed key biosynthetic intermediate, 1-(3'-oxodecanoyl)-D-xylulose (**9**), to syringolide 2 (**1**; n=2) as a single stereoisomer, provides circumstantial evidence that D-xylulose and the appropriate β-ketoacids are the primary biogenetic precursors of the syringolides (**1**), and that their biosynthetic conversion to the elicitor proceeds initially via esterification rather than intermolecular Knoevenagel condensation.

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