

\$0040-4039(96)00611-9

Biomimetic Synthesis of the Microbial Elicitor Syringolide 2

Julian P. Henschke and Rodney W. Rickards*

Research School of Chemistry, Australian National University Canberra, ACT 0200, Australia

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. Copyright © 1996 Published by Elsevier Science Ltd

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.



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 ourselves⁴ and others⁵ 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 studies⁵ 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



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 mixture⁹) 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.



Scheme 1. a) 5 -> 7: *p*-MeOC₆H₄CHO, ZnCl₂, sonnication, 6 h; b) 4, THF, reflux 6 h; c) AcOH, H₂ / 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 catalyst¹¹ in acetic acid, to furnish 1-(3'-oxodecanoyl)-D-xylulose (9) in 83% yield after radial chromatography. ¹H and ¹³C NMR spectroscopy¹² 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, Al₂O₃ : 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 ¹H and ¹³C NMR spectra (in CDCl₃) to authentic syringolide 2, and had $[\alpha]_D^{22}$ -82° (*c* 0.001, CHCl₃) {lit.² [\alpha]_D^{24}-75.91° (*c* 0.22, CHCl₃)}.

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.

Acknowledgements: We thank Professors J.J. Sims and N.T. Keen, University of California, for helpful discussions and for reference samples of syringolides 1 and 2.

REFERENCES AND NOTES

- Smith, M. J.; Mazzola, E. P.; Sims, J. J.; Midland, S. L.; Keen, N. T.; Burton, V.; Stayton, M. M. Tetrahedron Lett. 1993, 34, 223-226.
- Midland, S. L.; Keen, N. T.; Sims, J. J.; Midland, M. M.; Stayton, M. M.; Burton, V.; Smith, M. J.; Mazzola, E. P.; Graham, K. J.; Clardy, J. J. Org. Chem. 1993, 58, 2940-2945.
- Keen, N. T.; Tamaki, S.; Kobayashi, D.; Stayton, M.; Gerhold, D.; Shen, H.; Gold, S.; Lorang, J.; Thordal-Christensen, H. Characterization and function of avirulence genes from *Psudomonas syringae* pv. tomato. In NATO ASI Series, Vol. H 36, Signal Molecules in Plants and Plant-Microbe Interactions; Lugtenberg, B. J. J. Ed.; Springer-Verlag: Berlin, 1989; pp. 183-188. Keen, N. T.; Tamaki, S.; Kobayashi, D.; Gerhold, D.; Stayton, M.; Shen, H.; Gold, S.; Lorang, J.; Thordal-Christensen, H.; Dahlbeck, D.; Staskawicz, B. Mol. Plant-Microbe Interact. 1990, 3, 112-121. Keen, N.; Kobayashi, D.; Tamaki, S.; Shen, H.; Stayton, M.; Lawrence, D.; Sharma, A.; Midland, S.; Smith, M.; Sims, J. J. Adv. Mol. Genet. Plant-Microbe Interact. 1991, 1, 37-44.
- Rickards, R. W. Aspects of the Biomimetic Synthesis of Antibiotics and Their Bioregulators. In Progress in Chemistry of Medicinal Plants in Asia. Proceedings of the Sixth Asian Symposium on Medicinal Plants and Spices, Bandung, 1989; Achmad, S. A.; Makmur, L.; Harjanto, F.; Hakim, E. H.; Djajanti, S. D. Eds.; ITB University Press: Bandung, 1989; pp. 91-100.
- 5. Sakuda, S.; Higashi, A.; Tanaka, S.; Nihira, T.; Yamada, Y. J. Am. Chem. Soc. 1992, 114, 663-668.
- 6. Waring, R. B. Biomimetic Synthesis and Structural Revision of the Butanolide Microbial Autoregulators, PhD Thesis, Australian National University, 1994.
- 7. This work was reported at the 16th Annual Symposium of the Royal Australian Chemical Institute New South Wales Organic Group, Sydney, 1995 (Abstracts p. 5).
- 8. Schmidt, O. T.; Treiber, R. Ber. Deutsch. Chem. Ges. 1933, 66, 1765-1769.
- Hough, L.; Theobald, R. S. D-threo-Pentulose. In Methods Carbohydr. Chem. Vol. I. Analysis and Preparation of Sugars; Whistler, R. L.; Wolfrom, M. L. Eds.; Academic Press: New York, 1962; pp. 94-98.
- 10. Oikawa, Y.; Sugano, K.; Yonemitsu, O. J. Org. Chem. 1978, 43, 2087-2088.
- 11. Pearlman, W. M. Tetrahedron Lett. 1967, 1663-1664.
- NMR ¹H (CD₃OD) complex three component mixture; NMR ¹³C (CD₃OD) δ 14.4, 23.7, 24.4, 27.4, 30.1, 30.2, 32.9, 35.8, 43.6, 43.67, 43.72, 63.2, 64.2, 64.3, 65.48, 65.53, 66.2, 66.7, 69.5, 71.8, 73.7, 73.8, 75.2, 75.6, 77.0, 77.3, 77.9, 78.8, 81.7, 82.3, 102.7, 105.9, 168.7, 205.5, 207.7; MS (CI, NH₃) *m/z* 336 (MNH₄⁺), 318 (MNH₄⁺-H₂O), 301 (100%), 283, 258, 239, 204, 168, 150, 133, 127.
- 13. Vuorinen, T.; Serianni, A. S. Carbohydr. Res. 1990, 209, 13-31.
- 14. Keen, N. T. personal communication.
- 15. Wood, J. L.; Jeong, S.; Salcedo, A.; Jenkins, J. J. Org. Chem. 1995, 60, 286-287.
- Kuwahara, S.; Moriguchi, M.; Miyagawa, K.; Konno, M.; Kodama, O. Tetrahedron Lett. 1995, 36, 3201-3202. Kuwahara, S.; Moriguchi, M.; Miyagawa, K.; Konno, M.; Kodama, O. Tetrahedron 1995, 51, 8809-8814.

(Received in UK 6 March 1996; revised 27 March 1996; accepted 29 March 1996)