Tetrahedron Letters, Vol. 34, No. 2, pp. 223-226, 1993
Printed in Great Britain

0040-4039/93 $5.00 + .00
Pergamon Press Ltd

The Syringolides: Bacterial C-Glycosyl Lipids That Trigger Plant Disease Resistance

M.J. Smith* and E.P. Mazzola

Natural Products and Instrumentation Branch, Center for Food Safety and Applied Nutrition
Food and Drug Administration, Washington, DC 20204

J.J. Sims, S.L. Midland and N.T. Keen

Department of Plant Pathology, University of California, Riverside, CA 92521

V. Burton and M.M. Stayton

Department of Molecular Biology, University of Wyoming, Laramie, WY 82071

Abstract: The salient structural and bioorganic properties of this new class of signal molecules are reported.

The syringolides are signal transducers biosynthesized through the action of avirulence gene D (*avrD*) of *Pseudomonas syringae*.[1,2] Our spectral and biosynthetic analyses have disclosed that these novel natural products possess an unprecedented ring system, are prototypical in overall structure (as C-glycosyl lipids), and contain a remarkable number of highly deshielded NMR signals (due to an unusual ensemble of deshielding β-substituents). Several hallmarks of such biomolecules (that "elicit" the *gene-for-gene* hypersensitivity response of disease resistance in plants) are also highlighted.

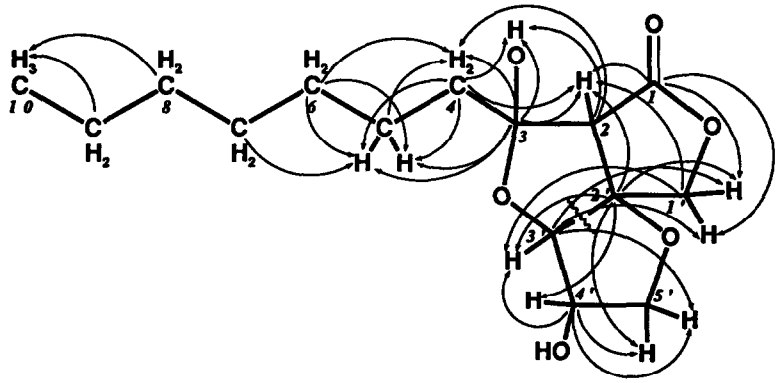

Figure 1. Decisive HMBC connectivities in *Ps-1*. (C-2' and C-3' are directly linked in the actual structure.)

The structure determination of syringolide *Ps-1* was brought to a penultimate stage *via* COSY, HMQC, selective INEPT (INAPT), and HMBC NMR correlations, which demonstrated that the proton (resonance) at 1.89 and the carbon at 39.5 ppm both belonged to the sidechain methylene (C-4) attached directly to the central ring system (Figure 1; NMR assignments, reference 3). COSY and HMQC spectra also revealed the existence of an isolated proton (3.08 ppm, methine), a 2-spin system (4.31, 4.66 ppm, methylene), and a 4-spin system

(4.48; 4.14; 3.82, 3.94 ppm; methine; methine; methylene), as well as certain hydrocarbon sidechain connectivities. The considerably deshielded nature of the isolated methylene protons (4.31, 4.66 ppm) suggested that they might be linked to the oxygen of an ester; a strong IR band at 1773 cm^{-1} defined the butanolide (γ-lactone) substructure. The existence of long-range connectivities between H-5'A and C-2', along with chemical shift considerations, suggested that they are linked by an oxygen. Such considerations also indicated that another oxygen is situated between carbons 3 and 3'. The two hydroxyl groups were placed on carbons 3 and 4', owing to weak COSY correlations between the (exchangeable) hydroxyl protons at 4.27 and 5.32 ppm and the resonances at 4.14 and 3.08 ppm, respectively. The detection of long-range connectivities between H-2 and carbons 1' and 2' required that carbons 2 and 2' be joined (which corresponds to the C-glycosyl linkage). These experiments also established the following connectivities: (1) C-4, C-3, C-2, C-1, oxygen, C-1', and C-2'; and (2) C-3', C-4', and C-5'.

Of particular interest are carbons 3', 2', and 3 at 92.8, 99.0, and 108.8 ppm, respectively. Their remarkably deshielded chemical shifts initially suggested a ketal/acetal/hemiketal substructure, *i.e.*, an oxygen was situated between carbons 2' and 3'. Yet several convergent lines of biosynthetic evidence imposed further refinements upon this structure. The putative acetal linkage fell into disfavor because both portions of the carbon skeleton were still evident (hence contiguous) in NMR spectra of crude syringolide bisulfite adducts (purified from bacterial cultures). And although the *avrD* gene was both essential and sufficient to generate the syringolides in *P. syringae* and in *Escherichia coli*,[4] persistent efforts to formulate a plausible mechanism failed until it was deduced that carbons 2' and 3' are linked, which concomitantly afforded the correct structure (Figure 1), reconciled the HRMS/EI data (300.157060, M^+, $C_{15}H_{24}O_6$, Δ 0.8 ppm), and disclosed the NMR chemical shift anomalies noted. With respect to the last, it is striking that in addition to their directly attached oxygens, carbons 3', 2', and 3 possess an unusually large ensemble of deshielding β-substituents. Similarly, carbon 2 has eight β-substituents, five of which are oxygen. The biosynthesis is thus restricted to substrates that are common metabolites and very few steps, *e.g.*, Figure 2.

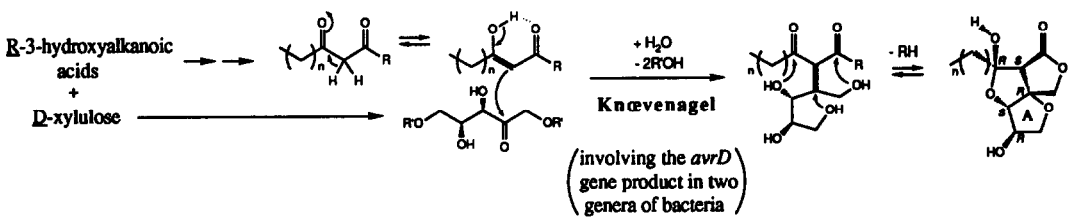

Figure 2. Plausible biosynthetic pathway and resultant configuration adapted by the syringolides; R = (thio)ester or OH, R' = H or (phospho)ester; *Ps-1*, n = 6; *Ps-2*, n = 4 methylenes.

The structure deduced on biosynthetic grounds was validated immediately using the original HMBC data; two relatively intense signals between (a) the proton at 4.48 and carbon at 75.6 ppm and (b) the proton at 4.66 and carbon at 92.8 ppm within individual traces could now be assigned as 3-bond carbon-proton connectivities (all 2-bond permutations had been ruled out previously). Their large magnitudes demonstrated that the penultimate ketal/acetal/hemiketal structure was no longer tenable, for both signals would have had to correspond to 4-bond connectivities. Every other structural permutation was, likewise, found to be incompatible with the cumulative NMR data, thereby providing overwhelming and definitive support for this deduction.

The relative stereochemistry of *Ps-1* was first determined from the NMR data in the following manner: (i) Dreiding models indicate that the dihedral angles between H-3' and C-1' and between H-1'A (4.66 ppm) and C-3' are very nearly 0°. The moderately intense, 3-bond connectivities between these nuclei support the conclusion that H-3', C-3', C-2', C-1', and H-1'A lie in a common plane. (ii) The absence of vicinal coupling between H-3' and H-4' suggests that the dihedral angle between them is *ca.* 90°, thereby conferring a *threo* configuration on the C-3'/-4' vicinal diols. (iii) The latter implication is supported by the intense 3-bond connectivity between H-5'A (3.94 ppm) and C-2', which requires that the dihedral angle between these nuclei be *ca.* 180°. (iv) Once H-5'A adapts such a conformation, the A-ring (Figure 2) becomes puckered so that the dihedral angle between H-3' and H-4' is *ca.* 90°, consistent with point ii. (v) Because the ring system of *Ps-1* occurs as one of two possible enantiomers, the relative stereochemistry of the molecule was thus defined by Dreiding models that accommodate the 1.8-Hz, 4-bond, NMR W-coupling between H-2 and OH-3 (see below).

The results and reasoning that afforded the proposed biosynthetic mechanism also offer the (predicted) absolute configuration of the molecule: (vi) *The decisive gene product*; the *avrD* gene codes for a protein that appears to catalyze the C-glycosylation of 3-ketoalkanoyl groups, thereby forming prototypical C-glycosyl lipids. Because the incorporation and expression of *avrD* by two dissimilar genera of bacteria lead to the secretion of *Ps-1* and *Ps-2*,[5] the essential components of this biosynthetic pathway are the *avrD* gene product and common metabolites. (vii) *The fatty acyl group*; within the genus *Pseudomonas*, R-3-hydroxydecanoic and R-3-hydroxyoctanoic acids abound as coupled polyesters.[5] Ordinary β-oxidations of these putative precursors by R-3-hydroxyalkanoyl dehydrogenases[6] could thus form both 3-ketoalkanoyl substrates. (viii) *The five-carbon moiety*; an equally common metabolite that would complete the carbon skeleton with the requisite *threo* configuration at carbons 3' and 4' is xylulose (*threo*-pentulose). D-xylulose is a normal product of D-glucose metabolism (the carbon source of the bacterial cultures) in *P. syringae* and *E. coli*, in stark contrast to L-xylulose, which is relatively rare in nature. (ix) *Biosynthetic precedence*; a Knœvenagel condensation (akin to Figure 2) between analogous 3-ketoalkanoyl precursors and a 1,2-ketol has, likewise, been proposed for the biosynthesis of the structurally related butanolides (Figure 3).[7] (x) *Related glycolipids*; aside from a partially characterized "lipopolysaccharide" from the cell envelope of *P. diminuta* that contains up to 15% w/w D-xylulose,[8] other reports of xylulolipids have not been encountered. (xi) *Absolute configuration*; Dreiding models that incorporate D-xylulose adapt a 2S, 3R, 2'R, 3'S, 4'R configuration; the corresponding relative stereochemistry and structure of *Ps-1* have now been reconfirmed by X-ray crystallography.[1]

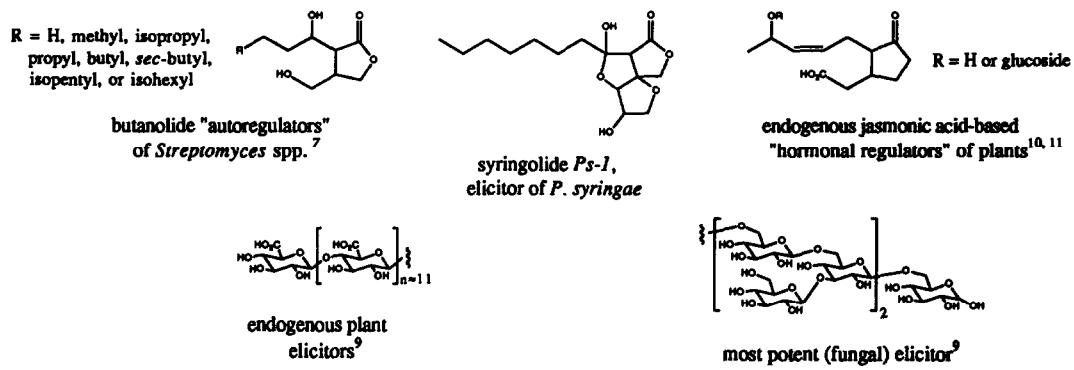

Figure 3. Pertinent signal molecules that possess comparable structures and/or bioactivities.

Lastly, because *P. syringae*, dissimilar pathogens, and their "elicitors" all trigger the aforementioned hypersensitivity response (HR) of plant defenses,[2,4,9] the chemistry that underlies such signal-transduction merits brief consideration. HR-based defenses are manifested by diverse, higher plants and involve a combination of localized cell death, lignification of cell walls, and the formation of phytoalexin antibiotics and jasmonic acid "hormonal regulators"[11] (Figure 3). Several "pathogenesis-related" β(1→3)glucanases, chitinases, and cinnamyl alcohol dehydrogenase are also induced.[9] This cascade of defenses is triggered by particular "avirulence" signals (elicitors) of pathogens when the plant possesses the corresponding disease "resistance" gene. Although the chemical mechanism by which the syringolides elicit this enigmatic *gene-for-gene* response (in plant lines expressing the *Rpg4* disease resistance gene) is unknown,[4] it is striking that diverse plants rapidly accumulate jasmonic acids as endogenous (secondary) signal molecules upon exposure to exogenous elicitors, *e.g.*, fungal cell wall fragments (Figure 3).[11]

In particular, the analogous structure/(pleiotropic) activity profiles displayed by the syringolides, jasmonic acids, and butanolides suggest that their modes of action (targets) may be very similar. For instance, several major processes under genetic regulation in *Streptomyces* spp. are induced by endogenous butanolides,[8] and particular receptor proteins have been isolated that bind these signal molecules and genomic DNA specifically and competitively.[12] Correspondingly, particular receptors (targets within the signal cascade of plant defenses) for the syringolides and jasmonic acids have also been implicated and are now being actively sought. The syringolides thus provide a valuable means to elucidate the molecular mechanisms of plant disease resistance.

REFERENCES AND NOTES

1. Reported at the proceedings of the 18th IUPAC Symposium on the Chemistry of Natural Products, Aug. 30, **1992**, Strasbourg, France. The helpful comments of B.A. Horenstein of the Albert Einstein College of Medicine, Koji Nakanishi of Columbia University, J.B. Neilands of U.C.-Berkeley, and S.W. Page and A.E. Pohland of the FDA are acknowledged. Ongoing studies concerning the isolation, characterization, and mode of action shall be reported in due course: Midland, S.L.; Keen, N.T.; Sims, J.J.; Midland, M.M.; Burton, V.; Stayton, M.M.; Smith, M.J.; Mazzola, E.P.; Graham, K.J.; Clardy, J.
2. Keen, N.; Tamaki, D.; Kobayashi, D.; Gerhold, D.; Stayton, M.M.; Shen, H.; Gold, S.; Lorang, J.; Thordal-Christensen, H.; Dahlbeck, D.; Staskawicz, B. *Mol. Plant Microbe Interact.* **1990**, *3*, 112.
3. **Syringolide *Ps-1*.** NMR data collected on a 500-MHz spectrometer, on 10 mg in $(CD_3)_2CO$, calibrated *vs.* TMS at 0.00 ppm, [**position**, ^{13}C shift, *n*H, ^{1}H shift (J)]: **1**, 172.6, 0, -; **2**, 59.7, 1, 3.08 d (1.8); **3**, 108.8, 0, -; **OH-3**, -, -, 5.32 d (1.8); **4**, 39.5, 2, 1.89 dd (8.4); **5**, 24.4, 2, 1.60 m/1.48 m; **6**, 30.4, 2, 1.28 m; **7**, 29.9, 2, 1.30 m; **8**, 32.5, 2, 1.32 m; **9**, 23.3, 2, 1.31 m; **10**, 14.3, 3, 0.87 t (7.0); **1'**,75.6, 2, 4.66 d (10.3)/4.31 d (10.3); **2'**, 99.0, 0, -; **3'**, 92.8, 1, 4.48 d (1.1); **4'**, 75.4, 1, 4.14 dd (4.1, 2.9); **OH-4'**, -, -, 4.27 d (4.1); **5'**, 74.9, 2, 3.94 dd (10.0, 1.1)/3.82 dd (10.0, 2.9).
4. Stayton, M.M.; Lawrence, D.; Hanekamp, T.; Burton, V.; Tamaki, S.; Kobayashi, D.; Smith, M.J.; Keen, N.T.; Sims, J. *Fourth Int. Symp. Biotech. Plant Protect.*, Kung, S; Hutcheson, S. Eds.; World Scientific: Singapore **1992**; in press.
5. Timm, A.; Steinbüchel, A. *Appl. Environ. Microbiol.* **1990**, *56*, 3360.
6. Dawes, E.A. *Microbial Energetics*, Chapman and Hall: New York, **1986**; pp. 158-163.
7. Sakuda, S.; Higashi, A.; Tanaka, S.; Nihira, T.; Yamada, Y. *J. Am. Chem. Soc.* **1992**, *114*, 663.
8. Wilkinson, S.G. *Carbohydrate Res.* **1981**, *98*, 247.
9. Ryan, C.A. *Annu. Rev. Cell Biol.* **1987**, *3*, 295.
10. Meyer, A.; Gross, D.; Vorkefeld, S.; Schimdt, J.; Sembdner, G.; Schreiber, K. *Phytochemistry* **1989**, *28*, 1007.
11. Gundlach, H.; Müller, M.J.; Kutchan, T.M.; Zenk, M.H. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 2389.
12. Kim, H.S.; Tada, H.; Nihira, T.; Yamada, Y. *J. Antibiotics* **1990**, *43*, 692.

(Received in USA 18 May 1992; accepted 12 October 1992)