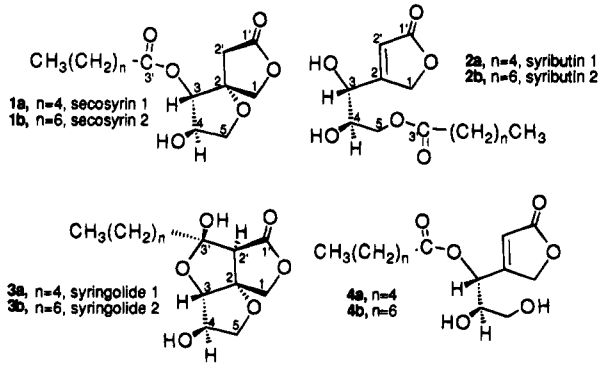
Secosyrins 1 and 2 and Syributins 1 and 2: Novel Structures Produced by Bacteria Expressing the *avrD* Gene

Sharon L. Midland, Noel T. Keen, and James J. Sims*

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

Received September 26, 1994 (Revised Manuscript Received January 27, 1995)

We describe here the isolation and structure elucidation of secosyrins 1 and 2 (**1a** and **1b**, respectively) and syributins 1 and 2 (**2a** and **2b**, respectively), the four major coproducts of the syringolide elicitors (**3a** and **3b**).



All of these unusual metabolites are produced by Gram-negative bacteria expressing the class I homology group of *avrD* alleles, genes from *Pseudomonas syringae* involved with formation of bacterial signal molecules or elicitors.[1] These compounds occur only in culture filtrates of bacterial cells carrying the *avrD* gene. We have reported the bacterial production[2] and structural elucidation[3] of the syringolides, the only known nonproteinaceous specific elicitors of the hypersensitive response. The secosyrins and syributins are not active elicitors, but they are of biosynthetic interest since they are coproduced with the syringolides. They are interesting new structures that provide clues to the nature of the *avrD* gene and the function of its protein product.

Fractionation of ethyl acetate extracts of culture fluids of *Pseudomonas syringae* pv. *tomato* or *Escherichia coli* carrying plasmid pAVRD12[2] by silica gel vacuum liquid chromatography and normal-phase HPLC (40% ethyl acetate–hexanes) gave pure oily samples of the secosyrins (**1a**, **1b**) and the syributins (**2a**, **2b**) in yields of about 1 mg each per liter of culture. By precise mass determination,[4] **1a** and **2a** were isomeric with syringolide 1 (**3a**); **1b** and **2b** were isomeric with syringolide 2 (**3b**).

The structures of the secosyrins and syributins were determined by spectroscopic and chemical methods. ^1H and ^{13}C NMR spectra of **1a** and **1b** were nearly identical, but **1b** contained two additional methylene units which were found by proton decoupling to be part of a hydrocarbon chain. This same homology was found for **2a** and **2b**. **1a** and **2a** are isomeric and comprised of an *n*-pentyl chain and $C_8H_9O_6$ moieties with four sites of unsaturation whereas **1b** and **2b** are also isomeric but contain an *n*-heptyl chain and the oxygenated moieties. ^1H and ^{13}C NMR chemical shift values and selective $^1H-^{13}C$ long-range correlation INAPT[5] (Table 1) showed that for both the secosyrins and the syributins the *n*-alkyl chain was attached to the carbonyl group of an ester.

The $C_8H_9O_6$ portion of **1a** and **1b** was identical and consisted of three methylenes, two methines, and three quaternary carbons, two of which were carbonyls. Thus, it is bicyclic. Infared bands at 2.9, 5.6, and 5.75 μm indicated an alcohol, a γ-lactone, and an aliphatic ester. ^{13}C NMR chemical shift values for all of the carbons except one methylene inferred α-oxygen substituents. Proton homonuclear spin–spin coupling experiments demonstrated that the two methines were adjacent and that one was bonded to an oxygenated methylene; the other was shown by INAPT to be attached to the aliphatic ester oxygen. The remaining two methylenes showed no vicinal proton–proton couplings, but they did have INAPT correlations to the lactone carbonyl and were therefore assigned as the γ- and α- carbons, respectively, of the lactone ring and separated by a quaternary center. Since carbon–hydrogen coupling constants indicated no three- or four-membered rings, the secosyrin skeleton was deduced to be as shown. Placement of the alcohol at the 4-position was further corroborated by synthesis of the acetate of **1b** and observation of a downfield acetylation shift of 0.9 ppm at H4 (Table 1).[6]

The secosyrins have three asymmetric carbons. The two methine protons in the tetrahydrofuran ring, H3 and H4, can be assigned as *trans* since their coupling constant is small. Determination of the relative stereochemistry at C2 is more difficult. NOE experiments define very close proximity for H1b, H4, and H2′b so these must be on the same face of the lactone ring. Further, small NOE is observed between H5b and H2′a and between H5a and H1a, indicating that H5a is on the same side of the tetrahydrofuran ring as the lactone oxygen and H5b is on the side of the lactone carbonyl. Thus the relative stereochemistry appears to be *2R*,3S*,4R** and completely analogous to the syringolides.

The $C_8H_9O_6$ portion of **2a** and **2b** was comprised of two methylenes, three methines, and three quaternary carbons. Both methylenes and two of the methines appeared in the oxygenated region of the NMR spectrum (Table 2). Infared spectra showed bands at 2.9, 5.57, 5.71, and 6.08 μm which indicated at least one alcohol, two ester carbonyls, and a double bond. An ultraviolet maximum at 222 nm (ϵ 4500 in MeOH) showed conjugation. The presence of an α,β-unsaturated γ-lactone which was protonated on the α-carbon but substituted at the β-carbon was inferred.[7] Decoupling of the α-proton identified the β-substituent as a methine appearing at 4.63 ppm in the ^1H NMR spectrum. This methine was

(1) Keen, N. T.; Buzzell, R. I. *Theor. Appl. Genet.* **1991**, *81*, 133.
(2) Keen, N. T.; Tamaki, S.; Kobayashi, D.; Gerhold, D.; Stayton, M.; Shen, H.; Gold, S.; Lorang, J.; Thordal-Christensen, H.; Dahlbeck, D.; Staskawicz, B. *Molec. Plant-Microbe Interact.* **1990**, *3*, 112.
(3) 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.
(4) Mass spectra were determined by desorption chemical ionization using NH_3 gas. **1a** and **1b** gave MNH_4^+ at 290.1610 and 290.1595 mass units, respectively, indicating $C_{13}H_{20}O_6$ as the best formula for their molecular ions (errors of 2.2 and 3.0 ppm). **2a** and **2b** gave 318.1925 and 318.1908, respectively, for MNH_4^+, corresponding to a best molecular ion of $C_{15}H_{24}O_6$ (errors 2.6 and 2.7 ppm).
(5) (a) Bax, A. *J. Magn. Reson.* **1984**, *57*, 314. (b) Johnson, L. F. *Relax. Times* **1990**, *7*, 4.
(6) Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*; Springer Verlag: Berlin, 1983.
(7) (a) LaLonde, R. T.; Peerakyla, H.; Hayes, M. P. *J. Org. Chem.* **1990**, *55*, 2847. (b) Chen, T.-B.; Galinis, D. L.; Wiemer, D. F. *J. Org. Chem.* **1992**, *57*, 862.

0022-3263/95/1960-1118$09.00/0

Table 1. 300 MHz NMR Data for Secosyrin 2 (1b) and Its Acetate in $CDCl_3$[a]

atom no.	**1b** δ^1H (multiplicity)	**1b** δ^{13}C (multiplicity)	**1b** INAPT correlations	4-acetyl **1b** δ^1H (multiplicity)	4-acetyl **1b** ^{13}C
1a	4.38 (d, J = 10.3)	76.1 (t, J = 153.7)	2, 3, 5, 1′	4.37 (d, J = 10.2)	75.2 (t)
1b	4.44 (d, 10.3)		2, 3, 1′	4.40 (d, 10.2)	
2		86.7 (s)			87.1 (s)
3	4.99 (bd, 2.0)	81.4 (d, 156.6)	1, 2, 4, 5, 3′	5.27 (ddd, 2.0, 0.5, 0.4)	77.8 (d)
4	4.31 (ddd, 5.1, 2.5, 2.0)	75.5 (d, 156.5)	1, 2, 3, 5	5.17 (ddd, 5.1, 2.3, 2.0)	77.1 (d)
5a	3.86 (ddd, 10.2, 2.5, 0.3)	73.0 (t, 148.9)	2, 3, 4	3.89 (ddd, 11.0, 2.3, 0.5)	71.2 (t)
5b	4.12 (ddd, 10.2, 5.1, 0.4)		1, 2, 3, 4	4.23 (ddd, 11.0, 5.1, 0.4)	
1′		174.7 (s)			173.7 (s)
2′a	2.58 (d, 18.0)	35.6 (dd, 140.8, 131.6)	1, 2, 1′	2.59 (d, 17.9)	35.2 (t)
2′b	2.78 (d, 18.0)		2, 3, 1′	2.73 (d, 17.9)	
3′		173.6 (s)			172.4 (s)
4′	2.36 (t, 7.5)	34.1 (t, 129.0)	3′, 5′, 6′	2.38 (dd, 7.9, 7.1)	33.9 (t)
5′	1.61 (bp, 7.5)	24.8 (t, 126.7)	4′, 7′	1.63 (bp, 7.5)	24.7 (t)
6′	1.30 (m)	29.0 (t, 124.0)	b	1.30 (m)	29.0 (t)
7′	1.30 (m)	28.8 (t, 124.0)	b	1.30 (m)	28.8 (t)
8′	1.30 (m)	31.6 (t, 126.5)	b	1.30 (m)	31.6 (t)
9′	1.30 (m)	22.5 (t, 125.2)	b	1.30 (m)	22.5 (t)
10′	0.88 (t, 6.8)	14.0 (q, 124.5)	8′, 9′	0.88 (t, 6.8)	14.0 (q)
Ac1					169.6 (s)
Ac2				2.10 (s)	20.7 (q)

[a] J values are given in Hz. Carbon–proton correlations were made by PSCSCM. All proton coupling constants were verified by homonuclear decoupling with resolution enhancement. [b] Overlapped peaks gave INAPT at 6′, 7′, and 8′.

Table 2. 300 MHz NMR Data for Syributin 1 (2a) and Its Acetonide in $CDCl_3$[a]

atom no.	**2a** δ^1H (multiplicity)	**2a** δ^{13}C (multiplicity)	**2a** INAPT correlations	**2a** acetonide δ^1H (multiplicity)	**2a** acetonide ^{13}C
1a	4.91 (ddd, J = 18.1, 1.9, 0.9)	71.7 (tdd, J = 153.6, 8.4, 1.9)	2, 3, 1′, 2′	4.86 (ddd, J = 18.0, 1.8, 1.1)	70.7 (t)
1b	4.99 (ddd, 18.1, 1.9, 0.6)			4.96 (ddd, 18.0, 1.8, 0.8)	
2		169.2 (bs)			165.8 (s)
3	4.63 (dddd, 3.1, 1.5, 0.9, 0.6)	68.8 (dt, 144.2, 3.4)	1, 2, 4, 5, 1′, 2′	4.73 (dddd, 8.1, 1.6, 1.1, 0.8)	74.6 (d)
4	3.96 (ddd, 6.4, 5.3, 3.1)	71.5 (dt, 148.0, 3.0)	2, 3	4.10 (ddd, 8.1, 4.8, 4.8)	78.0 (d)
5a	4.18 (dd, 11.7, 6.4)	64.8 (tdd, 149.8, 6.0, 2.5)	3, 4, 3′	4.27 (dd, 11.9, 4.8)	62.7 (t)
5b	4.32 (dd, 11.7, 5.3)			4.32 (dd, 11.9, 4.8)	
1′		173.5 (d, 8.8)			172.7 (s)
2′	6.08 (ddd, 1.9, 1.9, 1.5)	116.8 (dq, 182.1, 3.2)	1, 2, 3, 1′	6.11 (ddd, 1.8, 1.8, 1.6)	116.9 (d)
3′		174.7 (bs)			173.3 (s)
4′	2.36 (t, 7.6)	34.0 (tt, 127.2, 4.0)	3′, 5′, 6′	2.35 (t, 7.5)	33.9 (t)
5′	1.63 (m)	24.5 (bt, 125.4)	3′, 4′, 6′, 7′	1.64 (p, 7.5)	24.5 (t)
6′	1.31 (m)	31.2 (tp, 127.7, 4.2)	b	1.30 (m)	31.2 (t)
7′	1.31 (m)	22.3 (tq, 120.7, 4.2)	b	1.30 (m)	22.3 (t)
8′	0.90 (t, 6.9)	13.9 (qt, 124.2, 4.2)	6′, 7′	0.90 (t, 7.0)	13.9 (q)
Acd1				1.44 (s)	26.4 (q)
Acd2					111.1 (s)
Acd3				1.47 (s)	26.6 (q)

[a] See Table 1, footnote a. [b] Overlapped peaks were irradiated to give INAPT peaks 4′, 5′, 6′, 7′, and 8′.

found to be adjacent to a methine at 3.96 ppm which in turn was next to the methylene at 4.18/4.32 ppm. Both of these methines were determined by secondary deuterium isotope shift experiments[8] to bear hydroxyl groups. The ester bearing the aliphatic chain must then be attached to the methylene and the structure of the syributin skeleton is as shown . The relative configuration of the glycol is *threo*, since the chemical shift values of the methyl groups of the acetonide of **2a**, prepared with 2% H_2SO_4 in acetone and purified by HPLC, are nearly identical, reflecting the same number of *cis* interactions for each across the dioxolane ring.[9] The absolute configuration at C3 was determined by the exciton chirality method for allylic benzoates.[10] The 3-(*p*-bromobenzoate) of **2a**, prepared as a minor product from reaction of **2a** with *p*-bromobenzoyl chloride and purified by HPLC, gave a positive CD curve implying right-handed helicity at C3 and the *3R,4R* configuration for the syributins.

In conclusion, the secosyrins and the syributins appear to have stereochemistry which is like that of the syringolides. The absolute stereochemistry shown for the syributins is consistent with that of D-xylulose and thus supports its proposed role as a biosynthetic precursor . The secosyrins are formally related to the syringolides by reverse Claisen cleavage. The syributins might be formed by 1,3-acyl migration from intermediates like **4a** and **4b**, which could be derived by reverse Michael reaction from the secosyrins.

Acknowledgment. Mass spectra were determined by Ron New of the UCR ACIF. We thank the National Science Foundation and the U.S. Department of Agriculture for financial support.

Supplementary Material Available: ^{1}H NMR spectra of **1a**,**1b**, acetyl-**1b**, **2a**, **2b**, and the acetonide of **2a**, and the ^{1}H NMR and CD spectra of the *p*-bromobenzoate of **2a** (8 pages).

JO9416372

(8) Addition of small amounts of D_2O to a solution of **1b** in acetone-d_6 caused new peaks to appear 100 ppb upfield of the methine carbons. Similar effects were observed by: (a) Newmark, R. A.; Hill, J. R. *Org. Magn. Reson.* **1980**, *13*, 40. (b) Gagnaire, D.; Vincendon, M. *J. Chem. Soc., Chem. Commun.* **1977**, 509.

(9) (a) Dana, G.; Danechpajouh, H. *Bull. Soc. Chim. Fr. II* **1980**, 395. (b) El Ashry, E. S. H. *J. Chem. Soc., Chem. Commun.* **1986**, 1024. (c) Rychnovsky, S. D.; Rogers, B.; Yang, G. *J. Org. Chem.* **1993**, *58*, 3511.

(10) Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, B. *J. Am. Chem. Soc.* **1982**, *104*, 3775.