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# Synthesis of the Microbial Elicitor Syringolide 2 Multiply Labelled with Deuterium

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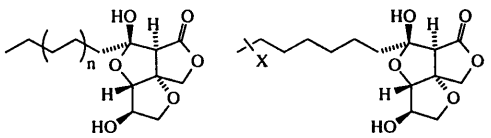
## SUMMARY

Multiply-deuterated syringolide 2 **3** was prepared from D-xylulose and 8-bromooctanoic acid by a convergent route based on our previously reported biomimetic synthesis of this microbial elicitor. Key steps were the thermal acylation of the anisylidene-protected sugar **6** with the C-octenoyl Meldrum's acid derivative **7b**, the one-pot triple-cyclisation of the xylulose β-ketoester **9b** to 9,10-dehydrosyringolide 2 (**10**), and the introduction of multiple deuterium labels in the side chain by catalytic exchange and reduction. The route is applicable to the synthesis of tritium labelled syringolide 2 **4**.

**KEYWORDS:** catalytic deuteration, microbial elicitor, receptor, syringolide.

## INTRODUCTION

In 1993 two homologous bacteria-plant signal compounds of unique structure were isolated from the culture fluids of the bacterial plant pathogen *Pseudomonas syringae* pv. *tomato* (1,2). These compounds, named syringolide 1 (**1**) and 2 (**2**), are produced by the action of avirulence gene D (*avrD*) in the bacterium, and elicit a hypersensitive defence response in soybean cultivars carrying the disease resistance gene, *Rpg4*. *Rpg4* is believed to encode a syringolide receptor protein (3,4), the isolation of which could aid in locating and identifying the resistance gene *Rpg4*. We have therefore modified our previously reported (5) biomimetic synthesis of syringolide 2 (**2**) to provide access to the isotopically labelled elicitor. The route has been validated by the synthesis of multiply deuterium-labelled syringolide 2 **3**, and is equally applicable to preparation of the multiply tritium-labelled analogue **4**. This route complements that recently proposed for the preparation of labelled syringolide 1 (**1**) (6), although the use of unlabelled hydrogen in that case concealed the advantageous multiple labelling which we now report.



**1**; n = 1
**2**; n = 2

**3**; X = $^{2}H_{0\text{-}10}$
**4**; X = $^{3}H_{n}$

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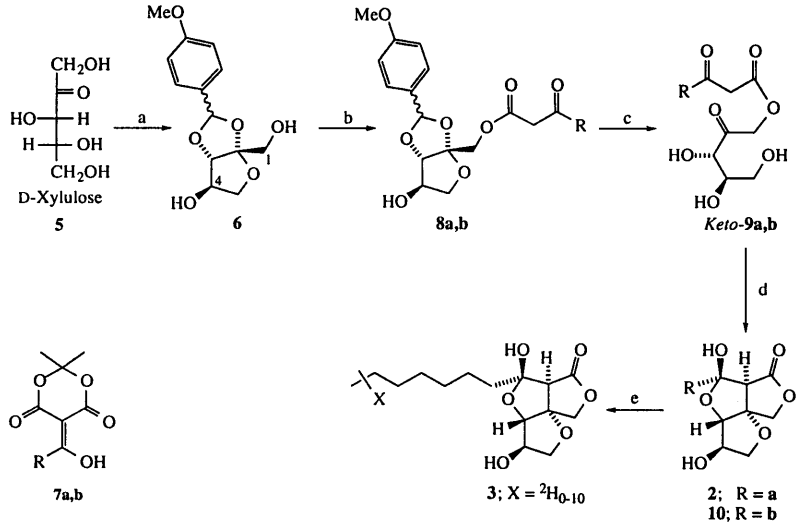
## RESULTS AND DISCUSSION

The four-step synthesis of syringolide 2 itself (Scheme 1) involved thermal acylation of the anisylidene acetal of D-xylulose (D-*threo*-pentulose) **6** with the C-octanoyl Meldrum's acid derivative **7a**, subsequent catalytic hydrogenolysis of the protecting group in the product ester **8a** releasing 1-*O*-3'-oxodecanoyl-D-xylulose as a mixture of its acyclic keto-form **9a** and cyclic α- and β-furanose anomers. This putative biosynthetic intermediate **9a** then underwent triple cyclisation to afford the elicitor **2** (5). Adaptation of this route to the preparation of the labelled analogue involved first replacing the acylating agent **7a** with its terminally unsaturated analogue **7b**, to give the new ester **8b**. Catalytic reduction as before, but with deuterium gas, would now affect both deprotection and simultaneous introduction of deuterium into the side-chain, forming the intermediate **9a** in labelled form which could be cyclised directly to the labelled elicitor **3**. This triple cyclisation although expedient is not high-yielding, however, and in order to facilitate the efficient and convenient use of isotopic hydrogen, particularly since high specific activity tritium might be needed, we have further modified the sequence so as to proceed via 9,10-dehydrosyringolide 2 (**10**) as the final intermediate.

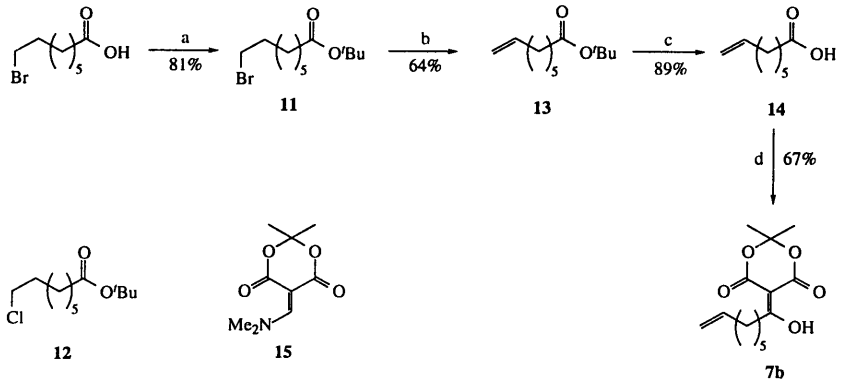



**Scheme 1** Synthesis of unlabelled **2** (5) and deuterium labelled **3** syringolide 2

The unsaturated acylating agent **7b** was prepared as shown in Scheme 2. Treatment of 8-bromooctanoic acid with the Vilsmeier reagent, prepared *in situ* (7) from oxalyl chloride and DMF in the presence of an excess of anhydrous NaBr and ethyl bromide (8), and then with *t*-butanol afforded the bromo-ester **11** in 81% yield. Omission of the added bromides resulted in a ~1:1 mixture of the bromo- and chloro-esters **11** and **12** (9), the latter product arising from halogen exchange. Use of oxalyl bromide in place of oxalyl chloride avoided this exchange, but gave the

bromo-ester **11** in only 45% yield. Dehydrohalogenation of the bromo-ester **11** with $^t$BuOK in DMSO furnished the olefinic ester **13** in 64% yield, accompanied by the substitution product *t*-butyl 8-*t*-butoxyoctanoate in 23% yield. Treatment of this ester mixture with TFA gave pure 7-octenoic acid (**14**) in 89% yield (from the ester **13**) following short-path distillation. The requisite unsaturated acylating agent **7b** was prepared in 67% yield from the acid **14** and Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) (10) using a novel procedure. If the acid chloride is readily available, the method of Oikawa and co-workers (11) is preferred for the preparation of this class of acylating agents. Our procedure avoids the need to prepare the acid chloride, and instead converts the acid into its reactive acyloxy-iminium chloride salt (7) which is then treated *in situ* with Meldrum's acid. 5-{(Dimethylamino)methylene}-2,2-dimethyl-1,3-dioxane-4,6-dione (**15**) and 7-octenoic anhydride were minor by-products of this preparation, but did not affect the subsequent acylation reaction and were not removed from the crude **7b**.




a) DMF, $(COCl)_2$, NaBr, EtBr, $^t$BuOH, Pyridine, MeCN; b) $^t$BuOK, DMSO; c) TFA, $CH_2Cl_2$; d) DMF, $(COCl)_2$, Meldrum's acid, Pyridine, MeCN

**Scheme 2** Preparation of the unsaturated acylating agent **7b**

The anisylidene acetal of D-xylulose **6** (Scheme 1) was best prepared (5) by ultrasound-promoted anisylidenation of the sugar mixture obtained upon pyridine-catalysed isomerisation (12,13) of D-xylose. This mixture contained ≥20% D-xylulose, following removal by crystallisation of some unreacted D-xylose (9). A twofold excess of the acetal **6** was reacted with the acylating agent **7b** to give the desired β-ketoester **8b** in 38% yield, accompanied by the 4-monoester (10%) and the 1,4-diester (14%). The latter two esters can be hydrolysed with aqueous NaOH in THF to regenerate the acetal **6**. The anisylidene protecting group was then removed by hydrolysis with 67% aqueous TFA, rather than by hydrogenolysis as previously (5), to yield 1-*O*-(3'-oxo-9'-decenoyl)-D-xylulose (**9b**) in 64% yield. $^1$H NMR spectroscopy revealed that this β-ketoester **9b** existed in $CD_3OD$ solution as a ring-chain tautomeric mixture of the β-furanose, α-furanose, and keto forms of the sugar moiety in a 6:3:1 ratio. Triple cyclisation of this ester **9b** promoted by basic alumina in THF gave (-)-9,10-dehydrosyringolide 2 (**10**) in 6% yield, with $^1$H and $^{13}$C NMR and CIMS data in agreement with those of syringolide 2 (1) apart from differences due

to the double bond. Deuterium labelled syringolide 2 **3** was then obtained in quantitative yield upon reduction with deuterium gas over Pd/C catalyst.

The $^1H$ NMR spectrum of the deuterated syringolide 2 **3** corresponded closely to that of syringolide 2 (**2**) itself in the region of the tricyclic nucleus. The $^2H$ NMR spectrum showed two broad singlets at δ 0.82 (C10-D) and 1.22 (C9-D, *etc.*), with relative intensities of 1.0:1.8 indicating that multiple incorporation of deuterium had occurred. This was confirmed by CIMS examination of the deuterated material, the $MNH_4^+$ cluster showing molecules carrying from 0 (*m/z* 318) to 10 deuterium atoms (*m/z* 328), and possibly more at low abundance. After correction for natural isotopic abundance, the CIMS data indicated an average incorporation of 3.9 deuterium atoms per molecule. Multiple and non-regiospecific labelling of olefins with isotopes of hydrogen during heterogeneous catalytic reduction results from allylic exchange and double bond migration prior to the reduction itself (14-17).

## CONCLUSION

Multiply-deuterated syringolide 2 **3** has been prepared in five steps from D-xylulose. Substitution of tritium for deuterium gas in the final catalytic step would provide the corresponding radiolabelled analogue for receptor-binding studies. The high level of isotope incorporation achieved, and consequent high specific radioactivity obtainable, would be advantageous for this application.

## EXPERIMENTAL

*General:*

$CH_2Cl_2$ was distilled from $CaH_2$. EtOAc and MeCN were stirred over $CaH_2$, decanted and distilled. DMF and DMSO were distilled under reduced pressure, and the latter was then dried over 4Å molecular sieves. THF was distilled from sodium wire in the presence of benzophenone. Pyridine was stored over KOH before distillation. Dry basic alumina was obtained by heating chromatographic grade basic alumina (Merck 1076, active basic 90, 70-230 mesh, activity I) at 140°C under high vacuum for 24 h. Anhydrous NaBr was prepared by heating AR grade NaBr at 140°C for 24 h under high vacuum. Merck Kieselgel 60 (Art 9385, 230-400 mesh) silica gel was used for column chromatography. Radial chromatography was performed on a Harrison Research 7924T Chromatotron with rotors coated with 1 or 2 mm of Merck Kieselgel 60 $PF_{254}$ gipshaltig (Art 7749) silica gel, or, for partition chromatography, with silica gel-poly(ethylene glycol) prepared as described in the instrument manual. $^1H$ (300 MHz) and $^{13}C$ (75 MHz) NMR spectra were recorded on a Varian Gemini-300 spectrometer, and were referenced to the solvents used; $CDCl_3$ $\delta_H$ 7.26 ppm and $\delta_C$ 76.9 ppm, $CD_3OD$ $\delta_H$ 3.40 ppm and $\delta_C$ 49.3 ppm. The $^2H$ NMR spectrum was recorded on a Varian XL-200 spectrometer and referenced to natural abundance $CDCl_3$ (7.26 ppm). EI, CI ($NH_3$) and high resolution EI mass spectra were recorded on VG-Micromass 7070F, AUTOSPEC or ZAB-2SEQ spectrometers with 70 eV electron beams. FAB and high resolution FAB spectra were recorded on a ZAB-2SEQ spectrometer. GC-MS spectra were recorded on a Hewlett Packard

instrument with a 5970 mass selective detector. Sonications were performed in a Branson B-2200 E4 ultrasonic bath operating at 47 kHz.

t-*Butyl 8-bromooctanoate* (**11**):

This procedure was adapted from that of Stadler (7). To a stirred mixture of anhydrous NaBr (4.64 g, 45.1 mmol), ethyl bromide (30 mL, 402 mmol), DMF (2.26 mL, 29.3 mmol), and MeCN (14.8 mL) at -18°C was added dropwise oxalyl chloride (860 μL, 9.86 mmol) in MeCN (860 μL). After further stirring for 40 min under nitrogen, 8-bromooctanoic acid (2.00 g, 8.96 mmol) in MeCN (2 mL) was added dropwise, and the resulting pale yellow mixture was stirred for 45 min. $^t$BuOH (2.12 mL, 22.5 mmol) in pyridine (2.24 mL, 27.7 mmol) was added dropwise, and after a further 0.5 h the reaction was allowed to warm to room temperature and stirred for 20 h. The reaction was diluted with EtOAc (160 mL), washed successively with 1 M HCl (40 mL), saturated solutions of $NaHCO_3$ (40 mL) and NaCl (40 mL), and then dried ($MgSO_4$) and evaporated to give a pale yellow liquid. Kugelrohr distillation gave the ester **11** (2.03 g, 7.27 mmol, 81%) as a colourless, aromatic liquid b.p. *ca.* 150°C/0.5 mmHg. $R_f$ 0.59 (50% $Et_2O$ in *n*-hexane). $^1$H NMR ($CDCl_3$) δ 1.27 (6H, m), 1.39 (9H, s), 1.53 (2H, m), 1.80 (2H, m), 2.15 (2H, t, *J*=7.5 Hz), 3.35 (2H, t, *J*=6.8 Hz). $^{13}$C NMR ($CDCl_3$) δ 24.7, 27.8, 27.9, 28.2, 28.6, 32.5, 33.6, 35.2, 79.7, 172.9. CIMS *m/z* 298 (9%, $MNH_4^+$), 296 (9, $MNH_4^+$), 281 (8), 280 (16), 279 (12), 278 (15), 242 (55), 240 (57), 225 (30), 223 (33), 207 (55), 205 (55), 143 (61), 125 (30), 97 (29), 57 (100). Analysis Calculated for $C_{12}H_{23}O_2Br$: C, 51.6, H, 8.3; found: C, 51.7, H 8.6.

t-*Butyl 7-octenoate* (**13**):

To a stirred solution of *t*-butyl 8-bromooctanoate (**11**) (5.00 g, 17.9 mmol) in DMSO (15 mL) was added dropwise a 1 M solution of $^t$BuOK (2.21 g, 19.7 mmol) in DMSO (20 mL). After 30 min the mixture was diluted with EtOAc (to *ca.* 400 mL) and washed with solutions of pH 7 buffer (100 mL) and saturated NaCl (50 mL). The aqueous washings were re-extracted with EtOAc (50 mL) and the organic layers combined, dried ($MgSO_4$), and evaporated to give a colourless oil which was chromatographed on a column of silica gel (25% $Et_2O$ in *n*-hexane). The olefin **13** (2.27 g, 11.4 mmol, 64%) eluted as a binary mixture (ratio determined by $^1$H NMR spectroscopy) with *t*-butyl 8-*t*-butoxyoctanoate (1.15 g, 4.2 mmol). $R_f$ 0.76-0.82 (50% $Et_2O$ in *n*-hexane). $^1$H NMR ($CDCl_3$) δ 1.15 (s), 1.29 (bm), 1.41 (s), 1.55 (m), 2.02 (q), 2.17 (t, *J*=7.5 Hz), 2.18 (t, *J*=7.5 Hz), 3.29 (t, *J*=6.6 Hz), 4.91 (m), 4.96 (m), 5.77 (m). $^{13}$C NMR ($CDCl_3$) δ 42.8, 24.9, 26.0, 27.4, 28.0, 28.4, 28.9, 29.5, 30.5, 33.4, 35.4, 35.45, 61.4, 72.2, 79.7, 79.74, 114.2, 138.7, 173.05. GC-MS ($R_t$=4.6 min, **13**) 142 ($M^+$-$C_4H_8$), 125, 124, 107, 96, 79, 57. GC-MS ($R_t$=15.0 min, *t*-butyl 8-*t*-butoxyoctanoate) 257 ($M^+$-$CH_3$), 219, 201, 159, 143, 125, 97, 57.

*7-Octenoic acid* (**14**):

To a stirred mixture of *t*-butyl 7-octenoate (**13**) (2.27 g, 11.4 mmol) and *t*-butyl 8-*t*-butoxyoctanoate (1.15 g, 4.2 mmol) in $CH_2Cl_2$ (13.7 mL) under argon was added TFA (9.6

mL). After 40 min at room temperature, the solvent was first evaporated under reduced pressure and then the remaining TFA under high vacuum. Purification by short path distillation furnished the acid **14** (1.44 g, 10.1 mmol, 89%) as a colourless aromatic oil, b.p. *ca.* 90°C/0.2 mmHg. $R_f$ 0.34-0.50 (5% MeOH in $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$) δ 1.38 (4H, bm), 1.64 (2H, m), 2.05 (2H, m), 2.35 (2H, t, *J*=7.5 Hz), 4.94 (1H, m), 4.99 (1H, m), 5.79 (1H, m), 10.97 (1H, bs). $^{13}C$ NMR ($CDCl_3$) δ 24.4, 28.4, 33.4, 33.9, 114.3, 138.6, 180.3. EIMS *m/z* 142 (1%, $M^+$), 124 ($M^+$-$H_2O$), 100 (15), 96 (34), 83 (36), 82 (61), 73 (25), 67 (28), 60 (54). HRMS Calculated for $C_8H_{12}O$ ($M^+$-$H_2O$): 124.0888; found: 124.0885.

*5-(1'-Hydroxy-7'-octenylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione* (**7b**):

This procedure was adapted from those of Stadler (7) and Oikawa *et al.* (11). To a stirred mixture of DMF (5.25 mL, 68.1 mmol) and MeCN (12.6 mL) at -18°C was added dropwise oxalyl chloride (2.01 mL, 23.0 mmol) in MeCN (2 mL). After 15 min, 7-octenoic acid (**14**), (3.00 g, 21.1 mmol) in MeCN (3 mL) was added dropwise, and the resulting colourless solution was stirred for a further 15 min before warming to 0°C. Pyridine (4.48 mL, 55.4 mmol) followed by Meldrum's acid (2.33 g, 16.2 mmol) in MeCN (3 mL) were then added. After stirring for 1 h at 0°C, the mixture was allowed to warm to room temperature and then stirred for another 3.5 h. The reaction was then diluted with EtOAc (to 120 mL) and washed with solutions of 1 M HCl (40 mL), pH 7 buffer (2 x 40 mL), and saturated NaCl (40 mL) before drying ($MgSO_4$) and evaporating to a deep red oil (4.53 g). $^1H$ NMR analysis showed that the crude product was a quaternary mixture of the dioxane **7b** (2.92 g, 10.9 mmol, 67% based on Meldrum's acid), 7-octenoic anhydride (670 mg, 2.51 mmol), 7-octenoic acid (458 mg, 3.22 mmol), and 5-{(dimethylamino)methylene}-2,2-dimethyl-1,3-dioxane-4,6-dione* (**15**), (482 mg, 2.42 mmol, 15%). It was unnecessary to purify the dioxane **7b** for acylation purposes. $^1H$ NMR ($CDCl_3$) δ 1.40 (bm), 1.70 (bm), 2.02 (m), 2.31 (t, *J*=7.5 Hz), 2.42 (t, *J*=7.4 Hz), 3.04 (t, *J*=7.6 Hz), 3.28 (s), 3.38 (s), 4.91 (bm), 4.96 (m), 5.77 (m). $^{13}C$ NMR ($CDCl_3$) δ 23.9, 24.4, 25.75, 26.4, 26.6, 28.1, 28., 28.3, 28.3, 28.6, 33.3, 33.65, 35.0, 35.5, 43.8, 48.6, 91.1, 102.8, 104.6, 114.2, 126.6, 138.5, 156.0, 160.9, 169.3, 170.4, 197.95. CIMS *m/z* 286 (14%, $MNH_4^+$), 284 (35), 269 (9, $MH^+$), 228 (100), 225 (39), 211 (57) 184 (15), 167 (20), 160 (14), 169 (16), 158 (28), 142 (43), 125 (24), 124 (14), 84 (19), 80 (27), 74 (69). HRMS Calculated for $C_{14}H_{21}O_5$ ($MH^+$): 269.1389; found: 269.1392.

*Crude D-xylulose* (**5**):

Impure D-xylulose (**5**) was prepared by isomerisation of dry D-xylose (200.0 g, 1.332 mol) in dry pyridine (2 L) (12,13) . After crystallisation of residual D-xylose (4 crops, 132.3 g, 0.881 mol, 66%), a tan-coloured syrup (67.7 g, 0.451 mol) was obtained which was dried over $P_2O_5$ under high vacuum for *ca.* 24 h before use. Isolation of the D-xylulose as its 2,3-*O*-isopropylidene derivative (12,18,19) indicated that the sugar mixture contained ≥20% D-xylulose (9).

* This was isolated and characterised following the acylation of **6**

*2,3-O-Anisylidene-β-D-*threo*-pentulofuranose* (**6**):

A $ZnCl_2$-4-methoxybenzaldehyde complex was formed by stirring freshly distilled 4-methoxybenzaldehyde (65 mL, 0.53 mol) and anhydrous $ZnCl_2$ (18.0 g, 132 mmol) for 10 min, followed by sonication for a further 5 min. The complex was immediately added to crude D-xylulose prepared as above (17.2 g, 115 mmol $C_5H_{10}O_5$) and sonicated for 6 h. The reaction mixture was diluted with EtOAc (500 mL), washed with saturated aqueous NaCl (4 x 50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Unreacted 4-methoxybenzaldehyde was removed by Kugelrohr distillation (140°C/0.5 mmHg) to give an immobile brown syrup which was chromatographed on a column of silica gel (5% MeOH in $CH_2Cl_2$). Further purification (2 g lots) through silica gel (1% MeOH in $CH_2Cl_2$ for 800 mL, then a gradient of 1% per 200 mL) gave an amber syrup which was purified by radial chromatography (300 mg lots on a 2 mm rotor: 2% MeOH in $CH_2Cl_2$ for 100 mL, then a gradient of 2% per 50 mL) giving a 1:1 diastereomeric mixture of the acetals **6** as a colourless syrup which solidified on standing (3.95 g, 14.7 mmol, 64% based on the estimated D-xylulose content of the sugar mixture). $R_f$ 0.49, 0.51 (10% MeOH in $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$) δ 3.80 (6H, s), 3.8-4.4 (10H, complex series of multiplets), 4.49 (1H, s), 4.55 (1H, s), 5.85 (1H, s), 6.11 (1H, s), 6.90 (4H, d, *J*=6.9 Hz), 7.35 (2H, *J*=8.8 Hz), 7.41 (2H, *J*=8.7 Hz). $^{13}C$ NMR ($CDCl_3$) δ 55.1, 62.4, 62.5, 74.4, 74.7, 76.8, 86.3, 104.8, 105.3, 113.7, 113.9, 127.7, 127.8, 128.3, 128.6, 160.5, 160.6. EIMS *m/z* 268 (17%, $M^+$), 267 (20), 237 (15), 161, 137 (53), 136 (64), 135 (100), 116 (25), 109 (13), 108 (22), 107 (12), 99 (12), 92 (13), 77 (29). Analysis Calculated for $C_{13}H_{16}O_6$: C, 58.2, H, 6.0; found: C, 58.1, H, 5.9.

*2,3-O-Anisylidene-1-O-(3'-oxo-9'-decenoyl)-β-D-*threo*-pentulofuranose* (**8b**):

To the crude dioxane **7b** (4.53 g containing 2.92 g, 10.9 mmol of **7b**) in THF (12 mL) was added anisylidene xylulose **6** (5.9 g, 22 mmol) in THF (40 mL). The reaction was heated under reflux under argon for 8 h. The solvent was evaporated and the product chromatographed in two portions (*ca.* 3.7 g each) on a column of silica gel (2% MeOH in $CH_2Cl_2$ for 2 L, then 5% MeOH in $CH_2Cl_2$). Following 2,3-*O*-anisylidene-1,4-di-*O*- and 2,3-*O*-anisylidene-4-*O*-(3'-oxo-9'-decenoyl)-β-D-*threo*-pentulofuranose, the ester **8b** eluted as a tan-coloured syrup (3.0 g) contaminated with some 4-monoester and 5-{(dimethylamino)methylene}-2,2-dimethyl-1,3-dioxane-4,6-dione (**15**). The 4-monoester was removed by chromatography on a column of silica gel (2% MeOH in $CH_2Cl_2$), further chromatography on silica gel (90% $Et_2O$ in *n*-hexane) affording the pure ester **8b** as a 1:1 diastereomeric mixture which solidified on standing (1.77 g, 4.1 mmol, 38% based on **7b**). $R_f$ 0.49 (5% MeOH in $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$) δ 1.33 (8H, bm), 1.58 (4H, m), 2.03 (4H, q), 2.21 (m), 2.53 (4H, q), 3.49 (2H, A,A' system), 3.54 (2H, s), 3.80 (6H, s), 4.05 (1H, dd, *J*=1.3 Hz, *J*=10.0 Hz), 4.09 (1H, d, *J*=10.2 Hz), 4.27 (1H, dd, *J*=3.5 Hz, *J*=10.4 Hz), 4.31 (1H, dd, *J*=10.0 Hz), 4.34 (1H, d, *J*=2.9 Hz), 4.43 (1H, d, *J*=2.9 Hz), 4.44 (1H, d, *J*=11.7 Hz), 4.50 (1H, s), 4.54 (1H, d, *J*=11.7 Hz), 4.54 (2H, s), 4.59 (1H, s), 4.93 (1H, m), 4.98 (1H, m), 5.78 (1H, bm), 5.83 (1H, s), 6.10 (1H, s), 6.89 (2H, d, *J*=8.9 Hz), 6.90 (2H, d, *J*=8.8 Hz), 7.34 (2H, d, *J*=8.8 Hz), 7.41 (2H, d, *J*=8.7 Hz). $^{13}C$ NMR ($CDCl_3$) δ 23.1, 28.25, 28.4, 33.35, 43.25, 43.3, 48.7, 48.8,

55.2, 63.9, 64.0, 74.35, 75.1, 75.35, 76.5, 86.5, 86.8, 104.7, 105.5, 112.0, 112.1, 113.7, 114.4, 127.6, 127.7, 127.8, 128.7, 138.6, 160.6, 160.8, 166.15, 166.2, 203.2. EIMS *m/z* 434 (8%, $M^+$), 433 (13), 268 (19), 267 (28), 237 (25), 167 (8), 137 (70), 136 (50), 135 (100), 125 (13), 121 (17), 116 (37), 108 (48), 99 (27), 86 (27), 86 (27), 84 (36), 77 (27), 71 (28), 69 (24). HRMS Calculated for $C_{23}H_{30}O_8$ ($M^+$): 434.1941; found: 434.1925. Analysis Calculated for $C_{23}H_{30}O_8$: C, 63.6, H, 7.0; found: C, 63.3, H, 7.3. Following the ester **8b**, the dioxane **15** (427 mg, 2.14 mmol, 13%), white platelets from acetone m.p. 122-124°C (*lit.* (20) m.p. 123-125°C and *lit.* (21) m.p. 124-125°C), was eluted from the same column with 5% MeOH in $CH_2Cl_2$.

*1-O-(3'-Oxo-9'-decenoyl)-D-*threo*-pentulose* (**9b**):

To a stirred solution of the ester **8b** (48 mg, 0.11 mmol) in aqueous THF (800 µL, $H_2O$:THF 1:7) under argon was added TFA (200 µL) dropwise. After 16.5 h, THF (20 mL) was added and the mixture concentrated under reduced pressure (to *ca.* 0.5 mL), diluted again with $CH_2Cl_2$ (0.5 mL) and partially purified by radial chromatography (1 mm rotor: eluted with 1% MeOH in $CH_2Cl_2$ with a gradient of 2% per 25 mL). Fractions containing the ester **9b** were concentrated (to 1 mL), then diluted (to *ca.* 30 mL) with THF and evaporated to give a colourless oil (57 mg). Removal of the remaining TFA by radial chromatography (1 mm rotor: 4% MeOH in $CH_2Cl_2$ with a gradient of 2% per 25 mL) gave the ester **9b** (21 mg, 0.07 mmol, 64%) as a white solid. $^1$H NMR spectroscopy showed that **9b** existed in $CD_3OD$ solution as a 6:3:1 mixture of the β-furanose, α-furanose and keto forms of the xylulose moiety, respectively. $R_f$ 0.37 (10% MeOH in $CH_2Cl_2$). $^1$H NMR ($CD_3OD$) δ 1.45 (bm), 1.67 (m), 2.14 (m), 2.31 (t, *J*=7.2 Hz), 2.68 (t, *J*=7.3 Hz), 2.69 (t, *J*=7.3 Hz), 2.74 (t, *J*=7.3 Hz), 3.66 (dd, *J*=4.4 Hz, *J*=9.1 Hz), 3.70 (dd, *J*=6.5 Hz, *J*=11.2 Hz), 3,93 (dd, *J*=6.5 Hz, *J*=11.8 Hz), 4.00 (d, *J*=4.9 Hz), 4.05 (d, *J*=2.4 Hz), 4.17-4.39 (complex series of multiplets), 4.97 (bs), 5.01 (m), 5.07 (m), 5.11 (d, *J*=17.7 Hz), 5.32 (d, *J*=17.7 Hz), 5.90 (bm). $^{13}$C NMR ($CD_3OD$) δ 24.6, 29.8, 30.1, 35.0, 43.8, 43.9, 44.0, 63.6, 65.9, 66.55, 67.0, 69.8, 72.1, 74.0, 74.2, 77.3, 77.6, 78.3, 79.1, 79.2, 82.6 , 103.0, 106.2, 115.2, 140.2, 169.0, 205.7, 205.9, 208.0. CIMS *m/z* 334 (27%, $MNH_4^+$), 316 (5), 300 (32), 299 (100), 281 (9), 257 (7), 216 (11), 185 (13), 167 (41), 125 (35), 107 (18), 97 (12), 87 (27), 85 (90), 83 (99), 64 (49). FABMS *m/z* 339 (100%, $MNa^+$), 299 (86, $MH^+$-$H_2O$). HRFABMS Calculated for $C_{15}H_{24}O_7Na$ ($MNa^+$): 339.1420; found: 339.1421. Analysis Calculated for $C_{15}H_{24}O_7$: C, 56.9, H, 7.6; found: C, 56.4, H, 7.9.

*(-)-9,10-Dehydrosyringolide 2* (**10**):

The ester **9b** (104 mg, 0.33 mmol) in THF (10 mL) was stirred with dry basic $Al_2O_3$ (1.00 g) under argon for 7.5 h. The solution was decanted and the remaining solids washed with THF (2 x 50 mL). The combined solvents were concentrated (to *ca.* 15 mL) and filtered through a 0.45 µm HPLC filter. Glacial acetic acid (50 µL) was added and the filtrate evaporated (to *ca.* 1 mL) before radial chromatography (1 mm rotor: EtOAc:*n*-hexane:MeOH 50:50:1 for 50 mL, then 70:30:2 for 50 mL, then 2% MeOH in EtOAc). Fractions containing the compound **10** were evaporated and immediately subjected to radial chromatography on a silica gel-poly(ethylene glycol) rotor (1 mm

rotor: 0% $Me_2CO$ in $Et_2O$ for 80 mL then a gradient of 1% per 50 mL). (-)-9,10-Dehydrosyringolide 2 (**10**) was obtained as a white solid (6 mg, 0.02 mmol, 6%). $R_f$ 0.43 (10% MeOH in $CH_2Cl_2$). $[\alpha]_{578}^{22}$ -64.6° (*c* 0.01, $CHCl_3$). $^1H$ NMR (deacidified $CDCl_3$) δ 1.38 (4H, bm), 1.46 (2H, m), 1.70 (bs), 1.91 (2H, t, *J*=8.0 Hz), 2.03 (2H, m), 3.07 (1H, s), 3.83 (1H, dd, *J*=2.8 Hz, *J*=10.5 Hz), 4.04 (1H, d, *J*=10.6 Hz), 4.30 (1H, d, *J*=2.6 Hz), 4.46 (1H, d, *J*=10.5 Hz), 4.54 (1H, s), 4.72 (1H, d, *J*=10.4 Hz), 4.94 (1H, m), 4.99 (1H, m), 5.79 (1H, bm). $^{13}C$ NMR ($CDCl_3$) δ 23.2, 28.5, 28.75, 33.5, 38.7, 59.0, 74.15, 74.6, 91.3, 97.5, 108.0, 114.4, 138.6, 172.1. CIMS *m/z* 316 (36%, $MNH_4)^+$, 300 (35), 299 (100), 281 (89), 256 (35), 239 (89), 237 (43), 221 (35), 209 (40), 195 (53), 191 (43), 167 (20), 138 (25), 125 (72), 124 (35), 107 (69), 95 (41), 83 (39), 82 (90), 81 (68), 80 (44), 69 (25), 67 (24). HRMS Calculated for $C_{15}H_{22}O_6$ ($M^+$): 298.1416; found: 298.1417.

*Deuterosyringolide 2* **3**:

9,10-Dehydrosyringolide 2 (**10**), (3 mg, 10 μmol) in EtOAc (200 μL) was stirred vigorously with Pd/C (5%, 0.5 mg) under an atmosphere of deuterium for 3.25 h at room temperature. The solution was passed through a 0.45 μm HPLC filter and evaporated to yield the deuterated product **3** as a white solid (3 mg, 100%). $R_f$ *ca.* 0.4 (10% MeOH in $CH_2Cl_2$). $^1H$ NMR ($CDCl_3$) δ 0.85 (2H, distorted multiplet), 1.26 (8H, bm), 1.45 (2H, m), 1.91 (2H, t, *J*=7.9 Hz), 3.07 (1H, s), 3.84 (1H, dd, *J*=2.8 Hz, *J*=10.4 Hz), 4.04 (1H, dd, *J*=1.2 Hz, *J*=10.4 Hz), 4.30 (1H, d, *J*=2.5 Hz), 4.46 (1H, d, *J*=10.4 Hz), 4.55 (1H, s), 4.72 (1H, d, *J*=10.5 Hz). $^2H$ NMR ($CHCl_3$) δ 0.82 (1.0D, bs), 1.22 (1.8D, bs). CIMS *m/z* (only the central ion of isotope clusters reported) 320 (35%, $MNH_4^+$), 304 (23), 288 (100), 259 (50), 245 (55), 229 (64), 215 (79), 199 (57), 150 (45), 128 (43), 98 (30), 69 (28), 60 (35), 59 (22), 58 (41). Deuterium distribution calculated from the $MNH_4^+$ cluster after correction for natural isotopic abundance: $^2H_0$ (10.3%), $^2H_1$ (14.6%), $^2H_2$ (14.5%), $^2H_3$ (12.6%), $^2H_4$ (10.5%), $^2H_5$ (8.6%), $^2H_6$ (7.6%), $^2H_7$ (6.9%), $^2H_8$ (5.6%), $^2H_9$ (4.5%), $^2H_{10}$ (4.2%).

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