Metabolites of the higher fungi. Part 33.[1] Grammicin, a novel bicyclic $C_7H_6O_4$ furanopyranol from the fungus *Xylaria grammica* (Mont.) Fr.

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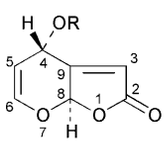
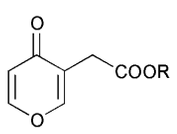
Grammicin **1** from *Xylaria grammica* is identified as 4-hydroxy-4*H*-furo[2,3-*b*]pyran-2(7a*H*)-one by chemical and physical methods. 4-Oxo-4*H*-pyran-3-acetic acid (γ-pyrone-3-acetic acid) **2** is a co-metabolite and the conversion of **1** into **2** is discussed.

Introduction

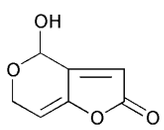
In part 31 of this series[2] a novel $C_7H_6O_4$ spiro lactone from the fungus *Xylaria longiana* was described and this has since been shown to originate biosynthetically from a phenolic precursor along a pathway similar to that of patulin.[3] Patulin[4] **3** and isopatulin[5] **4** are unique toxic lactones with complex chemistry for compounds of such a low molecular weight. From cultures of the fungus *Xylaria grammica* we now report the isolation, characterisation and chemistry of a new unique isomer of patulin, which we name grammicin **1**.



1 R=H
5 R=Ac



2 R=H
10 R=CH_3



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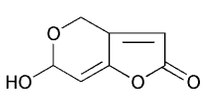

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The fungus genus *Xylaria* is now probably the largest within the family Xylariaceae comprising more than 500 species. *Xylaria grammica* occurs widely in the tropics where it is found growing on rotten wood. In the wild it typically produces cylindrical spore bearing stromata up to 24 cm long and 1.5 cm wide. However, in culture mature spore bearing stromata are not produced. *Xylaria* species are frequently misidentified but *Xylaria grammica* has been consistently interpreted from areas such as tropical America and tropical Africa.[6] For this investigation two different isolates (KC109 and 12EBV) were collected from wood in Cameroon and one (P114) from wood in Peru. In addition, three out of sixteen endophytic xylaria isolates (306, 307 and 536) from a teak leaf collected from a mixed deciduous forest at 900 metres above sea level in the Mae Rim District, Chiang Mai Provence, Thailand have yielded a similar metabolite mixture to that from the authenticated *grammica* strains.

Confirmation of their identity as *Xylaria grammica* was obtained by comparing the shape and appearance of the immature stromata with immature stromata material from an authenticated culture and by successfully inducing them to grow on wood and produce mature stromata bearing identifiable spores.[7] This work suggests that metabolite identification is useful in the identification of *Xylaria* endophytes and may lead to the discovery of new species.

Results and discussion

After surface culture over eight weeks on 3% malt extract containing 6% additional glucose the mycelium was extensively covered with long (5 cm) unbranched immature brown stromata, each with a colourless apex; small drops of a colourless liquid were exuded from the surface of the stromata. Solvent extraction of the medium and chromatography of the resulting gum in the solvent system toluene–ethyl acetate–acetic acid (50 : 49 : 1) gave grammicin **1** as a yellow oil [$C_7H_6O_4$; ν_{max} 3500–3300, 1795 cm^{-1}; λ_{max}(EtOH) 263 nm (ε/dm^3 mol^{-1} cm^{-1} 1540)] and γ-pyrone-3-acetic acid (4-oxo-4*H*-pyran-3-acetic acid) **2**. The ^{13}C NMR spectra of **1** in $CDCl_3$ show seven carbon resonances (Table 1), which comprise five methine plus two quaternary at δ 164.60 and 169.50. However, the latter is very weak, presumably due to a long relaxation time. The corresponding resonance is significantly stronger in $(CD_3)_2CO$ (Table 2) and confirms the presence of seven carbon atoms. Whilst $(CD_3)_2CO$ proved to be the best solvent for obtaining ^{13}C NMR data, and $^2J_{C-H}$ and $^3J_{C-H}$ long range correlation data using the FLOCK pulse sequence,[8] the ^1H NMR spectra were better resolved in $CDCl_3$. The IR absorption at 1795 cm^{-1} is indicative of a lactone and this is confirmed by the presence of the quaternary carbon resonance at δ 170.49. In the FLOCK spectrum [$(CD_3)_2CO$] this is seen as adjacent to the unsaturated methine carbon at δ 114.49, which in the ^{13}C–^1H COSY spectrum correlates with the uncoupled unsaturated proton at δ 6.13. There is also a long range correlation of the latter to a methine carbon resonance at δ 98.55. These data suggest the presence of an α,β-unsaturated γ-lactone. The infrared absorption is higher than would normally be expected for α,β-unsaturated γ-lactones and is more characteristic of β,γ-unsaturated systems as seen in angelica lactone (1800 cm^{-1}). However, other factors such as exocyclic unsaturation at the γ-position as in patulin, chelation as in γ-acetoxy-γ-valerolactone (1797 cm^{-1}) and spiro fusion at

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Table 1 ^{1}H and ^{13}C NMR data for grammicin in $CDCl_3$

Position	δ_H	δ_C	$\delta_C\ ^2J_{C-H}$	$\delta_C\ ^3J_{C-H}$
2	—	169.50		
3	6.20 (1 H, dd, *J* 2.25, 0.68 Hz)	115.63		
4	5.28 (1 H, ddd,[a] *J* 2.25, 2.29, 2.66 Hz)	63.26		
5	4.98 (1 H, dd, *J* 6.05, 2.66 Hz)	103.81	142.49	
6	6.36 (1 H, ddd, *J* 6.05, 2.29, 0.55 Hz)	142.49	103.81	
8	6.11 (1 H, d, *J* 0.54 Hz)	97.50		
9	—	164.60		
4-OH	2.49 (1 H, br s)			

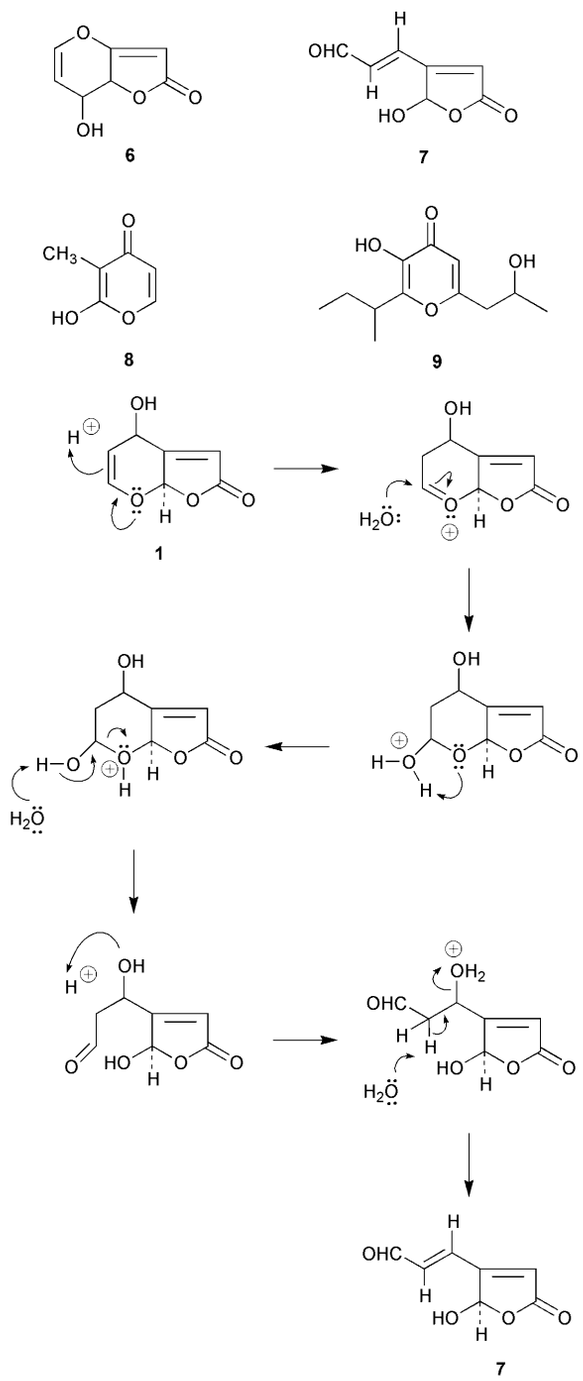
[a] Pseudo-quartet.

Table 2 ^{1}H and ^{13}C NMR data for grammicin in acetone-d_6

Position	δ_H	δ_C	$\delta_C\ ^2J_{C-H}$	$\delta_C\ ^3J_{C-H}$
2	—	170.49		
3	6.13 (1 H, dd, *J* 2.22, 0.78 Hz)	114.49	170.49	98.55
4	5.38 (1 H, br s)	63.43	167.91	
5	4.98 (1 H, dd, *J* 6.14, 2.56 Hz)	105.98	141.81	
6	6.40 (1 H, dd, *J* 6.14, 2.39 Hz)	141.81	105.98	
8	6.29 (1 H, s)	98.55		
9	—	167.91		
4-OH	3.0 (1 H, br s)			

the β-position as in the saturated γ-lactone longianone (1787 cm^{-1}) contribute to high frequency absorptions. Acetylation of **1** with acetic anhydride and pyridine yields a crystalline monoacetate **5** [$C_9H_8O_5$; ν_{max}1786 and 1736 cm^{-1}] and in its ^{1}H NMR spectrum ($CDCl_3$) the proton at δ 5.28 in the parent moves downfield $\Delta\delta$ 0.96, identifying it as a secondary alcohol methine proton. The proton at δ 5.28, which correlates in the ^{13}C–^{1}H COSY spectrum to the methine carbon at δ 63.26, is coupled to three other protons at δ 6.20, 4.98 and 6.36. The last two are *cis*-coupled to each other, (*J* 6.05 Hz) and in the ^{13}C–^{1}H spectrum they correlate respectively to the unsaturated methine carbons at δ 103.81 and 142.49; the latter from its position is adjacent to oxygen. This means that the methine carbon at δ 97.50 must be that at the γ-position of the lactone ring and the quaternary resonance at δ 164.60 that at the β-position; the low field position of the former favours structure **1** for grammicin rather than the alternative **6**. A NOESY spectrum ($CDCl_3$) of the acetate **5** suggests that the relative configuration of the methine proton at δ 6.24 (δ 5.28 in **1**) and the proton at δ 6.15 (δ 6.11 in **1**) is *trans* as no cross peak was detected.

When **1** is heated with dilute aqueous hydrochloric acid for 30 min an isomeric crystalline solid **7** [$C_7H_6O_4$; mp 153 °C; ν_{max}3223, 1768 and 1668 cm^{-1}; λ_{max}/nm 263 and 361 (ε/dm^3 mol^{-1} cm^{-1} 21668 and 3234)] is formed. The compound yields a red dinitrophenylhydrazone and the presence of a proton at δ 9.77 and a methine carbon at δ 194.21 identifies the compound as an aldehyde. The infrared absorption now at 1768 cm^{-1} indicates the retention of the lactone ring, which is now at a lower, more normal frequency. The aldehyde is unsaturated; the aldehyde proton is a doublet (*J* 7.51 Hz) and is coupled to an unsaturated methine proton at δ 6.72, which is *trans* coupled (*J* 16.12 Hz) to an unsaturated methine proton at δ 7.60. In the FLOCK spectrum the lactone α-methine proton at δ 6.61 correlates to the lactone methine carbon at δ 98.42 and an unsaturated methine carbon at δ 139.89; these bear a proton at δ 6.51 and the proton at δ 7.60 respectively. In addition, the unsaturated methine proton at δ 6.72 adjacent to the aldehyde group correlates to the lactone quaternary β-unsaturated carbon now at δ 159.60. A proton doublet at δ 7.14 is exchanged on addition of D_2O, identifying it as the proton of a hydroxy group situated at the γ-position of the lactone ring. The formation of this aldehyde **7** can be explained by an initial protonation of the pyran double bond, followed by the addition of water and then fission of the six-membered ring to yield a β-hydroxy aldehyde,

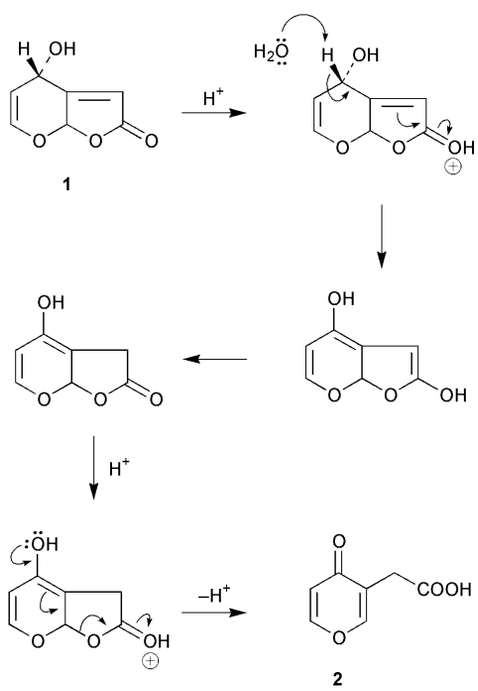


Scheme 1

which further dehydrates to yield the *trans* unsaturated aldehyde (Scheme 1).

When grammicin or its acetate is refluxed with aqueous 2% acetic acid in THF over 7 days a second crystalline isomer ($C_7H_6O_4$; ν_{max} 3600–2800, 1725 and 1638 cm^{-1}) is obtained in

addition to the aldehyde. This compound was identified as an acid by its reaction with diazomethane to produce a crystalline methyl ester. In this compound the infrared lactone absorption found in grammicin and the unsaturated aldehyde has been replaced by a low frequency carbonyl band at 1638 cm^{-1} (1660 cm^{-1} in the ester), which in the ^{13}C NMR spectrum can be associated with a new quaternary carbon at δ 177.51. This must be a conjugated ketone carbonyl because the positioning of two unsaturated methine carbons at δ 156.27 and 154.34 indicates they share attachment to the remaining oxygen atom. In the ^{13}C–^{1}H COSY spectrum these two unsaturated methine carbons correlate to two low field methine protons at δ 7.91 and 8.16 respectively; both are long range coupled (J 0.73 Hz) to each other and the former is also coupled to an adjacent methine proton at δ 6.47 (d, J 5.86 Hz) associated with an unsaturated methine carbon at δ 116.82. The molecule contains a methylene group whose protons at δ 3.74 are only long range W-coupled (J 0.73 Hz) and must connect only to quaternary carbons. The FLOCK spectrum identifies the compound as γ-pyrone-3-acetic acid **2** by showing the correlation of these methylene protons to an unsaturated quaternary carbon at δ 125.63, the quaternary carboxy and carbonyl carbons at δ 172.94 and 177.51, respectively, and the unsaturated methine carbon at δ 154.54. The high field position of the carbonyl carbon resonance is typical of γ-pyrone derivatives as exemplified by that of maltol[9] **8** at δ 177.2 and hyalopyrone[10] **9** at δ 180.1. A possible mechanism for this conversion is suggested (Scheme 2). The aldehyde- and acid-producing reactions are



Scheme 2

apparently competitive. In addition to grammicin the isolates from Cameroon and Peru also produced the acid **2**, and its methyl ester **10**, the former was quite readily isolated without chromatography. The methyl ester, was identified by comparison of its mp, mixed mp, IR, ^{13}C and ^{1}H NMR spectra with those of the ester formed by methylation of the acid with diazomethane. However, in the case of the endophytic isolates only the ester was produced.

The acid is known as xylaric acid and has quite recently been isolated[11] from the culture broth of an uncharacterised endophytic xylaria species isolated from the bark of a live oak in Columbia, South Carolina; interestingly, the compound has been shown to inhibit the interleukin-1β converting enzyme. In the report of this acid no mention was made of the formation of any other metabolite by this fungus and from the description of the growth characteristics the species is not *X. grammica*. The only reported synthesis of the acid[12] is from desoxypatulinic acid,[13] first described during early work on the identification of the structure of patulin, and its easy formation in culture from the widely available *X. grammica* and from grammicin offers a useful route to this difficultly accessible, interesting biologically active compound.

Experimental

General

Mps were determined on a Kofler hot-stage apparatus and are uncorrected, IR spectra on either a Perkin-Elmer 681 or a Nicolet 205 spectrophotometer; mass spectra (EI) and (FAB using 3-nitrobenzyl alcohol as matrix) on an AEI MS 902 spectrometer equipped with a MSS Data System for Windows (Data Version 2.03, Software Version 10.0). Optical rotations were recorded on a Perkin-Elmer 141 polarimeter and are reported in units of 10^{-1} deg cm^2 g^{-1}. Extracts were dried over Na_2SO_4. All chromatography columns, thick layer (PLC) and thin layer (TLC) glass plates were made up using Merck Kieselgel GF_{254}. Column sizes and solvent systems used are specified in each case. Flash chromatography was carried out using Fluka Kiesel gel 60 (239–400 mesh). ^{1}H and ^{13}C NMR spectra, using tetramethylsilane as internal standard, were determined at 270 and 67.8 MHz respectively with a JEOL GX270 spectrometer fitted with a dual 5 mm C/H probe. ^{1}H NMR spectra were acquired with 32 K data points over a spectrum width of 3001.2 or 6002.4 Hz; J values are given in Hz. Carbon atom types were established in the ^{13}C NMR spectrum by employing a combination of broad-band proton-decoupled and distortionless enhancement by polarisation transfer (DEPT) experiments with 32 K data points over a spectrum width of 17605.6 Hz. Assignments were established by employing a combination of 1-D and 2-D NMR experiments. 2-Dimensional spectra were acquired and processed by standard JEOL software; ^{1}H–^{1}H correlations by double quantum-filtered COSY (VDQFN), resolution 2.93 Hz in the $f1$ and $f2$ domains, PW1 = PW2 = $\pi/2$; $[^{1}J_{C-H}]^{13}C$–^{1}H correlations (VCHSHF), resolution $f2$ 17.19 and $f1$ 5.9 Hz, pulse delay 1, 2 or 3 s, J_{C-H} 140 Hz; and $[^{2}J_{C-H}$ and $^{3}J_{C-H}]^{13}C$–^{1}H correlations were established using the FLOCK pulse sequence of Reynolds *et al.*,[8] resolution $f2$ 17.19 and $f1$ 5.9 Hz, pulse delay 1, 2 or 3s, Δ^1 86.5 and Δ^2 46.5 ms or Δ^1 44.0 and Δ^2 24.0 ms.

Isolation of the metabolites

X. grammica (isolates no. KC 109, 12 EBV, P 114) was grown for eight weeks in subdued daylight at 23 °C in Thompson bottles (4 × 2 dm^3) each containing malt media (1 dm^3, 3%) containing additional glucose (6%). The mycelium was removed by filtration through muslin and the medium extracted with ethyl acetate (3 × 1 dm^3). The dried extract was evaporated to yield a semi-solid gum (6.1 g), which was triturated with a little ethyl acetate and the mixture filtered to yield a pale brown solid (0.68 g), which after crystallisation either from a mixture of acetone–hexane, or ethyl acetate, gave *4-oxo-4H-pyran-3-acetic acid* (γ-pyrone-3-acetic acid) **2** as needles (0.56 g), mp 172–173 °C (sublimes at 150 °C), *m/z* 154 (M^+, 5%), 136 (41), 110 (100) (Found C, 54.7; H, 3.8. Calculated for $C_7H_6O_4$ C, 54.55; H, 3.9%); ν_{max}(KBr)/cm^{-1} 3600–3300, 1725 and 1638; $\delta_H(C_5D_5N)$ 3.74 (2 H, d, J 0.73 Hz, 7-H), 6.47 (1 H, d, J 5.86 Hz, 5-H), 7.91 (1 H, dd, J 5.86, 0.73 Hz, 6-H), 8.16 (1 H, d, J 0.73 Hz, 2-H) and 10.75 (1 H, br s, OH); $\delta_C(C_5D_5N)$ 31.73 (7-CH_2), 116.82 (5-CH), 125.63 (3-C), 154.54 (2-CH), 156.27 (6-CH), 172.94 (8-C) and 177.51 (4-C).

The residue (5.33 g) remaining after evaporation of the triturating solvent was dissolved in the solvent mixture toluene–

ethyl acetate–acetic acid (60 : 40 : 1) and applied to a column of silica gel (3.5 × 46 cm). Fractions of 2 cm^3 were collected.

Tubes 113–214. Evaporation of the solvent yielded *4-hydroxy-4H-furo[2,3-b]pyran-2(7aH)-one* (grammicin) **1** as a yellow oil (2.49 g); $C_7H_6O_4$; *m/z* 154 (M^+, 4%), (CI[M + NH_4]$^+$ 172.0610, $C_7H_{10}O_4N$ requires 172.0610); $[\alpha]_D^{23}$ +23.89° (*c* 0.98 in EtOH); ν_{max}($CHCl_3$)/cm^{-1} 3400–3500 and 1790; λ_{max}(EtOH)/nm 213 (ε/dm^3 mol^{-1} cm^{-1} 9024) and 263 (ε 1540); δ_H and δ_C data are presented in Tables 1 and 2.

Tubes 381–499. These gave a solid, which crystallised from a mixture of acetone and hexane, or sublimed under vacuum at 90 °C, to yield *methyl 4-oxo-4H-pyran-3-acetate* **10** as diamond-shaped crystals (0.13 g); $C_8H_8O_4$; mp 92 °C; *m/z* 168 (M^+) (Found: C, 57.0; H, 4.8. $C_8H_8O_4$ requires C, 57.1; H, 4.8%); ν_{max}(KBr)/cm^{-1} 1738 and 1660; δ_H($CDCl_3$) 3.42 (2 H, d, *J* 1.10 Hz, 7-H), 3.72 (3 H, s, CH_3O), 6.39 (1 H, d, *J* 5.86 Hz, 5-H), 7.76 (1 H, dd, *J* 5.86, 1.10 Hz, 6-H) and 7.85 (1 H, d, *J* 1.10 Hz, 2-H); δ_C($CDCl_3$) 30.48 (7-CH_2), 52.29 (CH_3O), 116.75 (5-CH), 124.06 (3-C), 153.97 (2-CH), 155.54 (6-CH), 170.68 (8-C) and 177.40 (4-C).

The endophytic isolates no. 306, 307, and 536 were similarly cultured; all three gave the same products. Culture 307 gave the highest yield of grammicin **1** (2.49 g) and γ-pyrone-3-methyl acetate (methyl 4-oxo-4*H*-pyran-3-acetate) **10** (0.13 g). γ-Pyrone-3-acetic acid **2** was not produced by any of the three cultures.

Acetylation of grammicin 1

Grammicin **1** (2.0 g) was mixed with acetic anhydride (5 cm^3) and pyridine (3 drops). The mixture, which darkened and became warm, was set aside overnight and then poured on to ice. After 3 h at 5 °C the brown crystalline solid (1.6 g) was filtered and the filtrate neutralised with aqueous sodium hydrogen carbonate and extracted with ether (3×). Evaporation of the dried ether extract gave a gum, which crystallised immediately to yield additional solid (0.3 g). Recrystallisation of the combined solids from light petroleum (bp 80–100 °C) gave *4-acetoxy-4H-furo[2,3-b]pyran-2(7aH)-one* **5** as colourless needles (1.46 g); mp 81–83 °C; *m/z* 196 (M^+) (Found: C, 55.4; H, 4.0. $C_9H_8O_5$ requires C, 55.1; H, 4.1%); $[\alpha]_D^{23}$ +234° (*c* 1.06 in EtOH); ν_{max}(KBr)/cm^{-1} 1786, 1736 and 1637; δ_H($CDCl_3$) 2.19 (3 H, s, 11-CH_3), 4.96 (1 H, dd, *J* 6.23, 2.75 Hz, 5-H), 6.08 (1 H, dd, *J* 2.20, 0.73 Hz, 3-H), 6.15 (1 H, s, 8-H), 6.24 (1 H, ddd, *J* 2.20, 2.20, 2.75 Hz, 4-H) and 6.45 (1 H, ddd, *J* 6.23, 2.20, 0.73 Hz, 6-H); δ_C($CDCl_3$) 20.75 (11-CH_3), 64.36 (4-CH), 97.38 (8-CH), 100.16 (5-CH), 116.02 (3-CH), 143.69 (6-CH), 159.51 (9-C), 168.76 (2-C)* and 170.03 (10-C)* (* indicates assignments that may be interchanged).

3-(2-Hydroxy-5-oxo-2,5-dihydrofuran-3-yl)propenal 7

(a) A solution of grammicin **1** (500 mg) in aqueous hydrochloric acid (5 cm^3, 10%) was heated for 30 min on a boiling water bath. The orange–brown solution was evaporated to dryness under vacuum and the red crystalline residue (500 mg) was purified by flash chromatography in the solvent system toluene–ethyl acetate (50 : 50). Evaporation of the eluent gave a pale yellow, crystalline solid (200 mg), which after recrystallisation either from a mixture of acetone and light petroleum (bp 60–80 °C), or from water, yielded *3-(2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl)propenal* **7** as needles (170 mg); mp 151–153 °C; *m/z* 154 (M^+) (Found: C, 54.7; H, 4.0. $C_7H_6O_4$ requires C, 54.55; H, 3.9%); ν_{max}(KBr)/cm^{-1} 3223, 1768 and 1668; λ_{max}(EtOH)/nm 214 (ε/dm^3 mol^{-1} cm^{-1} 21267), 263 (ε 21668) and 361 (ε 3234); δ_H[$(CD_3)_2CO$] 6.51 (1 H, s, 5-H), 6.61 (1 H, d, *J* 0.73 Hz, 3-H), 6.72 (1 H, ddd, *J* 16.12, 7.51, 0.73 Hz, 7-H), 7.14 (1 H, d, *J* 8.43 Hz, OH), 7.60 (1 H, dd, *J* 16.12, 0.73 Hz, 6-H) and 9.77 (1 H, d, *J* 7.51 Hz, 8-H), δ_C[$(CD_3)_2CO$] 98.42 (5-CH), 125.04 (3-CH), 136.76 (7-CH), 139.89 (6-CH), 159.60 (4-C), 170.06 (2-C) and 194.21 (8-CHO).

(b) A solution of grammicin acetate **5** (200 mg) in aqueous acetic acid (6 cm^3, 5%) was refluxed for 1.5 h. The orange-coloured solution was evaporated to dryness under vacuum and the residue purified by PLC in the solvent system toluene–ethyl acetate–acetic acid (50 : 49 : 1). The products were detected by UV. Band 1 (R_f 0.9 blue) gave unchanged acetate **5** (52 mg) and band 2 (R_f 0.4 violet–blue) gave the propenal **7** (60 mg) as described above after recrystallisation from a light petroleum–acetone mixture.

4-Oxo-4*H*-pyran-3-acetic acid 2 from grammicin acetate 5

A solution of grammicin acetate **5** (200 mg) in aqueous acetic acid (1 cm^3, 2%) and tetrahydrofuran (5 cm^3) was refluxed for 7 days. The deep orange solution was evaporated to yield a brown semi-solid. The crude product, which gave a blue and two violet spots (TLC toluene–ethyl acetate–acetic acid, 50 : 49 : 1) when visualised at 366 nm, was triturated with ethyl acetate to give a brown solid (79 mg). Recrystallistion of the latter from ethyl acetate gave 4-oxo-4*H*-pyran-3-acetic acid **2** as rosettes of small needles; mp 172–173 °C. Evaporation of the trituration solvent yielded a gum which was purified by PLC to give unchanged grammicin acetate **5** (top blue spot, 70 mg), 3-(2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl)propenal **7** (middle violet spot, 20 mg) and additional 4-oxo-4*H*-pyran-3-acetic acid **2** (bottom violet spot close to baseline, 7 mg).

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References

1 Part 32. R. L. Edwards, D. J. Maitland, I. J. Scowen, A. J. T. De Sousa and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1*, 2001, 537.
2 R. L. Edwards, D. J. Maitland, C. L. Oliver, M. S. Pacey, L. Shields and A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1*, 1999, 715.
3 R. J. M. Goss, J. Fuchser and D. O'Hagan, *Chem. Commun.*, 1999, 2255.
4 F. M. Dean, *Naturally Occurring Oxygen Ring Compounds*, Butterworth, London, 1963, p. 518 and references cited therein.
5 J. Sekiguchi, G. M. Gaucher and Y. Yamada, *Tetrahedron Lett.*, 1979, 41.
6 R. W. G. Dennis, *Rev. Biol. (Lisbon)*, 1958, **1**, 194; R. W. G. Dennis, *Kew Bulletin*, 1956, **11**, 425.
7 S. Mekkamol, PhD Thesis, Liverpool John Moores University, 1998.
8 W. F. Reynolds, S. McLean, M. Perpick-Dumont and R. G. Enriquez, *Magn. Reson. Chem.*, 1989, **27**, 162.
9 R. P. Borris, K. Seifert, S. Johne and M. Hesse, *Helv. Chim. Acta*, 1982, **65**, 2481.
10 P. Venkatasubbaiah, C. G. Vandyke and W. S. Chilton, *Mycologia*, 1992, **84**, 719.
11 M. J. Salvatore, O. D. Hensens, D. L. Zink, J. Liesch, C. Dufresne, J. G. Ondeyka, T. M. Jurgens, R. P. Borris, S. Raghoobar, E. McCauley, L. Kong, S.E. Gartner, G. E. Koch, F. Pelaez, M. T. Diez, C. Cascales, I. Martin, J. D. Polishook, M. J. Balick, H. T. Beck, S. R. King, A. Hsu and R. B. Lingham, *J. Nat. Prod.*, 1994, **57**, 755.
12 B. G. Engel, W. Brzeski and Pl. A. Plattner, *Helv. Chim. Acta*, 1949, **32**, 1752.
13 P. M. Scott, B. Kennedy and W. Van Walbeek, *Experientia*, 1973, **28**, 1252.
14 The United Kingdom Chemical Database Service, D. A. Fletcher, R. F. McMeeking and D. J. Parkin, *J. Chem. Inf. Comput. Sci.*, 1996, **36**, 746.