Biologically Active Secondary Metabolites from the Ascomycete A111-95

2. Structure Elucidation

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(Received for publication July 30, 2001)

In the preceding paper[1)] the isolation and biological properties of eight lactones from the ascomycete A111-95 is described (compounds **3** and **4** were obtained as a mixture), while this part discusses the determination of their chemical structures. Seven of the compounds, **1**~**7**, are 5-membered lactones of what appears to be the same biosynthetic pathway, while 4-methoxy-6-pentylpyrone (**8**) appears not to be related with the others. The strongest nematicidal effect was demonstrated by the mixture of **3** and **4**, while galiellalactone (**7**) was shown to be a moderately cytotoxic agent.

Compounds **1**, **3** and **4** are new compounds while compound **2**[2)] and galiellalactone (**7**)[3)] previously have been reported from cultures of *Galiella rufa*, although the structure elucidation of **2** was never discussed (the structure of galiellalactone (**7**) was determined by X-ray crystallography). Pregaliellalactone (**5**)[4)], desoxygaliellalactone (**6**)[5)] and the pyrone **8**[6)] have been prepared synthetically, **5** and **6** in an effort to elucidate the biosynthesis of galiellalactone (**7**), but are reported here for the first time as natural products. As many of the spectroscopic data of the previously reported five-membered lactones **1**~**7** only can be found in doctoral theses, at least the NMR data recorded in this investigation are presented for all compounds **1**~**7** (see Experimental). The structure elucidation of all eight compounds was fairly straight-forward, as the molecules are small and the MS as well as NMR data are unambiguous. In all cases, except for compound **2** which was available in too low amounts for ^{13}C NMR spectroscopy, were the compounds subjected to 2D homo- as well as heteronuclear NMR experiments, and as an example the structure determination of compound **1** is discussed in greater detail. The high resolution MS data revealed that the composition of **1** is $C_{11}H_{14}O_2$, having 5 unsaturations, and together with the 1D NMR data this suggested that it contains one carbonyl group, three carbon–carbon double bonds and one ring. The two chains could be unfolded by following the COSY correlations starting from the signals of the two methyl groups, and their attachment to the γ-lactone ring was established by the long range ^{1}H-^{13}C correlations from 8-H_2 to C-6, C-7 and C-11 and from 4-H to C-5 and C-6. The structures of the other compounds were elucidated by parallel reasoning. The 5-H/7-H configuration in compound **4** was indicated by a strong NOESY correlation between the two protons. The absolute configuration of galiellalactone (**7**) was recently shown[7)] to be as depicted in Fig. 1, and as compounds **4**, **5** and **6** can be assumed to part of the same biosynthetic route they should have the same absolute configuration at C-7.

Experimental

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101). Mass spectra were recorded with a Jeol SX102 spectrometer, while the UV and the IR spectra were recorded with a Varian Cary 2290 and a Perkin Elmer 298 spectrometer. The melting point (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22 °C.

5-(*E*)-But-2-enylidene-3-propyl-5*H*-furan-2-one (**1**) was obtained as a yellowish oil. UV (MeOH) λ_{max} (ε): 320 nm (5,200). IR (KBr): 3350, 2920, 1735, 1640, 1470, 1385,

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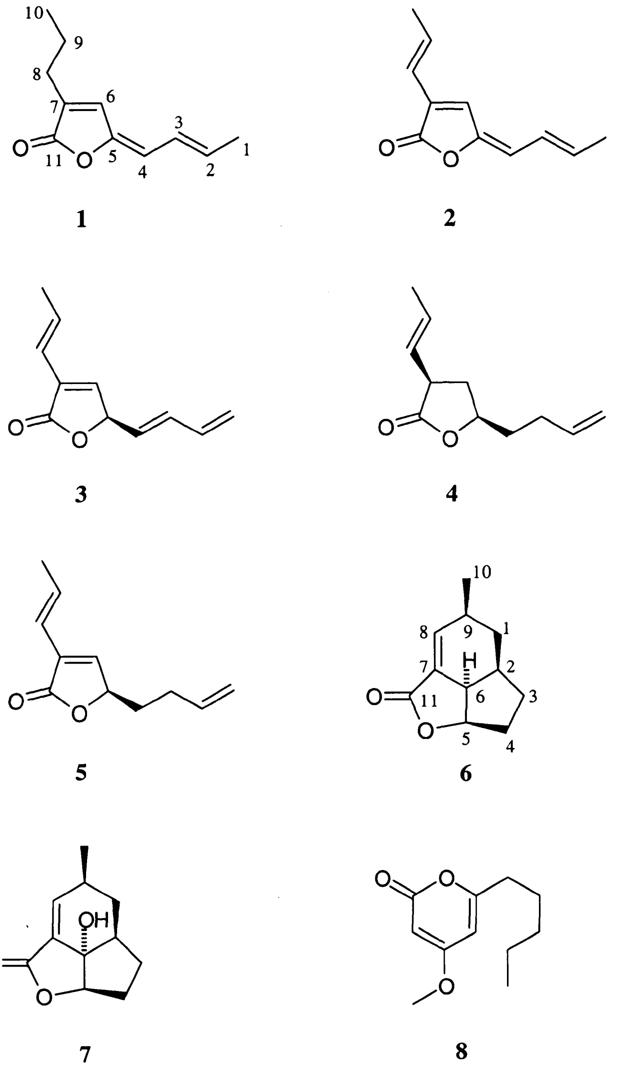
Fig. 1.



1280, 1105, 1040 and 745 cm^{-1}. ^{1}H (500 MHz) NMR data in CDCl$_3$ (δ; multiplicity; J): 6.99, t, 1.3, 6-H; 6.58, qdd, 1.6, 11.3, 15.2, 3-H; 5.99, qd, 7, 15, 2-H; 5.67, d, 11.3, 4-H; 2.36, t, 7.4, 8-H$_2$; 1.88, dd, 1.6, 6.9, 1-H$_3$; 1.62, tq, 7.4, 7.4, 9-H$_2$; 0.97, t, 7.4, 10-H$_3$. ^{13}C (125 MHz) NMR data in CDCl$_3$ (δ; multiplicity): 170.4 C-11, 146.4 C-5, 136.9 C-6, 135.5 C-2, 133.4 C-7, 124.9 C-3, 112.6 C-4, 27.2 C-8, 20.9 C-9, 18.8 C-1, 13.6 C-10. HREI-MS: 178.0989 (100%, M$^+$, calculated for $C_{11}H_{14}O_2$ 178.0994), 163 (22%), 149 (12%), 135 (33%), 121 (37%), 107 (16%), 82 (15%).

5-(*E*)-But-2-enylidene-3-(*E*)-propenyl-5*H*-furan-2-one (**2**) was obtained as a yellowish oil. UV (MeOH) λ_{max} (ε): 336 nm (7,400). IR (KBr): 3355, 2920, 1755, 1620, 1470, 1395, 1275, 1115, 1025 and 765 cm^{-1}. ^{1}H (500 MHz) NMR data in CDCl$_3$ (δ; multiplicity; J): 7.00, s, 6-H; 6.72, qd, 7, 16, 9-H; 6.51, dd, 7, 15, 3-H; 6.14, d, 16, 8-H; 5.98, qd, 7, 15, 2-H; 5.74, d, 11, 4-H; 1.83, d, 7, 1-H$_3$ and 10-H$_3$. Too small amounts were obtained for ^{13}C NMR spectroscopy. HREI-MS: 176.0833 (100%, M$^+$, calculated for $C_{11}H_{12}O_2$ 176.0837), 161 (15%) 135 (12%), 121 (24%), 107 (10%).

5-(*E*)-Buta-1,3-dienyl-3(*E*)-propenyl-5*H*-furan-2-one (**3**) and 5-(*E*)-But-3-enyl-3-(*E*)-propenyl-dihydrofuran-2-one (**4**) were obtained as a 1 : 1 mixture as a colourless oil. ^{1}H (500 MHz) NMR data in CDCl$_3$ (δ; multiplicity; J) for **3**: 6.95, m, 6-H; 6.82, dqd, 0.7, 6.8, 15.8, 9-H; 6.36, dd, 10, 16, 3-H; 6.31, ddd, 10, 10, 16, 2-H; 6.11, dqd, 0.7, 1.8, 15.8, 8-H; 5.49, m, 4-H; 5.35, d, 7.3, 5-H; 5.30, dd, 1.8, 15.9, 1-Ha; 5.21, dd, 1.8, 9.8, 1-Hb; 1.84, dm, 6.8, 10-H$_3$; and **4**: 5.79, ddd, 7, 10, 17, 2-H; 5.64, dqd, 1.3, 6.4, 15.4, 9-H; 5.48, m, 8-H; 5.05, ddd, 1.6, 1.8, 17.1, 1-Ha; 5.00, ddd, 1.2, 1.8, 10.2, 1-Hb; 4.38, dddd, 5, 5, 8, 10, 5-H; 3.26, m, 7-H; 2.47, ddd, 5.4, 8.6, 12.6, 6-Ha; 2.21, m, 3-H$_2$; 1.85, m, 4-Ha; 1.72, d, 6.4, 10-H$_3$; 1.72, m, 6-Hb; 1.71, m, 4-Hb. ^{13}C (125 MHz) NMR data in CDCl$_3$ (δ; multiplicity) for **3**: 171.8 C-11, 143.9 C-6, 135.2 C-2, 135.1 C-3, 134.1 C-9, 129.6 C-7, 126.6 C-4, 120.0 C-1, 119.6 C-8, 80.5 C-5, 18.9 C-10; and **4**: 177.3 C-11, 137.0 C-2, 129.8 C-9, 125.7 C-8, 115.6 C-1, 80.5 C-5, 44.2 C-7, 35.5 C-6, 34.6 C-4, 29.5 C-3, 17.9 C-10. A GC-MS analysis of the mixture showed that the components have the molecular weights 176 and 180, respectively.

Pregaliellalactone (**5**) was obtained as a yellowish oil, $[\alpha]_D^{22}$ $-40.8°$ (c 0.9 in CHCl$_3$). ^{1}H (500 MHz) NMR data in CDCl$_3$ (δ; multiplicity; J): 7.01; d; 0.7, 6-H; 6.78; qd; 6.8, 15.8, 9-H; 6.09; d; 15.8, 8-H; 5.77; ddd; 7, 10, 17, 2-H; 5.05; ddd; 1.6, 1.7, 17.1, 1-Ha; 5.01; ddd; 1.2, 1.8, 10.2, 1-Hb; 4.93; dd; 6, 6, 5-H; 2.22; m, 3-H$_2$; 1.80; d; 6.8, 10-H$_3$; 1.79; m, 4-Ha; 1.73; m, 4-Hb. ^{13}C (125 MHz) NMR data in CDCl$_3$ (δ; multiplicity): 172.0 C-11, 145.4 C-6, 136.8 C-2, 133.6 C-9, 129.7 C-7, 119.7 C-8, 115.9 C-1, 79.9 C-5, 32.8 C-4, 29.1 C-3, 18.8 C-10. HREI-MS: 178.0996 (58%, M$^+$, calculated for $C_{11}H_{14}O_z$ 178.0994), 163 (17%), 136 (75%), 133 (71%), 119 (32%), 95 (88%), 67 (100%), 55 (62%).

Desoxygaliellalactone (**6**) was obtained as a colourless oil, $[\alpha]_D^{22}$ $+61.9°$ (c 0.36 in CHCl$_3$). ^{1}H (500 MHz) NMR data in CDCl$_3$ (δ; multiplicity; J): 6.82; dd; 3, 3, 8-H; 5.03; m, 5-H; 3.04; m, 6-H; 2.43; m, 2-H; 2.14; m, 9-H; 2.10; m, 1-Ha; 1.84; m, 4-H$_2$; 1.76; m, 3-Ha; 1.15; m, 3-Hb; 1.14; d; 6.9, 10-H$_3$ 0.78; m, 1-Hb. ^{13}C (125 MHz) NMR data in CDCl$_3$ (δ; multiplicity): 170.5 C-11, 144.8 C-8, 129.9 C-7, 83.5 C-5, 44.2 C-6, 37.3 C-2, 34.5 C-1, 33.7 C-4, 31.7 C-3, 31.4 C-9, 20.1 C-10. HREI-MS: 178.0988 (89%, M$^+$, calculated for $C_{11}H_{14}O_z$ 178.0994), 149 (22%), 134 (71%), 119 (100%), 106 (83%), 105 (71%), 93 (49%), 91 (74%), 67 (26%).

Galiellalactone (**7**) was obtained as white crystals, mp

53~56°C, $[\alpha]_D^{22}$ −52.8° (*c* 0.2 in $CHCl_3$). ^1H (500 MHz) NMR data in $CDCl_3$ (δ; multiplicity; *J*): 6.99; d; 3.1, 8-H; 4.76; dd; 2.0, 7.5, 5-H; 3.39; s, 6-OH; 2.63; dqdd; 3, 7, 7, 8, 9-H; 2.43; dddd; 4.6, 7, 7, 10.6, 2-H; 2.24; ddd; 7.4, 7.4, 13.9, 1-Ha; 2.06; dddd; 7, 7, 11, 15, 4-Ha; 1.83; dddd; 3, 7, 7, 14, 3-Ha; 1.72; dddd; 2, 3, 7, 15, 4-Hb; 1.16; d; 7.3, 10-H_3; 1.15; m, 3-Hb; 1.04; ddd; 4.6, 8.0, 13.9, 1-Hb. ^{13}C (125 MHz) NMR data in $CDCl_3$ (δ; multiplicity): 170.1 C-11, 150.1 C-8, 130.7 C-7, 90.2 C-5, 81.7 C-6, 43.0 C-2, 33.0 C-1, 31.3 C-3, 31.3 C-4, 28.9 C-9, 20.8 C-10. HREI-MS: 194.0950 (37%, M^+, calculated for $C_{11}H_{14}O_3$ 194.0943), 176 (12%), 166 (100%), 150 (30%), 135 (39%), 122 (55%), 106 (67%), 94 (59%).

Acknowledgement

This work was supported by the Swedish Natural Science Research Council.

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