# New Macrocyclic Lactones from a *Penicillium* Species

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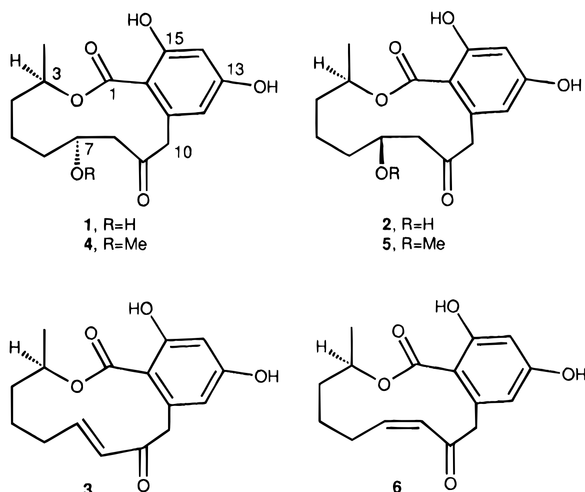
Six new macrocyclic lactones (**1**−**6**) were isolated from an extract of a *Penicillium* species. Structures were determined using a combination of spectroscopic techniques with emphasis on NMR spectroscopy.

In our search for low molecular weight ATP citrate lyase (ACL) inhibitors we recently isolated 2-chloro-1,3,8-trihydroxy-6-(hydroxymethyl)anthrone from a soil fungus of the genus *Penicillium*. This anthrone is a competitive ACL inhibitor with a $K_i$ of 283 ± 77 nM.[1] We now report the isolation of a series of new macrocyclic lactones from the same extract. These lactones (**1**−**6**) are structurally related to both curvularin[2−4] and the resorcyclic macrocyclic lactones,[5,6] but possess a modified carbon skeleton.




## Results and Discussion

The *Penicillium* sp. (preserved as Sterling Culture SC2193) was fermented and identified as previously described,[1] and the whole culture (1 L) was extracted with ethyl acetate (2 × 500 mL). The dried ethyl acetate extract (708 mg) was sequentially extracted with hexane (10 mL) and MeOH (5 mL). Further separation of the MeOH soluble fraction using reversed-phased HPLC gave lactones **1**−**6** as white solids. Photodiode array HPLC data showed that all six compounds had a UV maxima at approximately 300 nm, indicating that all compounds contained a structurally related aromatic moiety.

High-resolution mass spectrometry (HRMS) established the molecular formula for **3** as $C_{16}H_{18}O_5$. $^1H$, $^{13}C$, and COSY NMR spectral data (Tables 1 and 2) indicated the presence of an aromatic ring containing two meta-coupled hydrogens and two phenolic groups, an isolated methylene, a $CH_3CH(CH_2)_3CHCH$ system, a lactone carbonyl, and a ketone. An HMBC connectivities from H-8 to C-9 linked the aliphatic system to the ketone, indicating an α,β-unsaturated ketone. HMBC connectivities (Figure 1) from both H-10a and H-10b to C-9, C-11, and C-16, and from H-12 to C-10 placed the isolated methylene between the aromatic ring and the α,β-unsaturated ketone. Placing the lactone between C-3 and C-16, based on carbon chemical shift values, gave structure **3**. The double bond is in the *trans* configuration based on a 16.1 Hz coupling between H-7 and H-8.

HRMS, UV, CD, and NMR spectral data for **6** indicated that **6** differed from **3** only in double bond stereochemistry. A 11.9 Hz coupling between H-7 and H-8 indicated a *cis* configuration for **6**. The same HMBC connectivities were observed for **6** as were seen for **3**, indicating the structure as drawn. Further evidence for the carbon skeleton was provided by intense NOEs between H-12 and H-10a, and between H-12 and H-10b, placing H-12 close in space to both H-10a and H-10b.

COSY connectivities clearly established the presence of a $CH_3CH(CH_2)_3CH(OH)CH_2$ fragment in both compounds **1** and **2**. HMBC connectivities indicated that the carbon skeletons for compounds **1** and **2** was identical to that of **3**. Lactones **1** and **2** appeared to differ only in their relative stereochemistry. Lactones **4** and **5** differed from **1** and **2** by the presence of a methoxy instead of a hydroxy substituent at C-7. HPLC analysis of the original ethyl acetate extract confirmed the presence of lactones **1**, **2**, **4**, and **5** in relative quantities that were similar to the isolated ratios. Therefore lactones **1**, **2**, **4**, and **5** were not formed from **3** or **6** during the isolation procedure. Compounds **1**−**6** were inactive as ATP citrate lyase inhibitors.

Compound **3** had an $[\alpha]_D$ of +5.6°, while compound **6** had an $[\alpha]_D$ of +3.1°, indicating the same absolute configuration for the sole chiral centre at C-3 for both molecules. The absolute stereochemistry for compounds **1**−**6** was not determined, although comparison of the CD spectra for compounds **1**, **2**, **4**, and **5** with those of the macrocyclic zearalenones[6] indicated that the stereochemistry at the C-3 chiral center is probably *S*. This is deduced because the major influence on the n-π* transition band of the lactone at around 255−265 nm is the deviation from coplanarity of the C-12a C-1a C-1=O moiety,[7] which is probably determined by the configuration at C-3 for compounds **1**, **2**, **4**, and **5**.[6] The major influence on the sign of the n−π* band for these compounds is probably the chiral center at C-3 rather

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**Table 1.** ¹H NMR Data (δ, *J* in Hz) for Macrolactones **1**−**6** in $CD_3OD$

| position | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| 3 | 4.95 ddd (9.8, 6.0, 1.7) | 5.16 m | 5.13 m | 4.93 ddd (9.4, 6.0, 2.1) | 5.18 m | 5.02 bp (6.5) |
| 3-Me | 1.30 d (6.1) | 1.28 d (6.4) | | 1.31 d (6.1) | 1.28 d | 1.34 d (6.3) |
| 4a | 1.83 m | 1.65 m | 1.89 m | 1.78 m | 1.61 m | 1.84 m |
| 4b | 1.73 m | 1.65 m | 1.80 m | 1.78 m | 1.61 m | 1.66 m |
| 5a | 1.72 m | 1.58 m | 1.80 m | 1.65 m | 1.61 m | 1.66 m |
| 5b | 1.41 m | 1.58 m | 1.69 m | 1.39 m | 1.51 m | 1.66 m |
| 6a | 1.58 m | 1.80 m | 2.27 m | 1.70 m | 1.84 m | 2.50 m |
| 6b | 1.58 m | 1.52 m | 2.27 m | 1.49 m | 1.50 m | 2.22 m |
| 7 | 4.35 m | 4.34 m | 6.94 ddd (16.1, 8.7, 5.9) | 3.93 m | 3.88 m | 5.80 ddd (12.0, 10.0, 5.9) |
| 8a | 2.97 dd (13.2, 3.2) | 2.96 dd (15.4, 10.1) | 5.94 dt (16.1, 1.3) | 3.08 dd (13.4, 3.0) | 2.88 dd (15.7, 9.6) | 6.49 dt (11.8, 1.4) |
| 8b | 2.48 dd (13.2, 10.3) | 2.59 dd (15.4, 1.9) | | 2.40 dd (13.4, 10.1) | 2.70 dd (15.7, 1.8) | |
| 10a | 4.74 d (18.6) | 4.54 d (18.7) | 4.41 d (12.8) | 4.65 d (18.6) | 4.61 d (18.8) | 4.56 d (18.4) |
| 10b | 3.72 d (18.6) | 3.83 d (18.7) | 3.33 d (12.8) | 3.79 d (18.6) | 3.82 d (18.8) | 3.64 d (18.4) |
| 12 | 6.11 d (2.5) | 6.11 d (2.5) | 6.31 d (2.2) | 6.12 d (2.5) | 6.12 d (2.5) | 6.15 d (2.5) |
| 14 | 6.24 d (2.5) | 6.24 d (2.5) | 6.21 d (2.2) | 6.24 d (2.5) | 6.24 d (2.5) | 6.25 d (2.5) |
| OMe | | | | 3.40 s | 3.33 s | |

**Table 2.** ¹³C NMR Data (δ) for Macrolactones **1**−**6** in $CD_3OD$

| position | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| 1 | 172.54 | 172.27 | 171.08 | 172.45 | 172.26 | 172.47 |
| 3 | 76.16 | 73.94 | 73.73 | 76.08 | 73.85 | 76.54 |
| 3-Me | 21.19 | 18.62 | 20.66 | 21.06 | 18.57 | 21.44 |
| 4 | 33.77 | 33.64 | 34.99 | 33.90 | 33.51 | 32.05 |
| 5 | 22.04 | 19.40 | 25.47 | 21.69 | 19.03 | 26.06 |
| 6 | 36.75 | 36.89 | 32.61 | 34.34 | 34.00 | 27.43 |
| 7 | 67.18 | 68.01 | 151.96 | 77.16 | 78.17 | 139.87 |
| 8 | 53.56 | 51.34 | 131.30 | 49.56 | 47.44 | 132.44 |
| 9 | 206.18 | 207.85 | 201.75 | 208.74 | 208.14 | 204.38 |
| 10 | 50.88 | 52.95 | 44.27 | 51.02 | 53.17 | 51.44 |
| 11 | 139.64 | 139.87 | 136.50 | 139.57 | 139.90 | 139.59 |
| 12 | 114.00 | 113.74 | 110.52 | 113.94 | 113.76 | 113.80 |
| 13 | 163.72 | 163.86 | 158.40 | 163.66 | 163.90 | 163.61 |
| 14 | 102.94 | 103.02 | 102.67 | 102.94 | 103.04 | 102.94 |
| 15 | 166.17 | 166.76 | 161.14 | 165.95 | 166.84 | 166.76 |
| 16 | 106.86 | 106.49 | 114.73 | 107.10 | 107.15 | 105.95 |
| OMe | | | 56.69 | 56.48 | | |

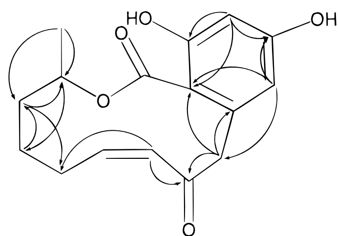


**Figure 1.** HMBC connectivities for compound **6**.

than the center at C-7 that is distant from the chiral chromophore. The n−π* band in the CD spectra of **1**, **2**, **4**, and **5** is of the same sign as that for the related zearalenones and zearalenols, which contain the same chiral chromophore with an *S* configuration at C-3. However, further data is needed to confirm this C-3 assignment for **1**, **2**, **4**, and **5**. Similar CD arguments were not applied to the assignment of the C-3 center for **3** and **6** because of conformational changes induced by the presence of a double bond. NMR comparisons indicated that **1** and **4** have the same relative configuration between C-3 and C-7, as do **2** and **5**, although the absolute configuration at C-7 for **1**, **2**, **4**, and **5** remains undefined.

## Experimental Section

**General Experimental Procedures.** One- and two-dimensional NMR spectra were recorded on a Bruker AMX 360 spectrometer. Chemical shifts are reported as δ values in ppm referencing $CHCl_3$ relative to TMS. UV spectra were recorded on a Shimadzu UV160U spectrophotometer. Optical rotation was determined on a Perkin-Elmer 241 polarimeter. CD spectra were recorded on as JASCO J-600 spectrophotometer. IR spectra were recorded on a Nicolet IBM IR/3X spectrometer. MS was performed on a Finnigan MAT TSQ 70 mass spectrometer, and HRMS data were obtained from M-Scan (Malvern, PA) using a VG ZAB 2-SE mass spectrometer. HPLC was performed on a Waters system with 990 photodiode array detection, 510 pump, 715 ultra WISP, automated gradient controller, and Powermate 386/20 computer.

**Organism.** The microbial culture was originally isolated from sandy loam soil, pH 5.9, taken from a roadside prairie near Pawnee Rock, KS. The organism was identified as a *Penicillium* sp. and the culture added to the Sterling culture collection as SC2193. Taxonomical identification was provided by Angela Belt (Blue Sky Research Service, Sonoma, CA) and was performed as previously described.[1] Briefly: The culture was incubated at 5, 25, and 37 °C and examined daily through day 7 and weekly thereafter for 4 weeks. The characteristics of monoverticilliate, nonvesiculate penicilli place the culture in the genus *Penicillium*. However, the greater than typical growth rate observed at 37 °C, the presence of a distinctively dark exudate and diffusible pigment coupled with the colony, and conidiophore morphology complicated final speciation of the organism.

**Fermentation.** Fermentation was performed as previously described.[1] Briefly: A seed culture was generated by inoculating a washed spore/mycelial suspension into previously described media. After 48 h, 1 mL of the seed culture was used to inoculate 30 mL of production media, which was incubated at 27 °C, 75% relative humidity, and 220 rpm in a New Brunswick Innova 4900 environmental shaker. Production cultures were harvested at 110 h, at which time the pH had dropped to 6.2−6.5.

**Isolation of Macrocyclic Lactones 1, 2, 3, 4, 5, and 6.** The whole culture (1 L) was extracted with ethyl acetate (2 × 500 mL). The dried ethyl acetate extract (708 mg) was then sonicated with hexane (10 mL) and centrifuged, and the hexane layer was decanted. Removal of hexane afforded 193 mg of an oil. MeOH (5 mL) was added to the hexane insoluble material and the sample sonicated and centrifuged. Removal of MeOH afforded 311 mg of a reddish/brown solid. The

remaining insoluble material (132 mg) was also reddish/brown. A portion of the MeOH soluble material (120 mg) was further separated using reversed-phase HPLC with a water/acetonitrile solvent gradient (70/30 to 30/70 over 30 min), yielding in order of elution, **1** (3.3 mg), **2** (2.4 mg), **3** (3.0 mg), **4** (5.2 mg), **5** (6.0 mg), and **6** (6.9 mg).

Macrolactone **1** was isolated as a redish/brown solid: mp 104−106 °C uncorrected; $[\alpha]_D$ = +21.4° (*c* 0.21, MeOH); CD (MeOH) max 264 ([$\vartheta$] 80 100), 228 (12 320), min 307 (−23 100), 235 (2770), 213 (−73 900) nm; HRFABMS *m/z* $[M + H]^+$ 309.1319, glycerol/thioglycerol ($C_{16}H_{21}O_6$ requires 309.1338); UV (MeOH) $\lambda$ max ($\epsilon$) 216 (12 940), 261 (8975), 302 (4710) nm; IR $\upsilon$ max 3312, 2923, 1713, 1602, 1355, 1308, 1262, 1209, 1174, 1097, 857, 799 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, refer to Tables 1 and 2.

Macrolactone **2** was isolated as a redish/brown solid: mp 128−132 °C uncorrected; $[\alpha]_D$ = +42.4° (*c* 0.15, MeOH); CD (MeOH) max 264 ([$\vartheta$] 77 000), 228 (36 960), min 311 (−9250), 238 (6150), 211 (−86 250) nm; HRFABMS *m/z* $[M + H]^+$ 309.1314, glycerol/thioglycerol ($C_{16}H_{21}O_6$ requires 309.1338); UV (MeOH) $\lambda$ max ($\epsilon$) 218 (14 612), 245 (14 512), 359 (12 081), 303 (6344) nm; IR $\upsilon$ max 3313, 2944, 1713, 1648, 1619, 1361, 1314, 1267, 1209, 1168, 1103, 849, 699 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, refer to Tables 1 and 2.

Macrolactone **3** was isolated as a redish/brown solid: mp 150−154 °C uncorrected; $[\alpha]_D$ = +5.6° (*c* 0.18, MeOH); CD (MeOH) max 281 ([$\vartheta$] −14 500), 230.0 (145 000), min 302 (−17 400), 252 (−116 000) nm; HRFABMS *m/z* $[M + H]^+$ 291.1798, glycerol/thioglycerol ($C_{16}H_{19}O_5$ requires 291.1796); UV (MeOH) $\lambda$ max ($\epsilon$) 222 (13 872), 296 (3971) nm; IR $\upsilon$ max 3169, 2944, 1613, 1590, 1344, 1262, 1168, 992, 845 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, refer to Tables 1 and 2.

Macrolactone **4** was isolated as a redish/brown solid: mp 92−94 °C uncorrected; $[\alpha]_D$ = +12.6° (*c* 0.34, MeOH); CD (MeOH) max 264 ([$\vartheta$] 86 300), min 306 (−22 900), 214 (−64 400) nm; HRFABMS *m/z* $[M + H]^+$ 323.1493, glycerol/thioglycerol ($C_{17}H_{23}O_6$ requires 323.1495); UV (MeOH) $\lambda$ max ($\epsilon$) 217 (14 780), 260 (11 123), 304 (7206) nm; IR $\upsilon$ max 3159, 2933, 1707, 1643, 1619, 1449, 1385, 1314, 1267, 1212, 1174, 1103, 854, 799, 758, 728 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, refer to Tables 1 and 2.

Macrolactone **5** was isolated as a redish/brown solid: mp 78−80 °C uncorrected; $[\alpha]_D$ = +4.6° (*c* 0.2, MeOH); CD (MeOH) max 263 ([$\vartheta$] 77 300), 226 (48 300), min 310 (−30 600), 236 (6450), 210 (−96 500) nm; HRFABMS *m/z* $[M + H]^+$ 323.1485, glycerol/thioglycerol ($C_{17}H_{23}O_6$ requires 323.1495); UV (MeOH) $\lambda$ max ($\epsilon$) 217 (15 168), 259 (11 110), 306 (8277) nm; IR $\upsilon$ max 3189, 2939, 1719, 1646, 1620, 1462, 1323, 1270, 1224, 1185, 1112, 845, 806 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, refer to Tables 1 and 2.

Macrolactone **6** was isolated as a redish/brown solid: mp 140−144 °C uncorrected; $[\alpha]_D$ = +3.1° (*c* 0.45, MeOH); CD (MeOH) max 265 nm ([$\vartheta$] 15 950), 225 (−300), min 316 (−4640), 232 (−3000), 212 (−10 150) nm; HRFABMS *m/z* $[M + H]^+$ 291.1235, glycerol/thioglycerol ($C_{16}H_{19}O_5$ requires 291.1233); UV (MeOH) $\lambda$ max ($\epsilon$) 219 (13 564), 263 (10 320), 303 (5762) nm; IR $\upsilon$ max 3383, 2929, 1641, 1607, 1458, 1322, 1254, 1159, 1105, 1058, 929, 854, 739 $cm^{-1}$; $^1H$ and $^{13}C$ NMR spectra, refer to Tables 1 and 2.

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