Naphthalenone and Phthalide Metabolites from *Lachnum papyraceum*[1]

R. Shan,[†] M. Stadler,[†] H. Anke,[‡] and O. Sterner*,[†]

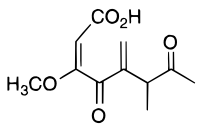
Division of Organic Chemistry 2, University of Lund, P.O.B. 124, S-221 00 Lund, Sweden, and Lehrbereich Biotechnologie der Universität Kaiserslautern, Paul Ehrlich-Strasse 23, D-67663 Kaiserslautern, Germany

Received February 28, 1997[⊗]

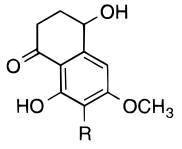
A new naphthalenone derivative (**2a**) and a phthalide (**6a**), as well as a methyl ester (**6b**), were isolated from submerged cultures of *Lachnum papyraceum*, together with known naphthalenone, phthalide, and chromanol derivatives. The structures of the new compounds, of which **2a** possesses weak antimicrobial and phytotoxic activities and weak cytotoxicity, were determined by spectroscopic methods.

The secondary metabolism of the ascomycete *Lachnum papyraceum* (Karst.) Karst (Hyaloscyphaceae) has, in previous studies, been shown to be based on chlorinated isocoumarin derivatives, which are further converted to potent nematicidal and antibiotic metabolites.[2] When this fungus was fermented in the presence of large amounts of $CaBr_2$ (100 mM), in an effort to obtain brominated metabolites, the secondary metabolism shifted completely, and the major metabolite was instead the penicillic acid analogue papyracillic acid (**1**).[3] In addition, smaller amounts of several chromanol, naphthalenone, and phthalide metabolites, of which compounds **2a**, **6a**, and **6b** are new, were produced. In this paper we describe the isolation and the chemical as well as biological characterization of the new metabolites.

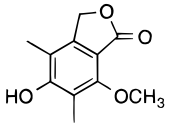
From the extracts of *L. papyraceum*, the naphthalenone derivatives **2a** and **2b**, the phthalides **3**, **4a**, **4b**, **6a**, and **6b**, and the chromanol **5** were isolated. The spectral data of the two naphthalenone derivatives **2a** and **2b** were very similar, and HRMS and 2D-NMR spectroscopy were used to determine their structures unambiguously. 6-*O*-Methylasparvenone (**2b**) has been reported previously from *Aspergillus parvulus*,[4] and biosynthetic studies[5] have indicated that it is, in fact, a pentaketide and that the ethyl group is attached at a later stage. This finding is supported by the isolation of both **2a** and **2b** from the same source in this investigation. The phthalide derivatives, on the other hand, appear to be tetraketides, derived from dimethylorsellinic acid. Compounds **3**, **4a**, **4b**, and **5** have been reported previously from *Aspergillus duricaulis*[6] and possess antibiotic activity. The carboxylic acid **6a** was isolated both as the free acid and as the methyl ester **6b**, and conversion of the acid to the ester by treatment with TMS−CH_2N_2 in MeOH confirmed their relationship. Neither compound showed a molecular ion in the EI MS due to the facile loss of the isopentenyl moiety, but chemical ionization with NH_3 gave MS data that were consistent with the NMR data obtained. The positions of the various functional groups were determined from the NOESY spectra, which showed that the isopentenyloxy group is attached to C-4 of the phthalide (strong NOESY correlation between 3-H_2 and 1′-H_2). Compound **2a** was shown to possess weak (MIC 100 μg/mL) antibacterial activity against *Micrococcus luteus* and *Enterobacter dissolvens* and antifungal activity against *Mucor miehei*, *Paecilomyces variotii*, and *Penicillium notatum*, while compound **6a** and its methyl ester **6b** were devoid of antimicrobial activity. All three compounds were weakly cytotoxic towards L-1210 cells, the concentration causing lysis of 50% of the cells after 48 h (IC_{50}) was 25 μg/mL for compounds **2a** and **6b** and 50 μg/mL for compound **6a**. A weak phytotoxic activity with compound **2a** was detected towards *Lepidium sativum* (IC_{50} 100 μg/disk), while **6a** and **6b** were inactive.



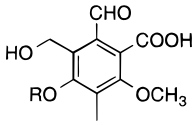
1



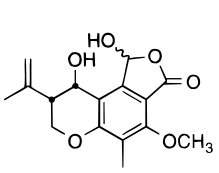
2a R=CH_3
2b R=CH_2CH_3



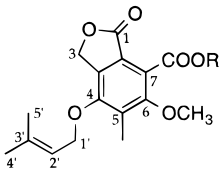
3



4a R=H
4b R=3-methyl-2-butenyl



5



6a R=H
6b R=CH_3

Although there is no apparent biogenetic relationship between the phthalide derivatives isolated in this investigation and the isocoumarins previously obtained from *L. papyraceum*, it is interesting to observe that papyracillic acid (**1**) theoretically could be derived from either group of compounds. A route that mimics the biosynthesis of the analogue penicillic acid could emanate from the isocoumarin 6-hydroxymellein,[3] but it is also reasonable to imagine that 2-*O*,3,5-trimethylorsellinic acid is converted to papyracillic acid (**1**) by the

* To whom correspondence should be addressed. Phone: +46-46 222 8213. Fax: +46-46 222 8209. E-mail: Olov.Sterner@orgk2.lth.se.
† University of Lund.
‡ Lehrbereich Biotechnologie der Uiversität Kaiserslautern.
⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1997.

S0163-3864(97)00145-6 CCC: $14.00

rearrangement of the C-3 methyl group to C-4 followed by the cleavage of the C-1/C-6 bond. However, this remains to be clarified by experiments with labeled precursors.

Experimental Section

General Experimental Procedures. (Trimethylsilyl)diazomethane used for methylation of compound **6a** was purchased from Aldrich, as a 2 M solution in hexane. The melting points, which are uncorrected, were determined using a Reichert microscope. Optical rotations were recorded with a Perkin-Elmer 141 polarimeter at 22 °C. UV spectra were obtained with a Perkin-Elmer λ 16 and IR spectra with a Bruker IFS 48. Mass spectra were recorded with a JEOL JMS-SX102 spectrometer, and NMR spectra were recorded at room temperature with a Bruker ARX 500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC, and HMBC experiments were performed with gradient enhancements using sine-shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH} = 145$ Hz and $^2J_{CH} = 10$ Hz. The raw data were transformed, and the spectra were evaluated with the standard Bruker UX-NMR software (rev. 941001).

Fermentation. *L. papyraceum* (Karst.) Karst (strain A48-88, mycelial cultures, deposited at LB Biotechnology, University of Kaiserslautern) was fermented as described previously,[3] with the addition of $CaBr_2$ (100 mM) after 10 days.

Extraction and Isolation. The mycelia were discarded, and the culture filtrate was extracted with EtOAc. The pure compounds were isolated from the extract by chromatography on SiO_2 eluted by mixtures of EtOAc and heptane. From a 40 L culture filtrate the following amounts of chromanol and phthalide derivatives were obtained: 37 mg of the naphtalenone **2a**, 8 mg of 6-*O*-methylasparvenone (**2b**), 3 mg of the phthalide **3**, 17 mg of the aldehyde **4a**, 138 mg of the aldehyde **4b**, 129 mg of compound **5**, 364 mg of the acid **6a**, and 80 mg of the ester **6b**.

3,4-Dihydro-4,8-dihydroxy-6-methoxy-7-methyl-1(2*H*)-naphthalenone (2a): white crystals; mp 161−163 °C; $[\alpha]_D$ +33° (*c* 1.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 223 (4.01), 288 (4.12) nm; IR (KBr) ν_{max} 3350 (OH), 2945 (CH), 1630 (C═O), 1595, 1575, 1495, 1420, 1355, 1320, 1290, 1220, 1155, 1145, 1080, 855 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.70 (1H, s, OH-8), 6.57 (1H, s, H-5), 4.66 (1H, dd, J = 3.9, 8.6 Hz, H-4), 3.79 (3H, s, OCH_3-6), 2,72 (1H, ddd, J = 4.6, 6.6, 17.7 Hz, Ha-2), 2.46 (1H, ddd, J = 4.8, 10.0, 17.7 Hz, Hb-2), 2.16 (1H, m, Ha-3), 1.94 (1H, m, Hb-3), 1.92 (3H, s, CH_3-7); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 202.6 (s, C-1), 163.8 (s, C-6), 161.4 (s, C-8), 146.0 (s, C-4a), 111.9 (s, C-7), 109.5 (s, C-8a), 100.2 (d, C-5), 67.3 (d, C-4), 55.4 (q, O*C*H$_3$-6), 34.7 (t, C-2), 31.4 (t, C-3), 6.9 (q, *C*H$_3$-7); EIMS (70 eV) *m/z* [M]$^+$ 222.0879 (100, $C_{12}H_{14}O_4$ requires 222.0892), 194 (20), 193 (18), 166 (61), 165 (62), 148 (19), 137 (17).

4-[(3-Methyl-2-butenyl)oxy]-6-methoxy-5-methyl-7-phthalidecarboxylic acid (6a): white crystals; mp 132−134 °C; UV (MeOH) λ_{max} (log ϵ) 213 (4.15), 249 (3.56), 294 (3.23); IR (KBr) ν_{max} 3385 (OH), 2940 (CH), 1765 (C═O), 1715 (C═O), 1400, 1360, 1320, 1225, 1115, 995 cm^{-1}; ^{1}H NMR ($CDCl_3$−CD_3OD, 95:5, 500 MHz) δ 5.34 (1H, tqq, J = 7.0, 1.4, 1.4 Hz, H-2′), 5.24 (2H, s, H_2-3), 4.27 (2H, d, J = 7.0 Hz, H_2-1′), 3.77 (3H, s, OCH_3-6), 2.18 (3H, s, CH_3-5), 1.68 (3H, s, H_3-4′), 1.58 (3H, s, H_3-5′); ^{13}C NMR ($CDCl_3$−CD_3OD, 95:5, 125 MHz) δ 168.9 (s, C-1), 166.8 (s, *C*OOH-7), 157.0 (s, C-6), 152.8 (s, C-4), 139.5 (s, C-3′), 132.2 (s, C-7a), 131.3 (s, C-5), 121.2 and 121.1 (s, C-3a) and (s, C-7), 118.9 (d, C-2′), 68.6 (t, C-1′), 67.9 (t, C-3), 61.8 (q, O*C*H$_3$-6), 25.4 (q, C-4′), 17.8 (q, C-5′), 10.2 (q, *C*H$_3$-5); EIMS (70 eV) *m/z* [M]$^+$ 238 (75), 220 (61), 191 (55), 69 (100), 41 (84); CIMS (NH_3) *m/z* 324 (M + NH_4^+, 71), 307 (M + H^+, 100), 256 (16), 212 (25).

4-[(3-Methyl-2-butenyl)oxy]-6-methoxy-5-methyl-7-phthalidecarboxylic acid methyl ester (6b): white crystals; mp 70−72 °C; UV (MeOH) λ_{max} (log ϵ) 215 (4.45), 250 (3.78), 292 (3.11); IR (KBr) ν_{max} 2950 (CH), 1770 (C═O), 1735 (C═O), 1455, 1430, 1325, 1295, 1210, 1180, 1115, 1030, 995, 975 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.37 (1H, tqq, J = 7.0, 1.4, 1.4 Hz, H-2′), 5.23 (2H, s, H_2-3), 4.47 (2H, d, J = 7.0 Hz, H_2-1′), 3.89 (3H, s, $COOCH_3$-7), 3.76 (3H, s, OCH_3-6), 2.19 (3H, s, CH_3-5), 1.71 (3H, s, H_3-4′), 1.62 (3H, s, H_3-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.4 (s, C-1), 165.8 (s, *C*OOCH$_3$-7), 157.4 (s, C-6), 153.3 (s, C-4), 139.9 (s, C-3′), 132.1 (s, C-7a), 131.2 (s, C-5), 122.2 and 120.4 (s, C-3a) and (s, C-7), 119.1 (d, C-2′), 68.8 (t, C-1′), 68.0 (t, C-3), 62.1 (q, O*C*H$_3$-6), 53.0 (q, COO*C*H$_3$-7), 25.8 (q, C-4′), 18.2 (q, C-5′), 10.5 (q, *C*H$_3$-5); EIMS (70 eV) *m/z* [M]$^+$ 252 (50), 221 (38), 220 (33), 191 (41), 69 (100), 41 (41); CIMS (NH_3) *m/z* 338 (M + NH_4^+, 37), 321 (M + H^+, 100), 256 (16), 212 (25).

Biological Assays. The assays for antimicrobial activity,[7] cytotoxicity,[8] and phytotoxicity[7] were carried out as described previously.

Acknowledgment. Financial support from the State of Rheinland Pfalz, the DAAD, Bonn, and the Swedish Science Research Council is gratefully acknowledged.

References and Notes

(1) New Metabolites with Nematicidal and Antimicrobial Activities from the Ascomycete *Lachnum papyraceum*. 10. Part 9: see Shan *et al.*[3]
(2) Anke, H.; Stadler, M.; Mayer, A.; Sterner O. *Can. J. Bot.* **1995**, *73*, S932−S939.
(3) Shan, R.; Anke, H.; Stadler, M.; Sterner, O. *Tetrahedron* **1996**, *52*, 10 249−10 254.
(4) Chao, P. D.; Schiff, P. L., Jr.; Slatkin, D. J.; Knapp, J. E.; Chao, M.; Rosenstein, R. D. *J. Nat. Prod.* **1975**, *38*, 213−217.
(5) Bartman, C. D.; Campbell, I. M. *Can. J. Microbiol.* **1979**, *25*, 130−137.
(6) Achenbach, H.; Muhlenfeld, A.; Brillinger, G. U. *Liebigs Ann. Chem.* **1985**, 1596−1628.
(7) Anke, H.; Bergendorff, O.; Sterner, O. *Food Chem. Toxicol.* **1989**, *27*, 393−398.
(8) Zapf, S.; Hossfeld, M.; Anke, H.; Velten, R.; Steglich, W. *J. Antibiot.* **1995**, *48*, 36−41. ‡Lehrbereich Biotechnologie der Universität Kaiserslautern.

NP970145S