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The Reactivity of the Fungal Toxin Papyracillic Acid[1]

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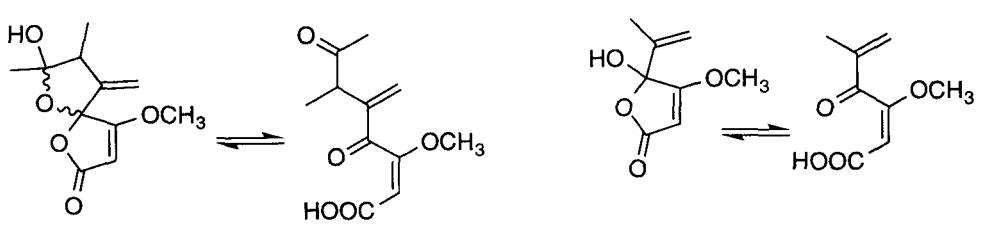
Abstract: Papyracillic acid (**1**), an antibiotic isolated from the ascomycete *Lachnum papyraceum*, owes its bioactivities to its reactivity towards nucleophiles. When reacted with cysteine and cysteine methyl ester, it exclusively added the thiol groups to the exomethylene double bond. Both papyracillic acid (**1**) and its analogue penicillic acid (**2**) react with pyridine to indolizine derivatives during acetylation conditions.

The antibiotic papyracillic acid (**1**) was recently isolated from extracts of submerged cultures of the ascomycete *Lachnum papyraceum*.[2] It is an analogue of penicillic acid (**2**), a classical mycotoxin produced by various fungi including strains of the genera *Penicillium* and *Aspergillus*. Together with patulin, isopatulin and ascladiol, penicillic acid (**2**) constitute a class of chemically relatively simple 5-membered cyclic lactones, which due to their toxicity and carcinogenicity are considered to be a potential health hazard to animals and man.[3] The toxic effects of penicillic acid (**2**) have been considered to be caused by its reaction with, for example, enzymes, and it has been shown to react with several amino acids to form less toxic products.[4] Although the chemical structure of such products has not been disclosed, it has been suggested that nucleophiles bind either to C-3 in the lactone ring or to the α,β-unsaturated keto function in the open-chain form shown in Figure 1.[4,5]

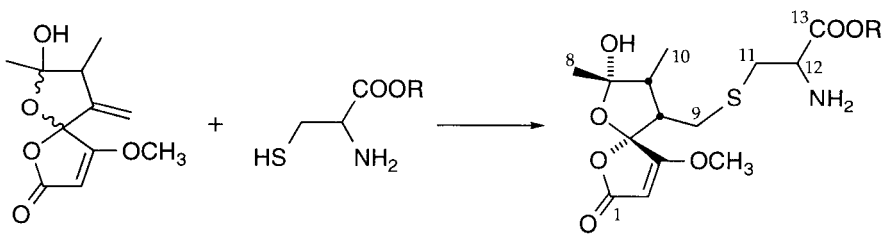


1 **2**

Figure 1.

Penicillic acid (**2**) is not mutagenic in the Ames' Salmonella assay,[6] and does not elicit DNA repair synthesis in primary hepatocytes,[7] although it induces single- and double-stranded DNA breaks in mammalian cells.[8] The molecular mechanism by which penicillic acid (**2**), and presumably also papyracillic acid (**1**), give rise to toxic effects therefore remains to be clarified. In this investigation, the reaction between papyracillic acid (**1**) and cysteine as well as cysteine methyl ester was studied, and the adducts formed were isolated and characterised. In addition, papyracillic acid (**1**) was found to react with pyridine during normal acetylation conditions, to a series of adducts that also were characterised.

The reaction between papyracillic acid (**1**) and cysteine and its methyl ester in phosphate buffer (pH 7.0) at 37 °C was fast (**1** was consumed after 15 minutes) and yielded essentially one product, respectively (see Figure 2). No attack on C-3 of papyracillic acid (**1**) was observed. Papyracillic acid (**1**) also reacted with glycine during the same conditions, but the reaction was slower (completed after approximately 24 hours) and yielded an unseparable mixture of products, underlining the potency of **1** as an electrophile. The structures of the adducts **3a** and **3b** were determined by the HMBC correlations observed between 9-H_2 and C-11, as well as between 11-H_2 and C-9, and the NOESY correlations between 6-H and 5-H as well as 8-H_3, between 9-H_2 and 10-H_3, and between 3-OCH_3 and 5-H.



1 **3**

Figure 2. a: R = H; b: R = CH_3.

During the structural study of papyracillic acid (**1**), before the structure was elucidated, attempts were made to acetylate the compound and it was observed that several products were formed (see Figure 3). The major product was found to be compound **4**, which together with compound **5a** may be formed by the addition of acetate to the electrophilic papyracillic acid (**1**). In addition, and unexpectedly, the three indolizine derivatives **6**, **7** and **8** were obtained, apparently formed after the nucleophilic attack by pyridine. The NMR chemical shifts of compounds **6**, **7** and **8** are in agreement with published data on indolizines,[9] and the structures of the compounds were determined by COSY, NOESY, HMQC and HMBC NMR experiments (data not given). Although pyridine is considered to be a weak nucleophile it can react with Michael-acceptors,[10] and the hypothetical compound **5b** could be a precursor of the indolizines. Compound **6** could then be formed after abstraction of 9-H of **5b**, formation of a bond between C-4 and C-2' of the pyridyl residue, followed by hydrolysis of the enol ether and decarboxylation. The acetyl groups at C-3 of compound **7** and **8** are probably added during the reaction, as indolizines are known to be acetylated in this position by pyridine/acetic anhydride.[11] However, the indolizine skeleton of compound **7** and **8** would appear to be formed after an attack by the conjugated enol of **5b** on C-2' of the pyridyl residue. In addition, a series of

transformations including deacetylation and oxidation would have to take place, and the methyl group at C-1 in compound **7** and **8** would be the C-6 methyl group of papyracillic acid (**1**). Interestingly, compound **8** possesses potent fibrinogen-lowering activity.[12]

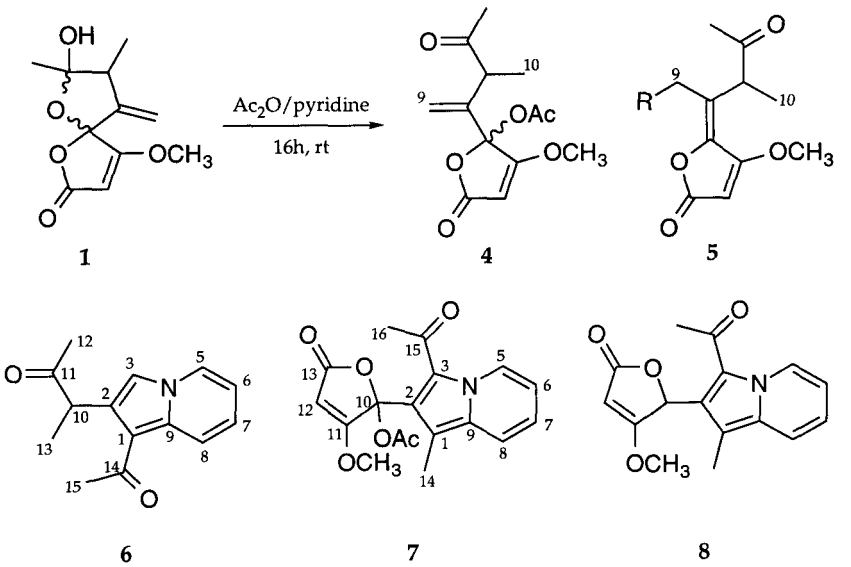


Figure 3. a: R = OAc; b: R = 1-pyridyl. Compound **5b** is hypothetical.

Compounds **4** and **5a** are not the precursors of the indolizines, both compounds were found to be stable in pyridine/acetic anhydride at room temperature overnight. Papyracillic acid (**1**) is perfectly stable in pure pyridine, even if heated to 80 °C overnight, so the formation of adducts requires the acetylating conditions. For a comparison, penicillic acid (**2**) was also acetylated, and the major products were isolated and characterised (see Figure 4). The corresponding products were obtained, compound **9** corresponds to compound **4**, **10** is similar to **6** except that **10** also was acetylated at C-3, and in **11a** the C-3 acetyl group has (as the enol) been acetylated while the hydrolysis/decarboxylation has not taken place. **11a** was obtained pure as the ethyl ester **11b**, formed during the evaporation of pyridine which was expedited by the addition of ethanol.

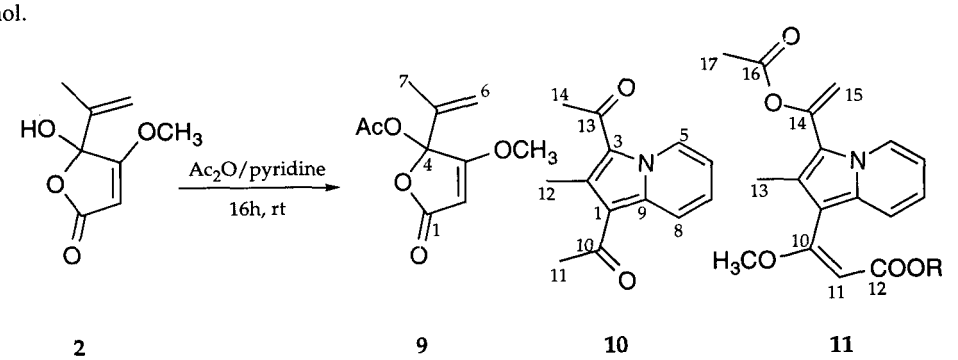


Figure 4. a: R = H; b: R = Et

Previous investigations have shown that the adducts formed after the reaction between penicillic acid (**2**) and cysteine as well as glutathione are considerably less toxic to mice or quails, but still toxic to chick embryos.[4] The chicken embryo test indicated that the glutathione adduct of penicillic acid was about 40 to 50% as toxic as a comparable dose of penicillic acid, while the cysteine adduct of penicillic acid was as toxic as penicillic acid itself. At the moment, little is known about the bioactivities of the various products formed from papyracillic acid (**1**) and penicillic acid (**2**) in this investigation, but they will be assayed in the near future.

EXPERIMENTAL

Papyracillic acid (**1**) was isolated from extracts of *Lachnum papyraceum*, as described earlier,[2] while penicillic acid (**2**) was purchased from Sigma. The NMR spectra were recorded with a Bruker ARX500 spectrometer with the solvent signals as reference ($^1H/^{13}C$: $CDCl_3$ 7.26/77.0; CD_3OD 3.31/49.2), the UV spectra with a Perkin Elmer λ16, the IR spectra with a Bruker IFS48, and the mass spectra with a Jeol SX102 spectrometer.

The cysteine adduct **3a** was obtained as a yellowish oil in 42 % yield after the reaction between papyracillic acid (**1**) (29 mg, 0.13 mmol) and L-cysteine (14 mg, 0.12 mmol) in 1 ml phosphate buffer (0.1M, pH=7.0), for 15 min at 37°C, and purification by reversed phase HPLC (0 to 30 % methanol in water during 60 min) and repeated silica gel chromatography (MTBE:MeOH 8:1). $[\alpha]_D$ -125 (*c* 1.0 in methanol). UV (methanol) λ_{max} (ε): 224 nm (6,400). IR (KBr): 3420, 1750, 1640, 1390 and 870 cm^{-1}. ^1H NMR, 500 MHz in CD_3OD (δ, mult., *J*): 5.26, s, 2-H; 3.96, s, 3-OCH_3; 3.70, dd, $J_{11a\text{-}12}$=3.6, $J_{11b\text{-}12}$=8.8, 12-H; 3.13, dd, $J_{11a\text{-}11b}$=14.6, $J_{11a\text{-}12}$=3.6, 11-Ha; 2.87, dd, $J_{11a\text{-}11b}$=14.6, $J_{11b\text{-}12}$=8.8, 11-Hb; 2.83, dd, $J_{5\text{-}9a}$=3.8, $J_{9a\text{-}9b}$=13.5, 9-Ha; 2.74, m, 5-H; 2.59, dd, $J_{5\text{-}9b}$=10.2, $J_{9a\text{-}9b}$=13.5, 9-Hb; 2.03, dq, $J_{5\text{-}6}$=11.5, $J_{6\text{-}10}$=6.7, 6-H; 1.47, s, 8-H_3; 1.11, d, $J_{6\text{-}10}$=6.7, 10-H_3. ^{13}C NMR, 125 MHz in CD_3OD (δ): 180.1 C-3; 172.8 and 172.7 C-1 and C-13; 110.0 C-4; 108.8 C-7; 91.3 C-2; 60.7 3-OCH_3; 54.8 C-12; 49.1 C-5; 47.6 C-6; 34.7 C-11; 29.8 C-9; 26.6 C-8; 12.2 C-10. MS (FAB, positive ions), m/z: 370 ($M + Na^+$) and 348.1129 ($M + H^+$, $C_{14}H_{22}NSO_7$ requires 348.1117).

The cysteine methyl ester adduct **3b** was obtained as a yellowish oil in 52 % yield after the reaction between papyracillic acid (**1**) (29 mg, 0.13 mmol) and cysteine methyl ester (16 mg, 0.12 mmol) in 1 ml phosphate buffer (0.1M, pH=7.0), for 15 min at 37°C, and purification as described for **3a**. $[\alpha]_D$ -106 (*c* 1.0 in chloroform). UV (methanol) λ_{max} (ε): 222 nm (8,200). IR (KBr): 3400, 2950, 1750, 1640, 1450, 1380, 1220, 1020 and 870 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 5.07, s, 2-H; 3.90, s, 3-OCH_3; 3.70, s, 13-OCH_3; 3.60, dd, $J_{11a\text{-}12}$=4.7, $J_{11b\text{-}12}$=7.2, 12-H; 2.83, dd, $J_{11a\text{-}11b}$=13.5, $J_{11a\text{-}12}$=4.7, 11-Ha; 2.70, dd, $J_{11a\text{-}11b}$=13.5, $J_{11b\text{-}12}$=7.2, 11-Hb; 2.67-2.52, m, 9-Ha, 5-H and 9-Hb; 2.00, dq, $J_{5\text{-}6}$=11.5, $J_{6\text{-}10}$=6.7, 6-H; 1.49, s, 8-H_3; 1.08, d, $J_{6\text{-}10}$=6.7, 10-H_3. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 177.1 C-3; 174.1 C-13; 169.6 C-1; 108.1 C-4; 107.2 C-7; 90.4 C-2; 59.7 3-OCH_3; 53.6 C-12; 52.3 13-OCH_3; 48.2 C-5; 46.0 C-6; 37.8 C-11; 29.5 C-9; 26.2 C-8; 11.7 C-10. MS (FAB, positive ions), m/z: 362.1248 ($M + H^+$, $C_{15}H_{24}NSO_7$ requires 362.1273).

Compounds **4-8** were obtained after the treatment of papyracillic acid (**1**) with acetic anhydride:pyridine (1:5) at room temperature for 16 hours, evaporation of the volatiles under reduced pressure by the repeated addition of ethanol, and fractionation of the residue on a silica gel column eluted by heptane:ethyl acetate. The yields were 63 % of **4**, 9 % of **5a**, 5 % of **6**, and 3 % of **7**, and 7 % of **8**.

5-Acetoxy-4-methoxy-5-(1-(1-methyl-2-oxopropyl)vinyl)-2,5-dihydro-2-furanone (**4**) was obtained as white crystals, m.p. 55-57 °C, as a 3:2 epimeric mixture. $[\alpha]_D$ +25 (*c* 1.1 in chloroform). UV (methanol) λ_{max} (ε): 230 nm (7,400). IR (KBr): 1776, 1716, 1343, 1197, 1178, 1070 and 1028 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 5.55 and 5.21, m, 9-H_2; 5.20 and 5.13, s, 3-H; 3.85 and 3.87, s, 2-OCH_3; 3.27 and 3.43, q, $J_{6\text{-}10}$=7.0, 6-H; 2.10 and 2.06, s, 8-H_3; 2.04 and 2.01, s, 4-OAc; 1.12 and 1.19, d, $J_{6\text{-}10}$=7.0, 10-H_3. ^{13}C

NMR, 125 MHz in $CDCl_3$ (δ): 207.1 and 207.6 C-7; 177.1 and 177.7 C-2; 168.2 and 168.2 C-1; 167.2 and 167.2 4-OAc; 142.2 and 142.7 C-5; 117.7 and 117.4 C-9; 101.0 and 100.9 C-4; 90.5 and 89.8 C-3; 59.8 and 59.9 2-CH_3; 46.5 and 47.7 C-6; 27.7 and 27.3 C-8; 21.1 and 21.1 4-OAc; 17.1 and 16.5 C-10. MS (EI, 70 eV), m/z: 226.0860 (M^+ - CH_2CO, 12 %, $C_{11}H_{14}O_5$ requires 226.0841), 209 (6 %), 208 (6 %), 166 (100 %), 151 (14 %), 139 (25 %), 123 (21 %). MS (CI, NH_3), m/z: 286 (M + NH_4^+, 100 %).

5-(1-Acetoxymethyl-2-methyl-3-oxo-(Z)-butylidene)-4-methoxy-2,5-dihydro-2-furanone (5a) was obtained as white crystals, m.p. 54-56 °C. $[\alpha]_D$ +250 (*c* 1.1 in chloroform). UV (methanol) λ_{max} (ε): 263 nm (11,400). IR (KBr): 1782, 1745, 1717, 1607, 1441, 1366, 1229, 1027 and 970 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 5.38, s, 2-H; 5.10, d, $J_{9a\text{-}9b}$=12.9, 9-Ha; 4.86, d, $J_{9a\text{-}9b}$=12.9, 9-Hb; 3.94, s, 2-OCH_3; 3.93, q, $J_{6\text{-}10}$=7.0, 6-H; 2.13, s, 8-H_3; 1.97, s, 4-OAc; 1.22, d, $J_{6\text{-}10}$=7.0, 10-H_3. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 206.0 C-7; 170.4 4-OAc; 170.1 C-2; 166.6 C-1; 143.1 C-4; 120.3 C-5; 92.0 C-3; 59.7 2-CH_3; 58.2 C-9; 47.6 C-6; 28.3 C-8; 20.5 4-OAc; 12.9 C-10. MS (EI, 70 eV), m/z: 268 (M^+, 2 %), 226.0852 (M^+ - CH_2CO, 33 %, $C_{11}H_{14}O_5$ requires 226.0841), 209 (3 %), 166 (100 %), 151 (11 %), 137 (14 %), 123 (16 %). MS (CI, NH_3), m/z: 286 (M + NH_4^+, 100 %).

1-Acetyl-2-(1-methyl-2-oxo-propyl)indolizine (6) was obtained as a greenish oil. $[\alpha]_D$ +428 (*c* 1.2 in chloroform). UV (methanol) λ_{max} (ε): 233 nm (20,200), 271 nm (4,000), 280 nm (4, 000), 350 nm (11,900). IR (KBr): 1711, 1622, 1501, 1426, 1352, 1238, 1158 and 963 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 7.97, d, $J_{5\text{-}6}$=6.8, 5-H; 7.84, d, $J_{7\text{-}8}$=9.2, 8-H; 7.18, d, $J_{3\text{-}10}$=0.8, 3-H; 7.08, dd, $J_{6\text{-}7}$=7, $J_{7\text{-}8}$=9, 7-H; 6.71, dd, $J_{5\text{-}6}$=$J_{6\text{-}7}$=7, 6-H; 4.62, dd, $J_{3\text{-}10}$=0.8, $J_{10\text{-}13}$=7.2, 10-H; 2.61, s, 15-H_3; 2.31, s, 12-H_3; 1.47, d, $J_{10\text{-}13}$=7.2, 13-H_3. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 209.9 C-11; 192.0 C-14; 136.3 C-9; 132.1 C-2; 126.4 C-5; 123.4 C-7; 119.0 C-8; 114.1 C-3; 112.2 C-6; 112.2 C-1; 45.0 C-10; 31.0 C-14; 28.8 C-12; 16.4 C-13. MS (EI, 70 eV), m/z: 229.1105 (M^+, 87 %, $C_{14}H_{15}O_2N$ requires 229.1103), 214 (10 %), 212 (8 %), 186 (100 %), 172 (65 %), 170 (28 %), 144 (79 %), 143 (39 %). MS (CI, NH_3), m/z: 230 (M + H^+, 100 %).

5-(3-Acetyl-1-methyl-2-indolizinyl)-5-acetoxy-4-methoxy-2,5-dihydro-2-furanone (7) was obtained as a greenish oil. $[\alpha]_D$ +84 (*c* 0.4 in chloroform). UV (methanol) λ_{max} (ε): 231 nm (28,400), 377 nm (6,700). IR (KBr): 1775, 1648, 1453, 1370, 1339, 1198, 1159 and 1013 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 8.95, d, $J_{5\text{-}6}$=7.3, 5-H; 7.43, d, $J_{7\text{-}8}$=9.0, 8-H; 6.94, dd, $J_{6\text{-}7}$=6.5, $J_{7\text{-}8}$=9.0, 7-H; 6.71, dd, $J_{5\text{-}6}$=$J_{6\text{-}7}$=7, 6-H; 5.33, s, 12-H; 3.94, s, 11-OCH_3; 2.69, s, 16-CH_3; 2.31, s, 14-H_3; 2.14, s, 10-OAc. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 192.9 C-15; 177.8 C-11; 168.4 C-13; 167.4 10-OAc; 133.6 C-9; 126.1 C-5; 122.7 C-2; 122.4 C-3; 121.1 C-7; 117.2 C-8; 113.8 C-6; 109.1 C-1; 101.0 C-10; 90.6 C-12; 60.0 11-OCH_3; 31.9 C-16; 21.5 10-OAc; 9.8 C-14. MS (EI, 70 eV), m/z: 343.1059 (M^+, 88 %, $C_{18}H_{17}O_6N$ requires 343.1056), 300 (8 %), 283 (20 %), 258 (79 %), 242 (100 %), 200 (45 %). MS (CI, NH_3), m/z: 344 (M + H^+, 59 %), 284 (100 %).

5-(3-Acetyl-1-methyl-2-indolizinyl)-4-methoxy-2,5-dihydro-2-furanone (8) was obtained as a greenish oil. $[\alpha]_D$ +239 (c 1.5 in chloroform). UV (methanol) λ_{max} (ε): 230 nm (17,400), 259 nm (7,900), 378 nm (4, 800). IR (KBr): 1754, 1632, 1458, 1375, 1235 and 1158 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 9.81, d, $J_{5\text{-}6}$=7.3, 5-H; 7.47, d, $J_{7\text{-}8}$=8.8, 8-H; 7.11, dd, $J_{6\text{-}7}$=7.7, $J_{7\text{-}8}$=8.8, 7-H; 6.84, dd, $J_{5\text{-}6}$=$J_{6\text{-}7}$=7, 6-H; 6.66, d, $J_{10\text{-}12}$=1.3, 10-H; 5.31, d, $J_{10\text{-}12}$=1.3, 12-H; 3.90, s, 11-OCH_3; 2.68, s, 16-CH_3; 2.21, s, 14-CH_3. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 186.3 C-15; 180.3 C-11; 172.1 C-13; 136.2 C-9; 128.3 C-5; 124.2 C-2; 123.3 C-7; 122.1 C-3; 116.5 C-8; 114.4 C-6; 111.2 C-1; 89.4 C-12; 74.8 C-10; 59.8 11-OCH_3; 31.2 C-16; 8.8 C-14. MS (EI, 70 eV), m/z: 285.1009 (M^+, 65 %, $C_{16}H_{15}O_4N$ requires 285.1001), 259 (11 %), 243 (100 %), 242 (70 %), 228 (48 %), 200 (17 %), 158 (14 %), 154 (15 %), 130 (20 %). MS (CI, NH_3), m/z: 303 (M + NH_4^+, 15 %), 284 (M + H^+, 100 %).

Compounds **9-11b** were obtained after acetylation of penicillic acid (**2**) (*vide supra*). The yields were 58 % of **9**, 2 % of **10** and 5 % of **11b**.

5-Acetoxy-5-(1-methyl-1-ethenyl)-4-methoxy-2,5-dihydro-2-furanone (9) was obtained as white crystals, m.p. 72-74 °C. UV (methanol) λ_{max} (ε): 230 nm (10,100). IR (KBr): 3125, 1765, 1640, 1460, 1370, 1350, 1270, 1225, 1200, 1120, 1100, 1030, 950, 910, 840 and 800 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ,

mult., *J*): 5.34, m, 6-Ha; 5.15, s, 2-H; 5.13, m, 6-Hb; 3.88, s, 3-OCH_3; 2.08, s, 4-OAc; 1.79, m, 7-H_3. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 178.0 C-3; 169.0 C-1; 167.7 4-OAc; 138.3 C-5; 116.0 C-6; 101.3 C-4; 89.7 C-2; 59.9 3-OCH_3; 21.3 4-OAc; 17.3 C-7. MS (EI, 70 eV), m/z: 212.0662 (M^+, 42 %, $C_{10}H_{12}O_5$ requires 212.0685), 169 (61 %), 152 (39 %), 142 (27 %), 126 (39 %), 124 (28 %), 100 (94 %), 68 (84 %), 43 (100 %).

1,3-Diacetyl-2-methylindolizine (10) was obtained as yellow oil. UV (methanol) λ_{max} (ε): 234 nm (8,300), 260 (8,600), 290 (5,100), 336 (6,400) and 348 (6,600). IR (KBr): 2920, 1770, 1640, 1610, 1490, 1410, 1390, 1200 and 910 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 10.00, d, $J_{5\text{-}6}$=7.1, 5-H; 8.30, d, $J_{7\text{-}8}$=9.0, 8-H; 7.38, dd, $J_{6\text{-}7}$=6.8, $J_{7\text{-}8}$=9.0, 8-H; 6.97, dd, $J_{5\text{-}6}$=$J_{6\text{-}7}$=7, 6-H; 2.87, s, 12-H_3; 2.65, s, 11-H_3; 2.65, s, 14-H_3. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 192.8 C-13; 189.1 C-10; 138.5 C-9; 135.3 C-2; 128.9 C-5; 127.8 C-7; 123.2 C-3; 118.7 C-8; 115.5 C-1; 114.8 C-6; 31.9 C-14; 31.6 C-11; 14.9 C-12. MS (EI, 70 eV), m/z: 215.0954 (M^+, 39 %, $C_{13}H_{13}NO_2$ requires 215.0946), 200 (100 %), 186 (6 %), 172 (10 %), 158 (11 %), 143 (8 %), 130 (15 %), 43 (13 %).

3-(3-(1-Acetoxy-1-ethenyl)-2-methyl-1-indozinyl)-3-methoxy-(*E*)-2-propenoic acid ethyl ester (11b) was obtained as a yellow oil. UV (methanol) λ_{max} (ε): 230 nm (21,100), 260 (15,000), 330 (8,700) and 352 (8,700). IR (KBr): 2975, 2930, 1760, 1710, 1605, 1520, 1490, 1370, 1195, 1140, 1125, 1100 and 1050 cm^{-1}. ^1H NMR, 500 MHz in $CDCl_3$ (δ, mult., *J*): 8.12, d, $J_{5\text{-}6}$=7.1, 5-H; 7.24, d, $J_{7\text{-}8}$=9.0, 8-H; 6.81, dd, $J_{6\text{-}7}$=6.6, $J_{7\text{-}8}$=9.0, 7-H; 6.55, dd, $J_{5\text{-}6}$=$J_{6\text{-}7}$=7, 6-H; 5.41, d, $J_{15a\text{-}15b}$=1.5, 15-Ha; 5.40, s, 11-H; 5.21, d, $J_{15a\text{-}15b}$=1.5, 15-Hb; 4.01, q, *J*=7.1, 12-OCH_2CH_3; 3.82, s, 10-OCH_3; 2.28, s, 13-H_3; 2.11, s, 17-H_3; 1.08, t, *J*= 7.1, 12-OCH_2CH_3. ^{13}C NMR, 125 MHz in $CDCl_3$ (δ): 168.8 C-16; 166.8 C-12; 166.0 C-10; 144.6 C-14; 132.6 C-9; 126.2 C-2; 124.2 C-5; 119.8 C-7; 118.0 C-8; 117.8 C-3; 111.1 C-6; 109.0 C-15; 107.7 C-1; 93.5 C-11; 59.4 12-OCH_2CH_3; 55.9 10-OCH_3; 20.8 C-17; 14.2 12-OCH_2CH_3; 11.1 C-13. MS (EI, 70 eV), m/z: 343.1431 (M^+, 100 %, $C_{19}H_{21}NO_5$ requires 343.1420), 314 (22 %), 300 (47 %), 286 (46 %), 284 (52 %), 272 (45 %), 256 (32 %), 240 (29 %), 228 (22 %), 212 (41 %), 198 (50 %), 182 (33 %), 168 (26 %), 154 (30 %).

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REFERENCES AND NOTES

1. Part 11 in a series on New metabolites with nematicidal and antimicrobial activities from the ascomycete *Lachnum papyraceum* (Karst.) Karst. Part 10, "Naphthalenone and phthalide metabolites from *Lachnum papyraceum*", is submitted for publication in *J. Nat. Prod.* For part 9, see reference 2.
2. Shan, R.; Anke, H.; Stadler, M.; Sterner, O. *Tetrahedron* **1996**, *52*, 10249.
3. Cole, R. J.; Cox, R. H. Handbook of toxic fungal metabolites.
4. Ciegler, A.; Mintzlaff, H.J.; Weisleder, D; Leistner, L. *Appl. Microbiol.* **1972**, *24*, 114.
5. Black, D.K. *J. Chem. Soc.* **1966**, 1123.
6. Ueno, Y.; Kubota, K.; Ito, T.; Nakamura, Y. *Cancer Res.* **1978**, *38*, 536.
7. Mori, H.; Kawai, K.; Ohbayashi, F.; Kuniyasu, T.; Yamazaki, M.; Hamasaki, T.; Williams, G.M. *Cancer Res.* **1984**, *44*, 2918.
8. Umeda, M.; Tsutsui, T.; Saito, M. *Gann.* **1977**, *68*, 619.
9. E. Pretsch, T. Clerc, J. Seibl, W. Simon; Tables of spectral data for structure determination of organic compounds, 2nd edition 1989. Springer Verlag, Berlin, pp C161 and H330.
10. Katritzky, A.R. Handbook of Heterocyclic Chemistry; Pergamon Press: Oxford, 1985; p. 156.
11. Katritzky, A.R. Handbook of Heterocyclic Chemistry; Pergamon Press: Oxford, 1985; p. 255.
12. European patent application No. PCT/ep96/04636, from October 25th, 1996.

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