### *3. Extraction, isolation and purification of the compounds*

Dry powdered roots (12 kg) were exhaustively extracted in soxhlet apparatus with $CHCl_3$. The dry extract (180 g) was fractionated over 360 g of silica gel with gradient light petroleum: $CHCl_3$ to give four fractions [14]. All fractions are kept in the refrigerator. The first fraction (50 g, light petroleum/$CHCl_3$, 1 : 1) was fractionated over 800 g of silica gel, and 15 fractions were obtained.

#### *3.1. Sanigerone (7,12-dihydroxy-1,5(10),6,8,12-abietapentaene-11,14-dione)* (***1***)

Fraction 3 (7.5% chloroform in petroleum ether) was subjected to column and preparative TLC (petroleum ether: ethyl acetate; 49 : 1) to give 60 mg of sanigerone (**1**). UV ($\lambda_{max}$, MeOH) 297, 427, 487 nm. IR (KBr, ν, $cm^{-1}$) 3479, 3330, 2963, 2928, 1662, 1645, 1612, 1449, 1439, 1396, 1372. EIMS m/z (rel. int.) 312 (100), 297 (87), 283 (43), 279 (75), 269 (77), 251 (52), 237 (14), 201 (32), 165 (35), 128 (29), 115 (16), 83 (16), 69 (13), 55 (10). $^1$H NMR ($CDCl_3$) δ 8.00 (1 H, dt, J = 10.0, 1.8 Hz, H-1), 6.31 (1 H, dt, J = 10.0, 4.7 Hz, H-2), 2.27 (2 H, dd, J = 1.8, 4.7 Hz, $CH_2$-3), 7.24 (1 H, s, H-6), 13.60 (1 H, s, OH-7), 7.90 (1 H, s, OH-12), 3.37 (1 H, hept., J = 7.1 Hz, H-15), 1.30 (6 H, d, J = 7.1 Hz, $CH_3$-16 and $CH_3$-17), 1.28 (6 H, s, $CH_3$-18 and $CH_3$-19). $^{13}$C NMR ($CDCl_3$) δ 124.51 (C-1), 133.73 (C-2), 37.65 (C-3), 34.86 (C-4), 155.36 (C-5), 122.62 (C-6), 162.14 (C-7), 112.76 (C-8), 121.98 (C-9), 129.63 (C-10), 183.05 (C-11), 153.75 (C-12), 126.56 (C-13), 190.57 (C-14), 23.87 (C-15), 19.75 (C-16 and C-17), 28.38 (C-18 and C-19).

#### *3.2. Saligerone (1,5(10),6,8,13-abietapentaene-3,11,12-trione)* (***2***)

Fraction 10 (45% chloroform in petroleum ether) was subjected to repeated column and preparative TLC (petroleum ether: ethyl acetate; 45 : 5) to give 25 mg of saligerone (**2**), UV ($\lambda_{max}$, MeOH) 269, 324, 381 nm. IR (KBr, ν, $cm^{-1}$) 3058: 2925, 1713, 1695, 1663, 1646, 1629, 1571, 1467, 1450, 1388. EIMS m/z (rel. int.) 296 [$M^{+2}$] (14), 294 (12), 266 (29), 251 (12), 238 (21), 223 (35), 195 (13), 43 (100). $^1$H NMR ($CDCl_3$) δ 8.98 (1 H, d, J = 10.7 Hz, H-1), 6.40 (1 H, d, J = 10.7 Hz, H-2), 7.66 (1 H, d, J = 9.7 Hz, H-6), 7.35 (1 H, d, J = 7.9 Hz, H-3), 7.13 (1 H, s, H-14), 3.04 (1 H, hept., J = 6.9 Hz, H-15), 1.18 (6 H, d, J = 6.9 Hz, $CH_3$-16 and $CH_3$-17), 1.48 (6 H, s, $CH_3$-18 and $CH_3$-19). $^{13}$C NMR ($CDCl_3$) δ 139.60 (C-1), 128.85 (C-2), 202.12 (C-3), 47.88 (C-4), 150.40 (C-5), 132.58 (C-6), 131.17 (C-7), 135.47 (C-8), 127.07 (C-9), 132.23 (C-10), 183.10 (C-11), 181.20 (C-12), 146 (C-13), 139.12 (C-14), 27.19 (C-15), 21.53 (C-16 and C-17), 27.61 (C-18 and C-19).

Acknowledgement: The author thanks Dr. Ihab Essa, Lecturer at the Department of Microbiology (Diary Products), Faculty of Agriculture, University of Alexandria, for measuring the antimicrobial activity of sanigerone.

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## Nosporins A and B, new metabolites from a filamentous fungus, VKM-3750

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*Received March 5, 2001*
*Accepted December 12, 2001*

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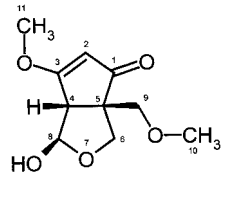
*Pharmazie 58: 76–77 (2003)*

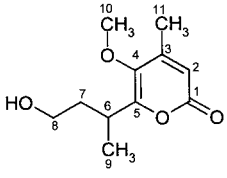
In the course of our screening for new metabolites from fungi [1] we investigated culture extracts of the taxonomically identified fungus VKM F-3750 using mass spectrometry and staining behaviour on TLC as a screening feature. This fungal strain was isolated from a forestal region near Pushchino (Moscow region, Russia). It was deposited as strain VKM-3750 in the All Russian Culture Collection (VKM) of the G. K. Skrjabin-Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (Pushchino near Moscow, Russia).

Colonies of the fungal strain VKM-3750 on potato dextrose agar (PDA) and malt-extract agar (MA) are growing rapidly attaining a diameter of 35–45 mm in subcentral areas, azonate or indistinctly zonate near colony margins. Vegetative hyphae are white to shightly brownish, passing towards greyish-brownish with aging. Often the hyphae display bulbous and other nonconidial structures. Conidiogenes were absent. The strain reversed usually in drab to brownish or blackish shades. Formation of spores was not observed as this was observed with representatives of the agromomycetes, hyphomycetes or mitosporic fungi.

Cultivation occurred for 120 h under submerged conditions in 750 ml Erlenmeyer flasks containing 150 ml of a medium composed of (g/L): mannitol 20, succinic acid 5.4, $MgSO_4 \cdot 7\ H_2O$ 0.3 and $KH_2PO_4$ 1 (24 °C, rotary shakers 220–240 r.p.m.). The culture broth was adjusted to pH 8.0–8.5 by $NH_4OH$ and extracted twice by $CHCl_3$ in ratio 1 : 1. The residue of the evaporated culture extract was fractionated using several subsequent chromatographic steps such as column chromatography on silica gel and preparative thin-layer chromatography on normal and reverse phase silica gel. Finally 15.8 mg of metabolite A (**1**) and 13.0 mg of B (**2**) were isolated as waxy mass from 3.5 L culture broth. **1** and **2** were given the name



**1**



**2**

nosporins A and B due to the missing sporulation of the producer strain.

Structures of **1** and **2** were assigned on the basis of optical spectroscopy, mass spectrometry and 1D and 2D NMR spectroscopy ($^1$H, $^{13}$C, DEPT, COSY, NOESY, HSQC, HMBC). UV absorbances ($\lambda_{max}$) at 255 nm for **1**, and 295 nm for **2**, attested to the presence of double bonds in conjugation to the keto group. This structural feature was confirmed, too, by IR absorbances ($\lambda_{max}$; cm$^{-1}$, in KBr) at 1670 cm$^{-1}$ and 1660 cm$^{-1}$, respectively.

HREI-MS showed for **1** m/z 214.0834 ($M^+$, calcd. 214.0838 for $C_{10}H_{14}O_5$) and for **2** m/z 212.1032 ($M^+$, calcd. 212.1049 for $C_{11}H_{16}O_4$). Optical rotation values ($[\alpha]_D$) +5.2° for **1** and +7.5° for **2** attested to the chiral nature of both compounds.

The $^1$H NMR spectra of **1** displayed a singlet signal of an olefinic proton (5.25 ppm) and two singlet methoxyl proton signals (3.80 ppm, 3.40 ppm). Moreover two oxygen-bonded methylene protons (3.73 ppm, 3.58 ppm) were visible.

The proton spectrum of **2** displayed one singlet olefinic proton signal (6.05 ppm), one methoxyl group (3.58 ppm), two methyl groups (1.26 ppm, doublet; 1.88 ppm, singlet) and one oxygen-bonded methylene group. The $^{13}$C NMR spectra of **1** and **2** displayed 10 and, respectively, 11 signals whereby the bonding type was clearly assignable due to the chemical shift and multiplicity pattern in the DEPT spectra.

For structural elucidation of **1** and **2** the HSQC and C,H long-range coupled NMR spectra (HMBC) were of pivotal importance. Thus the chemical constitution was assigned doubtlessly. The relative stereochemistry of **1** at C-4 and C-5 was determined on the basis of the observable NOE's between H-4 and H-9 in the NOESY spectrum. The small coupling constant $^3J_{H\text{-}4,\,H\text{-}8} = 0.5$ Hz in the $^1$H NMR spectrum of **1** suggested that H-8 is in the same position relative to H-4. Both **1** and **2** displayed moderate antibacterial activity against some Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 (MIC >50 μg/ml) as was determined by the agar diffusion assay [2]. No activity was found against Gram-negative Bacteria and fungi.

The bicyclic ring system of **1** was established so far in only some few fungal metabolites such as xenovulene A [3]. However, the six-membered diene lactone structure (2H-pyran-2-one) as found in **2** is frequently occurring in fungi [3].

## Experimental

### *1. Instruments*

HREI-MS was carried out on an AMD-402 sector-field mass spectrometer (AMD Intectra, Harpstedt, Germany). UV-VIS and IR-spectra were recorded on Beckman DU 601 and Shimadzu IR scanning spectrometers. NMR spectra were recorded on a Bruker Avance DRX 500 instrument. Optical rotation was measured on a Propol polarimeter (Dr. Kernchen Optics Seelze, Germany).

### *2. Nosporin A* (1)

Yield: 15.8 mg, wax. TLC: $R_f$ 0.45 silica gel 60, Merck, $CHCl_3$/MeOH/conc. $NH_4OH$ (90 : 10 : 0.1; v/v). MS (70 eV) m/z ($M^+$) 214.0834 (calcd. 214.0838 for $C_{10}H_{14}O_5$). UV-VIS (MeOH, $\lambda_{max}$) 220, 255 nm. IR ($\lambda_{max}$, cm$^{-1}$, KBr): 520, 595, 692, 779, 835, 892, 854, 944, 992, 1035, 1093, 1130, 1197, 1250, 1262, 1300, 1304, 1333, 1379, 1381, 1448, 1471, 1592, 1670, 2950, 2955, 3310. $^1$H NMR (500 MHz, in $CDCl_3$, δ in ppm): 3.21 (d, $^3J = 0.5$ Hz, 1 H, H-4), 3.41 (s, 3 H, H-10), 3.58 (d, $^3J = 1.5$ Hz, 2 H, H-9), 3.72 (d, $^3J = 10.5$ Hz, 1 H, $H_A$-6), 3.75 (d, $^3J = 10.5$ Hz, 1 H, $H_B$-6), 3.81 (s, 3 H, H-11), 4.10 (8-OH, broad), 5.25 (s, 1 H, H-2), 5.45 (d, $^2J = 0.5$ Hz, broad, 1 H, H-8). $^{13}$C NMR (125 MHz, in $CDCl_3$, δ in ppm): 58.2 (C-4, d), 59.2 (C-5, s), 59.2 (C-11, q), 59.5 (C-10, q), 69.2 (C-6, t), 71.4 (C-9, t), 97.6 (C-8, d), 104.3 (C-2, d), 187.7 (C-3, s), 203.9 (C-1, s).

### *3. Nosporin B* (2)

Yield: 13 mg, wax. TLC: $R_f$: 0.38, silica gel 60, Merck, $CHCl_3$/MeOH/conc. $NH_4OH$ (90 : 10 : 0.1; v/v). MS (70 eV) m/z ($M^+$) 212.1032 (calcd. 212.1049 for $C_{11}H_{16}O_4$). UV-VIS (MeOH, $\lambda_{max}$): 295 nm. IR ($\lambda_{max}$, cm$^{-1}$, KBr): 970, 1012, 1047, 1081, 1103, 1143, 1185, 1250, 1299, 1356, 1378, 1400, 1457, 1520, 1561, 89, 1637, 1600, 1660, 2925, 3395. $^1$H NMR (500 MHz, $CDCl_3$, δ in ppm): 1.26 (d, $^3J = 5.5$ Hz, 3 H, H-9), 1.75 (m, 1 H, $H_A$-7), 1.88 (s, 3 H, H-11), 1.95 (m, 1-H, $H_B$-7), 2.50 (8-OH), 2.78 (m, 1 H, H-6), 3.62 (m, 1 H, $H_A$-8), 3.65 (m; 1 H, $H_B$-8), 3.85 (s, 3 H, H-10), 6.05 (s, 1 H, H-2). $^{13}$C NMR (125 MHz, in $CDCl_3$, δ in ppm): 8.5 (C-11, q), 18.5 (C-9, q), 35.5 (C-6, d), 37.2 (C-7, t), 56.2 (H-10, q), 60.3 (C-8, t), 93.5 (C-2, d), 101.3 (C-3, s), 165.5 (C-4, s), 166.3 (C-5, s), 166.4 (C-1, s).

Acknowledgements: Support of this work by BMBF/DLR (Bonn, Germany, project RUS-98–167) and FCI (Frankfurt, Germany) is gratefully acknowledged.

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