New Naphthoquinone Derivatives from the Ascomycete IBWF79B-90A

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Bioactivity-guided fractionation of extracts from the fungus IBWF79B-90A resulted in the isolation of three known naphthoquinones, herbarin, dehydroherbarin, and *O*-methylherbarin and the azaanthraquinone scorpinone as well as three structurally related derivatives, *O*-phenethylherbarin and herbaridines A and B. All seven compounds exhibited cytotoxic activities against several cell lines.

Key words: Naphthoquinone, Herbaridine, Cytotoxic Activity

Introduction

Naphthoquinone metabolites are widespread in nature and show a wide range of biological activities. Closely related structures to those described herein could be found in plants, *e.g.* psychorubin isolated from *Psychotria rubra* (Hayashi *et al.*, 1987) as well as nanaomycin A isolated from *Streptomyces* species (Tanaka *et al.*, 1975). A considerable number of naphthoquinones have also been isolated from fungi. Species of the genus *Fusarium* produce a wide variety of related metabolites, *e.g.* fusarubins, which exhibit antimicrobial, cytotoxic (Kurobane *et al.*, 1986), and phytotoxic (Medentsev and Akimenko, 1992) activities. *Fusarium* species are able to produce azaanthraquinones like bostrycoidin, which was the first isolated natural 2-aza-anthraquinone (Arsenault, 1965); its structure is related to scorpinone isolated here from IBWF79B-90A and previously from a *Bispora*-like tropical fungus (Miljkovic *et al.*, 2001). The mechanism of the biosynthesis was studied and indicated the existence of a common precursor which is modified through reduction, dehydration or oxidation and leads to the variability of derivatives (Medentsev and Akimenko, 1998).

Seven structurally related naphthoquinones were isolated from the ascomycete IBWF79B-90A, and three of them are new. The known compounds herbarin, dehydroherbarin, and *O*-methylherbarin were first isolated from the fungus *Torula herbarum* (Kadkol *et al.*, 1971; Narasimhachari and Gopalkrishnan, 1974). Their biological activity is comparable to related, already known compounds like the fusarubins.

Material and Methods

Producing organism

The sterile filamentous ascomycete IBWF79B-90A could neither be identified by microscopic characteristics nor by ITS sequencing. The ITS sequence determined by us is available through GenBank (accession number EU848215), the fungus is deposited in the culture collection of the Institute of Biotechnology and Drug Research (IBWF e.V.), Kaiserslautern, Germany. For maintenance the fungus was grown on agar slants on YMG agar (4 g/l yeast extract, 10 g/l malt extract, 10 g/l glucose; the pH value was adjusted to 5.5 before autoclaving). Solid media contained 2% of agar.

Fermentation and isolation of the naphthoquinones

The fungus was grown in YMG medium in a 20-l fermenter (DCI-Biolafitte, St. Cloud, MN, USA) at 22–24 °C with agitation (130 rpm) and aeration (3 l/min). For inoculation a well grown shake culture (250 ml) in the same medium from a 500-ml Erlenmeyer flask was used. During the fermentation, the carbon source was depleted after 11 d. The highest metabolite concentration was reached after 19 d as judged by analytical HPLC. The fermentation was stopped and the culture fluid was separated from the mycelia through filtration. The culture fluid (17 l) was extracted twice with EtOAc and the combined extracts were dried. This crude extract (1.5 g) was applied onto silica gel (Merck 60, 0.063–0.2 mm, 75 g). Elution with cyclohexane/EtOAc (3:1) yielded intermediate I (56.8 mg), with cyclohexane/EtOAc (1:1) intermediate II (293.8 mg), and with EtOAc intermediate III (468.0 mg). The purification of the compounds was achieved by preparative HPLC (Merck, LiChroSorb RP18, 7 µm, 250 × 25 mm) with MeCN/H$_2$O gradients. Intermediate I furnished 0.6 mg herbarin (**4**) (RT 31.4 min) and 0.8 mg *O*-phenethylherbarin (**1**) (RT 51.9 min) with a MeCN/H$_2$O gradient (20% to 70% MeCN in 70 min; flow, 25 ml/min). Dehydroherbarin (**5**) (4.7 mg; RT 39.5 min; in 50 min from 35% to 70% MeCN; flow, 20 ml/min) was isolated from intermediate II. Intermediate III was separated with a MeCN gradient (10% to 50% in 50 min; flow, 25 ml/min) and yielded *O*-methylherbarin (**6**) (5.3 mg; RT 29.0 min), scorpinone (**7**) (2.5 mg; RT 31.2 min) and a mixture of herbaridines A (**2**) and B (**3**) (19.0 mg). This mixture was separated by semipreparative HPLC (Merck, LiChroSpher 100, RP18, 5 µm, 125 × 5 mm; 1% to 100% MeCN in 20 min; flow, 1 ml/min) and yielded herbaridine A (**2**) (1.9 mg; RT 8.7 min) and herbaridine B (**3**) (13.3 mg; RT 11.8 min).

Spectroscopic characterization

Melting points were determined with a Dr. Tottoli apparatus (Büchi) and are uncorrected. Optical rotations were measured with a Krüss P8000 polarimeter at 589 nm. UV and IR spectra were measured with a Perkin-Elmer Lambda-16 spectrophotometer and a Bruker IFS48 FTIR spectrometer, respectively. NMR spectra were recorded in CDCl$_3$ with a Bruker Avance II (400 MHz) or DRX-500 (500 MHz) spectrometer, the chemical shifts were referenced to the residual solvent signal (CDCl$_3$ δ_H = 7.26 ppm, δ_C = 77.16 ppm; Gottlieb *et al.*, 1997). APCIMS spectra were measured with a Hewlett Packard MSD1100 instrument. FAB mass spectra were measured with a Vacuum Generators VG70S spectrometer (Xe-FAB ionization) using *m*-nitrobenzyl alcohol or glycerol as matrix. HRFABMS data were determined using PEG 300 or 600 as the reference.

Physiochemical properties

O-Phenethylherbarin (**1**): Yellow solid, m.p. 143–144 °C. – $[a]_D^{25}$ +66.0 ° (*c* 0.043, CDCl$_3$). – UV (MeOH): λ_{max} (log ε) = 214 (4.51), 265 (4.11), 411 (3.48) nm; λ_{min} (log ε) = 230 (3.90), 318 (2.99) nm. – IR (KBr): ν = 3436, 2939, 1657, 1595, 1456, 1330, 1278, 1213, 1158, 1101, 1049, 701 cm^{-1}. – ^{1}H and ^{13}C NMR: see Tables I and II. – FABMS: *m/z* (%) = 245.1 [M–CH$_3$COO(CH$_2$)$_2$Ph+H]$^+$ (31), 287.1 [M–O(CH$_2$)$_2$Ph]$^+$ (22), 409.2 [M+H]$^+$ (14). – HRFABMS: *m/z* = 409.1656; [C$_{24}$H$_{24}$O$_6$+H]$^+$ requires *m/z* = 409.1646.

Table I. ^{1}H NMR data (400 MHz, CDCl$_3$) of **1**, **2a**, and **3**. Coupling constants (*J*) are given in Hz.

H	1	2a	3
1	4.61; ddd; 18.8, 2.6, 0.9	4.24; dd; 11.9, 10.6	4.10; dd; 11.9, 4.7
	4.14; ddd; 18.8, 4.2, 3.1	4.13; dd; 11.9, 4.7	3.88; dd; 11.9, 10.7
4	2.82; ddd; 18.6, 3.1, 0.9	2.37; dd; 13.7, 3.8	2.33; dd; 13.8, 3.8
	2.46; ddd; 18.6, 4.2, 2.6	1.72; dd; 13.7, 11.7	1.72; dd; 13.8, 11.6
4a	–	3.38; ddd; 13.2, 11.7, 3.8	3.37; ddd; 13.2, 11.6, 3.8
6	7.27; d; 2.5	7.14; d; 2.5	7.13; d; 2.4
8	6.72; d; 2.5	6.72; d; 2.5	6.71; d; 2.4
10a	–	2.92; ddd; 13.2, 10.6, 4.7	2.91; ddd; 13.2, 10.7, 4.7
3-CH$_3$	1.48; s	1.51; s	1.39; s
7-OCH$_3$	3.97; s	3.93; s	3.92; s
9-OCH$_3$	3.95; s	3.92; s	3.91; s
1'	3.69; m	–	3.23; s
2'	2.79; m	–	–
2"/6"	7.16; m	–	–
3"/5"	7.20; m	–	–
4"	7.16; m	–	–
3-OH	–	~2.0	–

Table II. ^{13}C NMR data (101 MHz, CDCl$_3$) of **1**, **2a**, and **3**. Multiplicities were determined indirectly by DEPT-135 and HSQC.

C	1	2a	3
1	58.67; t	60.29; t	60.27; t
3	97.12; s	95.47; s	97.79; s
4	32.75; t	34.70; t	35.79; t
4a	136.81; s	45.15; d	45.02; d
5	184.04; s	197.23; s	197.40; s
5a	136.13; s	139.00; s	139.00; s
6	103.53; d	102.08; d	102.03; d
7	162.04; s	164.79; s	161.29; s
8	104.13; d	104.84; d	104.92; d
9	164.72; s	161.36; s	164.74; s
9a	114.25; s	118.11; s	118.13; s
10	181.63; s	194.01; s	194.25; s
10a	143.02; s	48.86; d	48.71; d
3-CH$_3$	–	30.27; q	23.39; q
7-OCH$_3$	56.55; q	56.11; q	–
9-OCH$_3$	56.11; q	56.56; q	–
1'	62.50; t	–	48.08; q
2'	36.66; t	–	–
1"	138.93; s	–	–
2"/6"	129.13; d	–	–
3"/5"	128.42; d	–	–
4"	126.51; d	–	–

Table III. ^{1}H (400 MHz) and ^{13}C NMR data (101 MHz, CDCl$_3$) of **2b**. Coupling constants (J) are given in Hz. Multiplicities were determined indirectly by DEPT-135.

Position	δ_H	δ_C
1	–	194.01; s
2	3.21; ddd; 12.5, 4.7, 2.4	53.07; d
3	3.48; ddd; 12.5, 5.5, 4.3	45.24; d
4	–	196.82; s
4a	–	138.91; s
5	7.15; d; 2.4	102.18; d
6	–	165.04; s
7	6.73; d; 2.4	105.02; d
8	–	161.17; s
8a	–	118.34; s
6-OCH$_3$	3.92; s	56.11; q
8-OCH$_3$	3.94; s	56.02; q
1'	4.23, 3.67; m	59.95; t
1"	3.05; dd; 18.0, 4.3	40.47; t
	3.00; dd; 18.0, 5.5	
2"	–	206.56; s
3"	2.28; s	30.49; q

Herbaridine A (**2**): Yellow solid, m.p. 120 – 121 °C. – $[a]_D^{25}$ +45.8 ° (*c* 0.041, CDCl$_3$). – UV (MeOH): λ_{max} (log ε) = 235 (4.36), 252 (4.26), 279 (3.96), 337 (3.83) nm; λ_{min} (log ε) = 245 (4.23), 270 (3.93), 306 (3.46) nm. – IR (KBr): v = 3446, 1682, 1596, 1457, 1321, 1208, 1160, 1053, 1016 cm^{-1}. – ^{1}H and ^{13}C NMR of **2a**: see Tables I and II; of open-chain ketone **2b**: see Table III. – FABMS: *m/z* (%) = 289.1 [M–OH]$^+$ (80), 307.1 [M+H]$^+$ (53). – HRFABMS: *m/z* = 289.1069; [C$_{16}$H$_{18}$O$_6$–OH]$^+$ requires *m/z* = 289.1071.

Herbaridine B (**3**): Yellow solid, m.p. 153 – 154 °C. – $[a]_D^{25}$ +110.7 ° (*c* 0.058, CDCl$_3$) – UV (MeOH): λ_{max} (log ε) = 235 (4.34), 252 (4.24), 279 (3.94), 338 (3.81) nm; λ_{min} (log ε) = 245 (4.21), 270 (3.92), 306 (3.44) nm. – IR (KBr): v = 2939, 1683, 1596, 1457, 1324, 1254, 1222, 1160, 1046, 825 cm^{-1}. – ^{1}H and ^{13}C NMR: see Tables I and II: – FABMS: *m/z* (%) = 289.1 [M–OCH$_3$]$^+$ (54), 321.1 [M+H]$^+$ (58). – HR-FABMS: *m/z* = 321.1344, [C$_{17}$H$_{20}$O$_6$+H]$^+$ requires *m/z* = 321.1333.

Herbarin (**4**): Yellow solid, m.p. 205 – 207 °C (Lit. 192 – 193 °C; Kadkol *et al.*, 1971). – UV (MeOH): λ_{max} (log ε) = 212 (4.32), 263 (3.99), 404 (3.35) nm; λ_{min} (log ε) = 227 (3.70), 314 (2.62) nm. – IR (KBr):

v = 3378, 2954, 1656, 1639, 1598, 1561, 1469, 1324, 1282, 1219, 1166, 1095, 1069, 1039, 978, 939, 847, 814, 729 cm^{-1}. – ^{1}H NMR (500 MHz, CDCl$_3$): δ = 7.24 (d, J = 2.4 Hz, 1H, H-6), 6.70 (d, J = 2.4 Hz, 1H, H-8), 4.74 (ddd, J = 18.9, 2.7, 1.2 Hz, 1H, H$_a$-1), 4.68 (ddd, J = 18.9, 3.9, 3.0 Hz, 1H, H$_b$-1), 3.95 (s, 3H, OMe), 3.94 (s, 3H, OMe), 2.83 (ddd, J = 18.7, 3.0, 1.2 Hz, 1H, H$_a$-4), 2.54 (ddd, J = 18.7, 3.9, 2.7 Hz, 1H, H$_b$-4), 2.30 (s, 1H, OH-3), 1.61 (s, 3H, CH$_3$–3) (Paranagama *et al.*, 2007). – APCIMS pos.: *m/z* (%) = 287.1 [M–OH]$^-$ (100), 305.1 [M+H]$^+$ (56); APCIMS neg.: *m/z* (%) = 286.0 [M–H$_2$O]$^-$ (52), 304.1 [M]$^-$ (100).

Dehydroherbarin (**5**): Crimson solid, m.p. 192 – 193 °C (Lit. 189 – 190 °C; Kadkol *et al.*, 1971). – UV (MeOH): λ_{max} (log ε) = 215 (4.47), 249 (4.11), 277 (4.15), 332 (3.68), 390 (3.49), 483 (3.61) nm; λ_{min} (log ε) = 234 (4.07), 258 (4.08), 315 (3.60), 369 (3.45), 427 (3.42) nm. – IR (KBr): v = 1657, 1625, 1589, 1467, 1390, 1321, 1291, 1249, 1223, 1202, 1155, 1068, 1042, 964, 847, 748 cm^{-1}. – ^{1}H NMR (500 MHz, CDCl$_3$): δ = 7.26 (d, J = 2.5 Hz, 1H, H-6), 6.71 (d, J = 2.5 Hz, 1H, H-8), 5.84 (q, J = 0.8 Hz, 1H, H-4), 5.12 (s, 2H, CH$_2$), 3.95 (s, 3H, OMe), 3.94 (s, 3H, OMe), 2.00 (d, J = 0.8 Hz, 3H, CH$_3$–3) (Kesteleyn and De Kimpe, 2000). – APCIMS pos.: *m/z* (%) = 287.2 [M+H]$^+$ (100); APCIMS neg.: *m/z* (%) = 271.0 [M–CH$_3$]$^-$ (13), 286.0 [M]$^-$ (25).

O-Methylherbarin (**6**): Yellow solid, m.p. 183–184 °C (Lit. 188–190 °C; Narasimhachari and Gopalkrishnan, 1974). – $[a]_D^{25}$ +59.5 ° (*c* 0.033, CDCl$_3$). – UV (MeOH): λ_{max} (log ε) = 212 (4.33), 262 (4.01), 406 (3.32) nm; λ_{min} (log ε) = 230 (3.83), 316 (2.92) nm. – IR (KBr): ν = 3414, 2940, 1658, 1595, 1565, 1465, 1426, 1331, 1277 1213, 1184, 1161, 1100, 1052, 941, 851, 832 cm^{-1}. – ^{1}H NMR (500 MHz, CDCl$_3$): δ = 7.25 (d, *J* = 2.4 Hz, 1H, H-6), 6.58 (d, *J* = 2.4 Hz, 1H, H-8), 4.71 (ddd, *J* = 18.8, 2.6, 0.8 Hz, 1H, H$_a$-1), 4.45 (ddd, *J* = 18.8, 4.2, 3.3 Hz, 1H, H$_b$-1), 3.95 (s, 3H, OMe), 3.94 (s, 3H, OMe), 3.29 (s, 3H, OCH$_3$–3), 2.80 (ddd, *J* = 18.7, 3.3, 0.8 Hz, 1H, H$_a$-4), 2.56 (ddd, *J* = 18.7, 4.2, 2.6 Hz, 1H, H$_b$-4), 1.51 (s, 3H, CH$_3$–3) (Narasimhachari and Gopalkrishnan, 1974). – APCIMS pos.: *m/z* (%) = 245.1 [M–CH$_3$COOCH$_3$+H]$^+$ (58), 287.1 [M–OCH$_3$]$^+$ (100), 319.1 [M+H]$^+$ (20); APCIMS neg.: *m/z* (%) = 318.1 [M]$^-$ (60).

Scorpinone (**7**): Yellow solid, m.p. 213–217 °C (Lit. 214–215 °C; Cameron *et al.*, 1980). – UV (MeOH): λ_{max} (log ε) = 233 (4.43), 277 (4.13), 395 (3.65) nm; λ_{min} (log ε) = 253 (4.06), 348 (3.31) nm. – IR (KBr): ν = 3415, 1660, 1598, 1451, 1394, 1330, 1294, 1221, 1169, 1128, 1059, 1017, 949, 853, 723 cm^{-1}. – ^{1}H NMR (500 MHz, CDCl$_3$): δ = 9.41 (s, 1H, H-1), 7.81 (s, 1H, H-4), 7.43 (d, *J* = 2.4 Hz, 1H, H-6), 6.84 (d, *J* = 2.4 Hz, 1H, H-8), 4.02 (s, 3H, OMe), 3.99 (s, 3H, OMe), 2.74 (s, 3H, CH$_3$–3) (Kesteleyn and De Kimpe, 2000). – APCIMS pos.: *m/z* (%) = 284.1 [M+H]$^+$ (100); APCIMS neg.: *m/z* (%) = 268.0 [M–CH$_3$]$^-$ (10), 283.0 [M]$^-$ (14).

Biological assays

The minimal inhibitory concentrations (MICs) against bacteria and fungi were determined as described previously (Anke *et al.*, 1989).

Cytotoxicity was assayed as described previously (Schoettler *et al.*, 2006). The cell lines Jurkat (ATCC TIB 152), Colo-320 (DSMZ ACC 144) and L-1210 (DSMZ ACC 123) were grown in RPMI 1640 medium (Invitrogen). Neuro-2A (DSMZ ACC 148) was grown in DMEM medium (Invitrogen). All media were supplemented with 10% heat-inactivated fetal calf serum (Invitrogen), 65 µg/ml of penicillin G and 100 µg/ml of streptomycin sulfate.

Results and Discussion

Isolation and structures

The naphthoquinones (Fig. 1) were isolated by cytotoxicity-guided fractionation using Jurkat cells for the biological assay. The UV data implied that they are structurally related. *O*-Phenethylherbarin (**1**) has a nominal mass of 408.2, and HRMS measurements gave an elemental composition of C$_{24}$H$_{24}$O$_6$, requiring 13 unsaturations. NMR spectra showed the presence of a benzene ring with two *meta*-coupled aromatic protons attached to carbon atoms resonating at high field. HMBC correlations revealed an 1,3-dimethoxybenzene partial structure. The proton at 7.27 showed an HMBC correlation to one of two carbonyl groups, which should be located in *ortho*-position. The
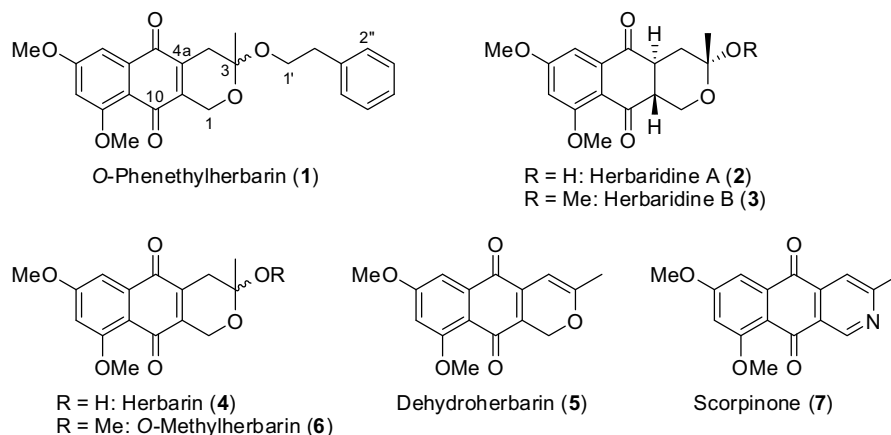

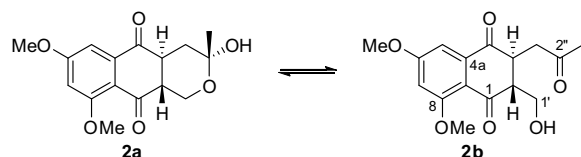Fig. 1. Chemical structures of the isolated compounds.

Fig. 2. Ring-opening equilibrium between hemiacetal **2a** and ketone **2b**.

same carbonyl group showed HMBC correlations to two methylene protons which exhibited homoallylic ^5J coupling to the protons of another methylene group over a double bond formed by two quaternary carbon atoms. The latter methylene group gave HMBC correlations to the other carbonyl group, establishing the structure of a 5,7-dimethoxy-1,4-naphthoquinone. The two methylene groups are part of an anellated dihydropyran unit, forming the 2-phenylethyl acetal of the known pyranonaphthoquinone herbarin (**4**), and NMR data were well consistent with those reported for **4** (Paranagama *et al.*, 2007) which also exhibited homoallylic ^5J couplings.

Herbaridine A (**2**) has a nominal mass of 306.1, and HRMS measurements gave an elemental composition of $C_{16}H_{18}O_6$, requiring 8 unsaturations. NMR analysis indicated the presence of a similar naphthoquinone scaffold, although no ^5J coupling was observed. All methylene protons exhibited dd multiplicity, and the double bond connecting the carbonyl groups was no longer present. Instead, two vicinal methine carbon atoms were found, and each of the corresponding protons showed coupling with the respective adjacent methylene group. As only a weak NOESY contact but a large ^3J coupling constant of 13.2 Hz was observed, both protons should occupy axial positions in the *trans*-anellated tetrahydropyran ring. The resulting structure is that of a cyclic hemiacetal and almost identical to that

of **4**, apart from the absence of the central double bond. In the tetrahydropyran ring, the axial protons of the methylene groups could be identified by their upfield chemical shifts and the large axial-axial couplings to the adjacent methine protons. The protons of the methyl group bound to the hemiacetal carbon atom showed nearly equipotent NOESY correlations to both protons of the neighbouring methylene group, so it should be located equatorially. The NOESY spectrum also exhibited exchange with a minor component which was identified as the ring-opened ketone **2b** (Fig. 2). Although interconversion of **2a** and **2b** was fast enough to be detected in the NOESY experiment, rendering the anomeric centre permanently labile, the stereoelectronically disfavoured epimer with an axial methyl group was not found in the NMR spectra.

Herbaridine B (**3**) has a nominal mass of 320.1, an elemental composition of $C_{17}H_{20}O_6$ according to HRMS measurements and thus 8 unsaturations. NMR analysis quickly revealed it to be the methyl acetal of herbaridine A (**2**). Again, the vicinal protons at positions 4a and 10a were found in *trans*-axial position by evaluation of coupling constants and NOESY data. The same holds true for the configuration of the acetal carbon atom which carries the electron withdrawing substituent in axial position, since characteristic NOE contacts were found between the methyl group and the methylene protons at position 4. Due to the acetal structure, ring opening was not observed for **3**.

Biological properties

The cytotoxic activities of the compounds are modest. The IC_{50} values are listed in Table IV. No data on the cytotoxicity of the known compounds **4 – 7** could be found; therefore they were included in the testing.

Table IV. Cytotoxicity of the naphthoquinones.

	IC_{50} [μg/ml]						
Cell line	*O*-Phenethyl-herbarin (**1**)	Herbaridine A (**2**)	Herbaridine B (**3**)	Herbarin (**4**)	Dehydro-herbarin (**5**)	*O*-Methyl-herbarin (**6**)	Scorpinone (**7**)
Jurkat	7.5	1.5	2	1	0.5	6	10
Colo-320	2.5	2.5	0.5	0.5	0.5	2.5	5
Neuro-2A	15	5	2.5	2.5	2.5	7.5	20
L-1210	10	2	0.5	2	2.5	7.5	> 20

Table V. MICs of the naphthoquinones.

Organism	O-Phene-thyl-herbar-in (**1**)	Herbaridine A (**2**)	Herbaridine B (**3**)	Herbarin (**4**)	Dehydro-herbarin (**5**)	O-Methyl-herbarin (**6**)	Scorpinone (**7**)
	MIC [μg/ml]						
Micrococcus luteus	> 50	> 50	> 50	> 50	> 50	> 50	> 50
Bacillus brevis	> 50	20 s	20 s	5 z	20 s	20 z	5 z
Bacillus subtilis	> 50	20 s	20 s	5 z	20 s	20 z	5 z
Mycobacterium phlei	> 50	20 s	20 s	5 s	20 s	20 s	5 s
Enterobacter dissolvens	> 50	> 50	> 50	> 50	> 50	> 50	> 50
Escherichia coli	> 50	20 s	20 s	5 s	10 s	20 s	5 s
Nematospora coryli	> 50	> 50	> 50	30	20	30	> 50

s, Bacteriostatic; z, bactericidal.

The naphthoquinone derivatives showed moderate antibacterial activity except for O-phenethylherbarin (**1**) which showed no activity up to 50 μg/ml. None of the tested filamentous fungi (*Paecilomyces variotii*, *Penicillium notatum*, *Mucor miehei*) was sensitive up to 50 μg/ml against compounds **1**–**7**. Herbarin (**4**), dehydroherbarin (**5**) and O-methylherbarin (**6**) showed moderate activity against the yeast *Nematospora coryli*. Antimicrobial activity for a mixture of **4** and **5** could be found in the literature (Kadkol *et al.*, 1971). For *Bacillus subtilis* and *Micrococcus luteus*, the MIC was 20 and 75 μg/ml, respectively. For fungal species, the MIC exceeded 50 μg/ml except for *Alternaria solani* with 50 μg/ml. Our findings correspond to the literature data with a MIC for *Bacillus subtilis* of 5 μg/ml for **4** and 20 μg/ml for **5**. The MICs for *Micrococcus luteus* and filamentous fungi exceeded 50 μg/ml which is consistent with the published data. The MIC values are listed in Table V.

Medentsev and Akimenko (1998) suggested that naphthoquinones are classical secondary metabolites of fungi and that their production is preferred under growth inhibition conditions. This was confirmed by the production of the metabolites of IBWF79B-90A eight days after complete consumption of glucose in the medium when the growth had already ceased. The structural relationship and the presumption of a common precursor of all the described compounds are obvious. The values for cytotoxicity, which ranged between 0.5 and 7.5 μg/ml for the closely related compounds herbaridine A (**2**), herbaridine B (**3**), herbarin (**4**), dehydroherbarin (**5**), and O-methylherbarin (**6**) were slightly higher than for O-phenethylherbarin (**1**) and scorpinone (**7**). For fusarubin and related compounds, the IC$_{50}$ values for L-1210 cells range from 1.7 to 6.2 μg/ml (Kurobane *et al.*, 1986). This cytotoxicity of the fusarubin derivatives corresponds to those of the herbarin derivatives. The antimicrobial activity with values of 5 to 30 μg/ml against several bacteria and the yeast *Nematospora coryli* is modest. Herbarin (**4**) and scorpinone (**7**) showed the highest bioactivity with values of 5 μg/ml against the *Bacillus* species, *Escherichia coli* and *Mycobacterium phlei*. Kurobane *et al.* (1986) investigated fusarubin derivatives and the values of the MICs for *Bacillus subtilis* (12.5–50 μg/ml), *Micrococcus luteus* (50–100 μg/ml) and two different *Candida albicans* (25–50 μg/ml) agree with those of the structurally related herbarin derivatives. The impact of quinones like bostrycoidin and fusarubin on the respiratory chain was investigated by Bironaité *et al.* (1992) for bovine heart mitochondria and by Medentsev and Akimenko (1992) for pea seedlings – both suggested that the biological activity is caused by oxidative stress.

Anke H., Bergendorff O., and Sterner O. (1989), Assays of the biological activities of guaiane sesquiterpenoids isolated from the fruit bodies of edible *Lactarius* species. Food Chem. Toxicol. **27**, 393–397.

Arsenault G. P. (1965), The structure of bostrycoidin, a *β*-aza-anthraquinone from *Fusarium solani* D$_2$ purple. Tetrahedron Lett. **45**, 4033–4037.

Bironaité D. A., Čénas N. K., Anusevičius Ž. J., Medentsev A. G., Akimenko V. K., and Usanov S. A. (1992), Fungal quinone pigments as oxidizers and inhibitors of mitochondrial NADH:ubiquinone reductase. Arch. Biochem. Biophys. **297**, 253–257.

Cameron D. W., Deutscher K. R., and Feutrill G. I. (1980), Synthesis of bostrycoidin and 8-*O*-methylbostrycoidin. Tetrahedron Lett. **21**, 5089–5090.

Gottlieb H. E., Kotlyar V., and Nudelman A. (1997), NMR chemical shifts of common laboratory solvents as trace impurities. J. Org. Chem. **62**, 7512–7515.

Hayashi T., Smith F. T., and Lee K.-H. (1987), Antitumor agents. 89. Psychorubin, a new cytotoxic naphthoquinone from *Psychotria rubra* and its structure-activity relationships. J. Med. Chem. **30**, 2005–2008.

Kadkol M. V., Gopalkrishnan K. S., and Narasimhachari N. (1971), Isolation and characterization of naphthoquinone pigments from *Torula herbarum* (Pers.). Herbarin and dehydroherbarin. J. Antibiot. **4**, 245–248.

Kesteleyn B. and De Kimpe N. (2000), Synthesis of two naphthoquinone antibiotics, dehydroherbarin and 6-deoxybostrycoidin. J. Org. Chem. **65**, 640–644.

Kurobane I., Zaita N., and Fukuda A. (1986), New metabolites of *Fusarium martii* related to dihydrofusarubin. J. Antibiot. **39**, 205–214.

Medentsev A. G. and Akimenko V. K. (1992), Mechanism of phytotoxic action of naphthoquinone pigments of the fungus *Fusarium decemcellulare*. Phytochemistry **31**, 77–79.

Medentsev A. G. and Akimenko V. K. (1998), Naphthoquinone metabolites of the fungi. Phytochemistry **47**, 935–959.

Miljkovic A., Mantle P. G., Williams D. J., and Rassing B. (2001), Scorpinone: a new natural azaanthraquinone produced by a *Bispora*-like tropical fungus. J. Nat. Prod. **64**, 1251–1253.

Narasimhachari N. and Gopalkrishnan K. S. (1974), Naphthoquinone pigments from *Torula herbarum*: structure of *O*-methylherbarin. J. Antibiot. **27**, 283–287.

Paranagama P. A., Wijeratne E. M. K., Burns A. M., Marron M. T., Gunatilaka M. K., Arnold A. E., and Gunatilaka A. A. L. (2007), Heptaketides from *Corynespora* sp. inhabiting the cavern beard lichen, *Usnea cavernosa*: first report of metabolites of an endolichenic fungus. J. Nat. Prod. **70**, 1700–1705.

Schoettler S., Bascope M., Sterner O., and Anke T. (2006), Isolation and characterisation of two verrucarins from *Myrothecium roridum*. Z. Naturforsch. **61c**, 309–314.

Tanaka H., Koyama Y., Nagai T., Marumo H., and Omura S. (1975), Nanaomycins, new antibiotics produced by a strain of *Streptomyces*. J. Antibiot. **28**, 868–875.