Total Synthesis of the Toxin Oosponol and of Structural Analogues and Investigation of Their Antibiotic Activities

Tibor Kovács, Isolde Sonnenbichler, and Johann Sonnenbichler*

Max-Planck-Institut für Biochemie,
D-82152 Martinsried, Germany

Received October 14, 1996

Keywords: *Gloeophyllum abietinum* / Oosponol / Isocoumarin / Structure-activity relationships / Antibiotics

The toxic metabolite of the basidiomycete *Gloeophyllum abietinum*, oosponol (**6b**), and the structural analogues (Figure 1) were synthesised in order to investigate which partial structures of the molecules are responsible for their biological activities. Different organisms were employed to test the antibiotic activities of the analogues. From the results obtained with the synthetic analogues of oosponol (**6b**), it became evident that the toxicity of this fungal metabolite can be attributed to a vinylogous acid anhydride structure, which in nature is produced by dehydrogenation of the nontoxic precursor oospoglycol (**7**).

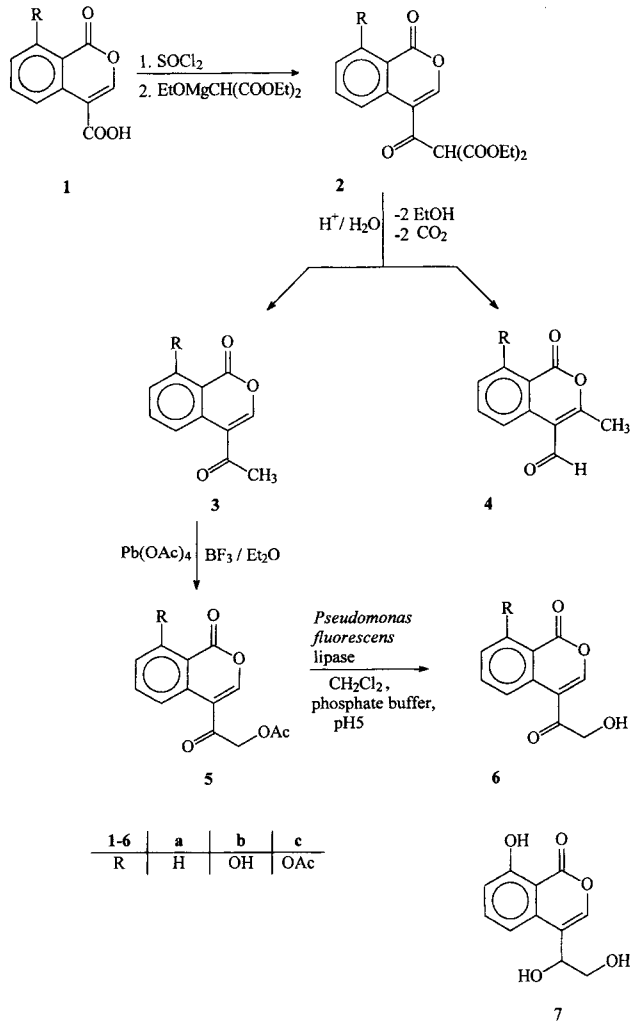
Introduction

In conifer forests, the basidiomycete *Gloeophyllum abietinum* competes with other fungi, such as the wood rot-causing *Armillaria ostoyae*, and produces a series of secondary metabolites[1] some of which are toxic to the antagonistic fungi. The isocoumarins oosponol (**6b**)[2] and oospoglycol (**7**) (Figure 1) are the most important metabolites from *G. abietinum*. Already Nozawa et al.[3] reported that oosponol (**6b**) exerts a strong antifungal activity against various plant-pathogenic moulds. Oosponol (**6b**) also causes severe skin rash, bronchitis and pneumonia and inhibits dopamine β-hydroxylase[4]. In contrast to oosponol (**6b**), the reduced form, oospoglycol (**7**), is not toxic[5]. In order to understand the interaction of oosponol with the molecular biology of the target cells, we were interested in those partial structures of the fungal metabolite that are responsible for its biological activity. For this purpose, oosponol (**6b**) was synthesised, and analogues (Figure 1) with structural similarities to the natural metabolite were also prepared. The fungicide, bactericidal and phytotoxic properties of the compounds were tested.

Results and Discussion

Before this work, a total synthesis of oosponol had not been performed. In order to develop a suitable method to construct the molecule, we first tried to prepare 4-[(hydroxy)-acetyl]-isocoumarin (**6a**), the 8-deoxy derivative of oosponol (**6b**). For this purpose, we used isocoumarin-4-carboxylic acid (**1a**) as a starting material, synthesised according to the Wolfbeis[6] method. Then the acetyl side-chain of the 4-acetyl-isocoumarin (**3a**) was introduced in 38% yield via reaction of the corresponding acid chloride with diethyl ethoxymagnesiummalonate, followed by an acid-catalysed hydrolysis and a decarboxylation step (Figure 1). Besides **3a** also 3-methyl-4-isocoumarincarbaldehyde (**4a**) was formed as a by-product. The ratio of **3a** to **4a** was 13:5, which can be explained by an acyl-lactone rearrangement[7].

Figure 1. Total synthesis of oosponol (**6b**) and analog isocoumarins and structure of oospoglycol (**7**)



A more complicated step further on in the synthesis involved the introduction of the hydroxy group at C-10. For

  0947–3440/97/0404–0773 $ 17.50+.50/0

this purpose, we successfully adopted a method from steroid chemistry[8,9] to introduce an acetoxy group at C-10 via an oxidative acetylation. We obtained here as the product the acetoxy-acetyl compound (**5a**) in 69% yield. The final step to produce **6a** had to be the cleavage of the protecting acetyl group. Due to the very high hydrolytic reactivity[10] of oosponol (**6b**), the hydrolysis under basic conditions would have been unsuccessful. Earlier attempts[11] to remove such acetyl groups by acid-catalysed hydrolysis also failed. We, therefore, applied an enzyme-catalysed hydrolysis at pH 5.0 using *Pseudomonas fluorescens* lipase in 93% yield to split off the acetyl moiety.

The described method of synthesis seemed suitable to also prepare oosponol (**6b**), using 8-hydroxy-isocoumarin-carboxylic acid (**1b**) as the starting material. In this case, however, the phenolic hydroxy group had to be protected before the condensation step with ethoxymagnesiummalonate and also during the oxidative acetylation. As a protecting group, we chose again acetate, since this group can be removed by the same enzymatic method with *P. fluorescens* lipase, as described before. Using this method we obtained a product in a total yield of 10%, whose melting point and spectroscopic data[10,12] were identical to that of oosponol (**6b**). The structures of all intermediates were identified and characterised using mass-, ^{1}H- and ^{13}C-NMR, IR and UV spectra, as described in the Experimental Section. Besides oosponol (**6b**) and deoxyoosponol (**6a**), we also obtained a series of isocoumarins with similar structures. This enabled us to investigate the relationship between structures and biological activities.

For the biological assays, the purity of all compounds was checked by NMR. The described derivatives (Table 1) were tested for their ability to inhibit the growth of *Escherichia coli*, the Gram-negative bacteria *Pseudomonas aeruginosa*, Gram-positive bacteria such as *Staphylococcus aureus*, the fungal basidiomycetes *Gloeophyllum abietinum* and *Heterobasidion annosum* and, moreover, eucaryotic cells in cultures of *Picea abies*. From the evolutionary point of view, these are very different organisms.

The tests were carried out using the agar diffusion technique[13] in which the organisms are grown in Petri dishes and the compounds applied onto the agar using small filter papers.

Table 1 shows the results from the biological tests. One can state that the antibiotic activities look very similar both with procaryotic and eucaryotic organisms.

None of the test compounds is active against the Gram-negative bacteria *Pseudomonas aeruginosa*. The 4-acetyl derivatives, however, have antibiotic activity against the Gram-positive bacteria *Staphylococcus aureus* and against *Escherichia coli*. We found that oosponol, like all the 4-acetyl substituted isocoumarins, possesses a fairly strong antibiotic activity. The resistance of *Pseudomonas aeruginosa* can be understood by considering its capability to use aromatic and heterocyclic compounds as a carbon source[14]. The phytotoxicities of the compounds against the plant cells *Picea abies* parallel those of the antibiotic activities against bacteria. Oosponol (**6b**) and all the 4-acetyl-substituted derivatives are highly toxic against *Picea abies* cells.

Table 1. Tests on the bacterial, fungicide and phytotoxic properties of the *Gloeophyllum abietinum* metabolites Oosponol, Oospoglycol and their synthetic analogues; the compounds were applied on filter papers (100 µg/disk) on *Escherichia coli* (**A**), *Staphylococcus aureus* (**B**), *Pseudomonas aeruginosa* (**C**); near to the growing mycelia of *Heterobasidion annosum* (**D**) and *Gloeophyllum abietinum* (**E**) or *Picea abies callus* (**F**) − no effect, +, ++, +++ increasing growth inhibition or necrosis

Compound	A	B	C	D	E	F
Isocoumarin-carboxylic acid (**1a**)	-	-	-	-	-	-
4-Acetyl-isocoumarin (**3a**)	++	+	-	++	+	++
3-Methyl-isocoumarin-4-carbaldehyde (**4a**)	+	-	-	+	+	+
4-[(Acetoxy)-acetyl]-isocoumarin (**5a**)	+	+++	-	+++	+	+++
4-(Hydroxyacetyl)-isocoumarin (**6a**)	+++	+++	-	+++	+	+++
Oosponol (**6b**)	+++	+++	-	+++	+	+++
Oospoglycol (**7**)	-	-	-	-	-	-

The compounds **3a**, **4a**, **5a**, **6a** and **6b** show also a strong fungitoxic activity against *Heterobasidion annosum*. The biological activity of these compounds against the fungus *Gloeophyllum abietinum* is rather weak, which can be explained by considering that oosponol is a metabolite of this fungus and in *Gloeophyllum abietinum*, enzymes are present that catalyze *in vivo* the oxidation equilibrium between the biological inactive precursor oospoglycol (**7**) and the toxic oosponol (**6b**)[15].

Examining the structures of oosponol (**6b**) and oospoglycol (**7**), it is apparent that the different side chains of the metabolites must be responsible for the biological activities. Indeed, it can be concluded from the data in Table 1 that the toxicity is related to the presence of an acetyl group in position 4 of the isocoumarin ring. The phenolic (C-8) and also the aliphatic (C-10) hydroxy or acetoxy groups, in general, are not important for the antibiotic effects, but they do increase the biological activity.

Recently, Dietrich et al.[10] have described the high chemical reactivity of oosponol. This supports the statement that the vinylogous acid anhydride moiety of the compounds (involving a high reactivity to nucleophiles) is responsible for the biological activity.

The authors wish to thank Prof. *A. Bauernfeind* (Max von Pettenkofer Institut, Universität München), for generously providing the *Pseudomonas aeruginosa* and *Staphylococcus aureus* cultures, Prof. *W. Schäfer* and *I. Buhrow* (MPI für Biochemie, Martinsried) for measuring and interpreting the mass spectra.

Experimental Section

Melting points: Büchi-Tottoli apparatus (uncorrected). − ^{1}H and ^{13}C NMR: Bruker AMX 500 and AM 400 spectrometer, respectively, with TMS as internal standard in $CDCl_3$ or in $[D_6]$DMSO. − IR: Perkin-Elmer 882. − MS: Finnigan MAT 312 (70 eV). − UV: Perkin-Elmer Lambda 5 in ethanol. − GC Carlo-Erba HRGC 5160, capillary column DB1 (0.25 mm × 30 m, film thickness 25 µm) temperature programme: 60°C (5°C/min). − All reactions

were monitored by TLC. The solutions for workup were dried ($NaSO_4$) and concentrated in vacuo. – Compound **7** was isolated according to a procedure described in ref.[13].

Synthesis of Oosponol and Other Isocoumarin Derivatives: 1-Oxo-1H-2-benzopyran-4-carboxylic Acid (Isocoumarin-4-carboxylic Acid) (**1a**): Synthesis according to ref.[6]; yield 70%; m.p. 240°C (dec.). – IR (KBr): $\tilde{\nu}$ = 3300–2800 cm^{-1} (COOH), 1740 (CO), 1699 (COOH). – UV: λ (log ε) = 212 nm (4.30), 286 (4.00), 297 (4.00), 325 (3.97). – ^1H NMR ([D$_6$]DMSO): δ = 7.67 (ddd, *J* = 8.0 and 8.0 and <1.0 Hz, 1H, 7-H), 7.93 (ddd, *J* = 8.0 and 8.0 and 1.0 Hz, 1H, 6-H), 8.21 (dd, *J* = 8.0 and 1.0 Hz, 1H, 8-H), 8.37 (s, 1H, 3-H), 8.63 (dd, *J* = 8.0 and <1.0 Hz, 1H, 5-H), 13.35 (s, 1H, COOH). – ^{13}C NMR ([D$_6$]DMSO): δ = 109.5 (C-4), 119.9 (C-8a), 125.1 (C-5), 128.9 (C-7), 129.2 (C-8), 133.5 (C-4a), 135.4 (C-6), 153.0 (C-3), 160.4 (C-1), 165.3 (COOH). – MS; *m/z* (%): 190 (100) [M$^+$], 172 (18), 162 (55), 144 (28), 134 (53), 105 (58), 89 (28), 77 (19). – $C_{10}H_6O_4$ (190.2): calcd. C 63.16, H 3.18; found C 63.15, H 3.14.

4-Acetyl-1H-2-benzopyran-1-one (4-Acetylisocoumarin) (**3a**) *and 3-Methyl-1-oxo-1H-2-benzopyran-4-carbaldehyde (3-Methylisocoumarin-4-carbaldehyde)* (**4a**): 1.0 g (5.26 mmol) isocoumarin-4-carboxylic acid (**1a**) in dry benzene was refluxed in thionyl chloride (0.5 ml) until HCl was formed. The benzene and the thionyl chloride were removed under reduced pressure. – Diethyl ethoxymagnesium malonate was prepared by heating (with exclusion of moisture) a mixture of 570 mg (24.8 mmol) magnesium turnings, 1.45 ml (24.8 mmol) dry ethanol and 0.3 ml carbon tetrachloride. Then after the reaction was started, a mixture of 40 ml dry diethyl ether and 3.76 ml (24.8 mmol) diethyl malonate was added. The mixture was gently refluxed till the magnesium was nearly dissolved. To this mixture, an ethereal suspension of the acid chloride prepared above was added with stirring and the mixture heated for 2 h further until the magnesium complex separated as a yellow jelly. The reaction mixture was decomposed with 1 M sulphuric acid and extracted with diethyl ether. After evaporation of the solvent, the residual oil was refluxed with a mixture of 10 ml acetic acid, 2 ml sulphuric acid and 5 ml water for 3 h. On cooling the solution was diluted with water and extracted with diethyl ether. Chromatographic separation [TLC, silicagel 60 with toluene acetone (4:1)] gave 380 mg of **3a** (38%), and 146 mg (15%) **4a**. – **3a**: m.p. 173°C, R_f = 0.65 (toluene acetone 4:1). – IR (KBr): $\tilde{\nu}$ = 3097 cm^{-1} (CH aromat.), 2929 (CH methylene), 1740 (CO lactone), 1680 (free CO). – UV: λ (log ε) = 248 nm (3.89), 310 (3.29). – ^1H NMR ($CDCl_3$): δ = 2.55 (s, 3H, CH_3), 7.60 (ddd, *J* = 8.0 and 8.0 and 1.0 Hz, 1H, 7-H), 7.82 (ddd, *J* = 8.0 and 8.0 and 1.5 Hz, 1H, 6-H), 8.12 (s, 1H, 3-H), 8.37 (dd, *J* = 8.0 and 1.5 Hz, 1H, 8-H), 8.76 (dd, *J* = 8.0 and 1.0 Hz, 1H, 5-H). – ^{13}C NMR ($CDCl_3$): δ = 28.6 (CH_3), 118.3 (C-4), 121.8 (C-8a), 126.8 (C-8), 130.1 (C-7), 130.4 (C-5), 134.7 (C-4a), 136.4 (C-6), 155.4 (C-3), 161.5 (C-1), 197.2 (CO). – GC: t_{ret} = 22.5 min. – MS; *m/z* (%): 188 (100) [M$^+$], 173 (59), 160 (41), 145 (33), 118 (54), 90 (25), 89 (44). – $C_{11}H_8O_3$ (188.2): calcd. C 70.21, H 4.28; found C 70.45, H 4.26. – **4a**: m.p. 170–171°C, R_f = 0.70 (toluene acetone 4:1). – IR (KBr): $\tilde{\nu}$ = 1675 cm^{-1} (CO aldehyde), 1740 (CO lactone). – UV: λ (log ε) = 222 nm (4.39), 249 (4.08), 280 (3.83). – ^1H NMR ($CDCl_3$): δ = 2.70 (s, 3H, CH_3), 7.57 (ddd, *J* = 8.0 and 8.0 and 1.0 Hz, 1H, 7-H), 7.83 (ddd, *J* = 8.0 and 8.0 and 1.5 Hz, 1H, 6-H), 8.31 (dd, *J* = 8.0 and 1.5 Hz, 1H, 8-H), 8.84 (dd, *J* = 8.0 and 1.0 Hz, 1H, 5-H), 10.40 (s, 1H, CHO). – ^{13}C NMR ($CDCl_3$): δ = 18.0 (CH_3), 113.8 (C-4), 125.4 (C-8), 129.5 (C-7), 130.4 (C-5), 130.6 (C-8a), 134.4 (C-4a), 136.4 (C-6), 161.0 (C-3), 169.2 (C-1), 189.0 (CHO). – GC: t_{ret} = 25 min. – MS; *m/z* (%): 188 (100) [M$^+$], 173 (66), 160 (44), 145 (36), 118 (60), 90 (31), 89 (62). – $C_{11}H_8O_3$ (188.2): calcd. C 70.21, H 4.28; found C 70.29, H 4.38.

4-[(Acetoxy)acetyl]-1H-2-benzopyran-1-one (4-[(Acetoxy)acetyl]isocoumarin) (**5a**): A solution of 100 mg (0.53 mmol) 4-acetylisocoumarin (**3a**) and 241 mg (0.64 mmol) lead tetra-acetate in 5 ml acetic acid containing (0.54 ml) boron trifluoride-diethyl ether complex was stirred at 25°C. The reaction was followed by TLC (toluene acetone 10:1). After 3 hours the reaction was completed and the starch-iodide test for lead tetra-acetate was negative. The reaction mixture was diluted with water, extracted with diethyl ether, and the crude product was purified by preparative TLC on silica gel (toluene acetone, 10:1), which gave 90 mg (69%) yellow oil. – R_f = 0.42. – IR (KBr): $\tilde{\nu}$ = 3087 cm^{-1} (CH aromat.), 2940 (CH methylene), 1750, 1740 and 1690 (CO), 1240 (O–CO acetate). – UV: λ (log ε) = 218 nm (4.32), 310 (3.48). – ^1H NMR ($CDCl_3$): δ = 2.24 (s, 3H, CH_3), 5.08 (s, 2H, CH_2O), 7.60 (ddd, *J* = 8.0 and 8.0 and <1.0 Hz, 1H, 7-H), 7.80 (ddd, *J* = 8.0 and 8.0 and 1.5 Hz, 1H, 6-H), 8.05 (s, 1H, 3-H), 8.31 (dd, *J* = 8.0 and 1.5 Hz, 1H, 8-H), 8.52 (dd, *J* = 8.0 and <1.0 Hz, 1H, 5-H). – ^{13}C NMR ($CDCl_3$): δ = 21.1 (CH_3), 66.6 (CH_2O), 116.1 (C-4), 121.5 (C-8a), 126.3 (C-8), 130.3 (C-7), 130.7 (C-5), 133.4 (C-4a), 136.3 (C-6), 152.2 (C-3), 160.6 (C-1), 171.0 (OCO), 191.2 (CO). – MS; *m/z* (%): 246 (10) [M$^+$], 204 (26), 173 (100), 145 (6), 117 (5), 89 (28). – $C_{13}H_{10}O_5$ (246.2): calcd. C 63.42, H 4.09; found C 63.37, H 4.04.

4-(Hydroxyacetyl)-1H-2-benzopyran-1-one (4-(Hydroxyacetyl)-isocoumarin) (**6a**): To a solution of 100 mg (0.41 mmol) of **5a** in 8 ml of dichloromethane a solution of 4 ml phosphat buffer (pH 5)[16] and 100 mg *Pseudomonas fluorescens* lipase (3592U) was added, and the mixture was stirred at room temperature for 10 h. After filtration of the lipase the filter cake was washed with dry dichloromethane (3 × 2 ml). The filtrate was extracted with dichloromethane and purified by preparative TLC on silicagel 60 with toluene acetone (4:1) yielding 77 mg of **6a** (93%) as colourless prisms; m.p. 146–147°C; R_f = 0.44 (toluene acetone 4:1). – IR (KBr): $\tilde{\nu}$ = 3430 cm^{-1} (OH), 3100 (CH aromat.), 2910 (CH methylene), 1740 (CO lactone), 1675 (CO free). – UV: λ (log ε) = 219 nm (4.26), 311 (3.49). – ^1H NMR ($CDCl_3$): δ = 3.44 (s, 1H, OH), 4.70 (s, 2H, CH_2O), 7.64 (ddd, *J* = 8.1 and 8.1 and 1.0 Hz, 1H, 7-H), 7.86 (ddd, *J* = 8.1 and 8.1 and 1.5 Hz, 1H, 6-H), 8.1 (s, 1H, 3-H), 8.36 (dd, *J* = 8.1 and 1.5 Hz, 1H, 8-H), 8.71 (dd, *J* = 8.1 and 1.0 Hz, 1H, 5-H). – ^{13}C NMR ($CDCl_3$): δ = 66.0 (CH_2OH), 115.5 (C-4), 121.4 (C-8a), 126.3 (C-8), 130.4 (C-7), 130.9 (C-5), 133.3 (C-4a), 136.5 (C-6), 152.8 (C-3), 160.5 (C-1), 197.0 (CO). – MS; *m/z* (%): 204 (36) [M$^+$], 188 (38), 173 (100), 145 (19), 118 (34), 117 (13), 89 (45), 63 (17). – $C_{11}H_8O_4$ (204.2): calcd. C 64.71, H 3.95; found C 64.68, H 3.98.

8-Hydroxy-1-oxo-1H-2-benzopyran-4-carboxylic Acid (**1b**): From 3-Hydroxyhomophthalic acid, according to ref.[6]; yield 78%; – m.p. 247°C (dec.) (Nitta 1963). – IR (KBr): $\tilde{\nu}$ = 3397 cm^{-1} (OH), 3300–2800 (COOH), 1687 (COOH) 1682 (CO chelat. lactone). – UV in acetonitrile: λ (log ε) = 210 nm (4.16), 333 (3.58). – ^1H NMR ([D$_6$]DMSO): δ = 7.10 (d, *J* = 8.0 Hz, 1H, 7-H), 7.81 (dd, *J* = 8.0 and 8.0 Hz, 1H, 6-H), 8.06 (d, *J* = 8.0 Hz, 1H, 5-H), 8.35 (s, 1H, 3-H), 10.95 (s, 1H, phen. OH), 13.38 (s, 1H, COOH). – ^{13}C NMR ([D$_6$]DMSO): δ = 105.9 (C-4), 110.7 (C-8a), 115.6 (C-7), 115.8 (C-5), 133.9 (C-4a), 137.9 (C-6), 152.3 (C-3), 160.7 (C-8), 164.1 (C-1), 165.1 (COOH). – MS; *m/z* (%): 206 (100) [M$^+$], 189 (6), 188 (25), 178 (59), 160 (72), 150 (26), 132 (16), 105 (26), 104 (47), 77 (25). – $C_{10}H_6O_5$ (206.2): calcd. C 58.26, H 2.93; found C 58.21, H 2.89.

8-Acetoxy-1-oxo-1H-2-benzopyran-4-carboxylic Acid (**1c**): To a solution of 1500 mg (7.3 mmol) **1b** in 10 ml dry pyridine, 4.5 ml

acetic anhydride were added and the mixture was allowed to stand for 12 h. The workup gave a crude product, which crystallized from isopropanol to yield 1470 mg (81%) **1c** as yellow crystals of m.p. 201 °C (dec.). – IR (KBr): $\tilde{\nu}$ = 3300–2800 cm^{-1} (COOH), 1750 and 1747 (CO), 1680 (COOH), 1210 (O–CO acetate). – UV in acetonitrile: λ (log ε) = 209 nm (4.25), 223 (4.25), 318.1 (3.62). – ^1H NMR ([D$_6$]DMSO): δ = 2.35 (s, 3H, CH_3), 7.39 (d, *J* = 8.0 Hz, 1H, 7-H), 7.97 (dd, *J* = 8.0 and 8.0 Hz, 1H, 6-H), 8.39 (s, 1H, 3-H), 8.58 (d, *J* = 8.0 Hz, 1H, 5-H), 13.39 (s, 1H, COOH). – ^{13}C NMR ([D$_6$]DMSO): δ = 20.8 (CH_3), 109.3 (C-4), 112.9 (C-8a), 123.0 (C-7), 123.6 (C-5), 135.7 (C-4a), 136.6 (C-6), 151.3 (C-8), 153.6 (C-3), 156.9 (C-1), 165.2 (COOH). – MS; *m/z* (%): 248 (12) [M^+], 220 (4), 206 (100), 188 (28), 178 (53), 160 (56), 150 (20), 134 (5), 132 (7), 106 (4), 105 (13), 104 (19). – $C_{12}H_8O_6$ (248.2): calcd. C 58.07, H 3.25; found C 57.96, H 3.21.

4-Acetyl-8-hydroxy-1H-2-benzopyran-1-one (4-Acetyl-8-hydroxyisocoumarin) (**3b**) *and 8-Hydroxy-3-methyl-1-oxo-1H-2-benzopyran-4-carbaldehyde (8-Hydroxy-3-methylisocoumarin-4-carbaldehyde)* (**4b**): 1.0 g (4.03 mmol) 8-Acetoxy-1-oxo-1H-2-benzopyran-4-carboxylic acid (**1c**) in dry benzene was refluxed in thionyl chloride (0.5 ml) until HCl was formed. The benzene and the thionyl chloride were removed under reduced pressure. – Diethyl ethoxymagnesiummalonate (24.8 mmol) was prepared as described under **3a** and the ethereal suspension of the acid chloride prepared above was added under stirring. The mixture was heated for further 2 h until the magnesium complex separated as a yellow jelly. The reaction mixture was decomposed with 1 M sulphuric acid and extracted with diethyl ether. After evaporation of the solvent, the residual oil was refluxed with a mixture of 10 ml acetic acid, 2 ml sulphuric acid and 5 ml water for 3 h. On cooling the solution was diluted with water and extracted with diethyl ether. Chromatographic separation [TLC, silicagel 60 with toluene acetone (10:1)] gave 296 mg of **3b** (36%), and 115 mg (14%) **4b**. – **3b**: m.p. 138 °C, R_f = 0.57 (toluene acetone 10:1). – IR (KBr): $\tilde{\nu}$ = 3440 cm^{-1} (OH), 3100 (CH aromat.), 2923 (CH methylene), 1695 (CO chelat. lactone), 1685 (CO free). – UV: λ (log ε) = 214.5 nm (4.29), 334.0 (3.68). – ^1H NMR ($CDCl_3$): δ = 2.55 (s, 3H, CH_3), 7.06 (d, *J* = 8.0 Hz, 1H, 7-H), 7.69 (dd, *J* = 8.0 and 8.0 Hz, 1H, 6-H), 8.00 (s, 1H, 3-H), 8.10 (d, *J* = 8.0 Hz, 1H, 5-H), 10.90 (s, 1H, phen. OH). – ^{13}C NMR ($CDCl_3$): δ = 28.9 (CH_3), 106.6 (C-8a), 117.4 (C-7), 117.6 (C-5), 119.4 (C-4), 133.8 (C-4a), 139.0 (C-6), 152.3 (C-3), 162.6 (C-8), 165.3 (C-1), 195.6 (CO). – MS; *m/z* (%): 204 (100) [M^+], 189 (31), 176 (59), 161 (36), 134 (50), 105 (40), 77 (19). – $C_{11}H_8O_4$ (204.2): calcd. C 64.71, H 3.95; found C 64.70, H 3.89. – **4b**: m.p. 124–126 °C, R_f = 0.64 (toluene acetone 10:1). – IR (KBr): $\tilde{\nu}$ = 3440 cm^{-1} (OH), 2920 (CH_3), 1700 (CO chelat. lactone), 1680 (CO aldehyde). – UV: λ (log ε) = 217.9 nm (4.54), 235.6 (4.29), 258.5 (4.10), 335.9 (3.98). – ^1H NMR ($CDCl_3$): δ = 2.70 (s, 3H, CH_3), 7.05 (d, *J* = 8.0 Hz, 1H, 7-H), 7.70 (dd, *J* = 8.0 and 8.0 Hz, 1H, 6-H), 8.20 (d, *J* = 8.0 Hz, 1H, 5-H), 10.35 (s, 1H, CHO), 10.80 (s, 1H, phen. OH). – ^{13}C NMR ($CDCl_3$): δ = 17.3 (CH_3), 105.9 (C-8a), 114.4 (C-4), 115.7 (C-7), 117.0 (C-5), 134.5 (C-4a), 139.2 (C-6), 162.5 (C-3), 165.1 (C-8), 167.6 (C-1), 188.7 (CHO). – MS; *m/z* (%): 204 (100) [M^+], 189 (30), 176 (57), 161 (36), 134 (51), 105 (36), 77 (20). – $C_{11}H_8O_4$ (204.2): calcd. C 64.71, H 3.95; found C 64.75, H 4.00.

8-Acetoxy-4-acetyl-1H-2-benzopyran-1-one (8-Acetoxy-4-acetylisocoumarin) (**3c**): To a solution of 250 mg (1.22 mmol) **3b** in 4 ml dry pyridine, 2 ml acetic anhydride was added and the mixture was allowed to stand for 12 h. The workup gave 246 mg crude product, which was purified by preparative TLC on silicagel 60 with toluene acetone (4:1) yielding 245 mg (81%) of **3c** as yellow oil; R_f = 0.63 (toluene acetone 4:1). – IR (KBr): $\tilde{\nu}$ = 3120 cm^{-1} (CH aromat.), 2929 (CH methylene), 1750, 1740 and 1670 (CO), 1210 (O–CO acetate). – UV: λ (log ε) = 215 nm (4.26), 318 (3.56). – ^1H NMR ($CDCl_3$): δ = 2.38 (s, 3H, $OCOCH_3$), 2.50 (s, 3H, CH_3), 7.19 (d, *J* = 8.0 Hz, 1H, 7-H), 7.77 (dd, *J* = 8.0 and 8.0 Hz, 1H, 6-H), 8.07 (s, 1H, 3-H), 8.61 (d, *J* = 8.0 Hz, 1H, 5-H). – ^{13}C NMR ($CDCl_3$): δ = 21.6 ($OCOCH_3$), 28.6 (CH_3), 114.2 (C-8a), 117.7 (C-4), 124.5 (C-7), 124.6 (C-5), 136.0 (C-4a), 136.9 (C-6), 152.5 (C-8), 154.2 (C-3), 157.3 (C-1), 170.1 (OCO), 195.6 (CO). – MS; *m/z* (%). 246 (9) [M^+], 204 (100), 189 (19), 176 (53), 161 (12), 134 (24), 105 (12). – $C_{13}H_{10}O_5$ (246.2): calcd. C 63.42, H 4.09; found C 63.21, H 4.12.

8-Acetoxy-4-[(acetoxy)acetyl]-1H-2-benzopyran-1-one (**5c**): A solution of 60 mg (0.24 mmol) 8-Acetoxy-4-acetylisocoumarin (**3c**) and 140 mg (0.32 mmol) lead tetra-acetate in 5 ml acetic acid containing (0.54 ml) boron trifluoride-diethyl ether complex was stirred at 25 °C. The reaction was followed by TLC (toluene acetone 4:1). The workup given for **5a**, followed by a purification by preparative TLC on silica gel (toluene acetone 4:1) yielded 48 mg (66%) yellow oil; R_f = 0.61. – ^1H NMR ($CDCl_3$): δ = 2.23 (s, 3H, $ROCOCH_3$), 2.40 (s, 3H, $ArOCOCH_3$), 5.05 (s, 2H, CH_2O), 7.25 (dd, *J* = 8.0 and <1.0 Hz, 1H, 7-H), 7.82 (dd, *J* = 8.0 und 8.0 Hz, 1H, 6-H), 8.01 (s, 1H, 3-H), 8.43 (dd, *J* = 8.0 and <1.0 Hz, 1H, 5-H). – ^{13}C NMR ($CDCl_3$): δ = 21.1 ($ArOCOCH_3$), 21.7 ($ROCOCH_3$), 66.8 (CH_2O), 114.6 (C-8a), 115.8 (C-4), 124.2 (C-7), 125.0 (C-5), 135.6 (C-4a), 137.2 (C-6), 152.4 (C-3), 152.8 (C-1), 170.1 (ArOCO), 171.0 (ROCO), 191.1 (CO). – MS; *m/z* (%): 304 (6) [M^+], 262 (60), 231 (5), 220 (35), 202 (25), 189 (100), 161 (7), 105 (18).

8-Hydroxy-4-(hydroxyacetyl)-1H-2-benzopyran-1-one (Oosponol) (**6b**): 100 mg (0.33 mmol) of **5c** was hydrolysed as **5a**. The workup yielded 90 mg crude product, which was purified by preparative TLC on silicagel 60 with toluene acetone (4:1) yielding 62 mg (85%) of **6b** of m.p. 172 °C (ref.[12] 172 °C); R_f = 0.32. – IR (KBr): $\tilde{\nu}$ = 3450 cm^{-1} (OH), 3135–3085 (CH aromat.), 2925 (CH methylene), 1695 (CO chelat. lactone), 1675 (CO free). – UV: λ (log ε) = 209 nm (4.32), 332 (3.58). – ^1H NMR ($CDCl_3$): δ = 4.66 (s, 2H, CH_2O), 7.14 (dd, *J* = 8.1 and <1.0 Hz, 1H, 7-H), 7.75 (dd, *J* = 8.1 and 8.1 Hz, 1H, 6-H), 7.92 (s, 1H, 3-H), 8.11 (dd, *J* = 8.1 and <1.0 Hz, 1H, 5-H), 10.88 (s, 1H, phen. OH). – ^{13}C NMR ($CDCl_3$): δ = 66.3 (CH_2OH), 116.7 (C-8a), 116.9 (C-7), 118.3 (C-4), 118.4 (C-5), 133.1 (C-6), 139.4 (C-4a), 151.2 (C-3), 162.8 (C-8), 164.8 (C-1), 196.8 (CO). – MS: *m/z* (%) = 220 (25) [M^+], 202 (21), 191 (13), 189 (100), 161 (11), 105 (27), 77 (9). – $C_{11}H_8O_5$: calcd. 220.03717, found 220.03677 (MS).

Biological Materials: Heterobasidion annosum (FR.) Bref./Synonym: *Fomes annosus* (FR.) (P. Karst) Line L1, Forstamt Freising (9.1.1980), *Gloeophyllum abietinum* (Bull. ex FR.) P. Karst: Forstamt Freising (22.3.1981). The basidiomycete *G. abietinum* and *H. annosum* were cultivated according to ref.[1]. – *Escherichia coli:* ATCC 25922, *Staphylococcus aureus:* ATCC 29213, *Pseudomonas aeruginosa:* ATCC 27853. – Callus tissue cultures of *Picea abies* were grown as described in ref.[13].

Test Systems: The tests for antibiotic and fungitoxic activity of the isolated compounds were performed using mainly the agar diffusion technique according to ref.[13]. A sterile filter paper (0.9 cm diameter) was loaded with each compound (0.1–100 μg) and placed onto the agar of a Petri dish which was inoculated with the test organisms: *G. abietinum, H. annosum, E. coli, S. aureus, P. aeruginosa.* In the case of *Picea abies* callus, the plant material was spread with a sterile spatula on agar areas that contained the tested compound. Growth inhibition was determined semiquantitatively by measuring the areas of inhibition radiating from the impreg-

nated filter sheets or by estimating the extent of necrosis compared to controls.

[1] H. Peipp, J. Sonnenbichler, *J. Biol. Chem. Hoppe Seyler* **1992**, *373*, 675–683.
[2] K. Nitta, J. Imai, I. Yamamoto, Y. Yamamoto, *Agr. Biol. Chem.*, **1963**, *27*, (12) 817–821.
[3] K. Nozawa, M. Yamada, Y. Tsuda, K. Kawai, S. Nakajima, *Chem. Pharm. Bull.* **1981**, *29*, 2689–2691.
[4] H. Umezawa, *Pure Appl. Chem.* **1973**, *33*, 129–144.
[5] H. Peipp, **1992**, Doctoral Thesis, University of Munich, p. 103.
[6] O. S. Wolfbeis, *Liebigs Ann. Chem.* **1981**, 819–827.
[7] F. Korte, H. Machleidt, *Chem. Ber.* **1955**, *88*, 136–143.
[8] H. B. Henbest, D. N. Jones, G. P. Slater, *J. Chem. Soc.* **1961**, 4472–4478.
[9] J. D. Cocker, H. B. Henbest, G. H. Phillips, G. P. Slater, D. A. Thomas, *J. Chem. Soc.* **1965**, 6–11.
[10] J. Dietrich, J. Sonnenbichler, T. Kovács, I. Zetl, *Biol. Chem. Hoppe-Seyler* **1994**, *375*, 629–633.
[11] C. Bhakta, *J. Indian Chem. Soc.* **1985**, *52*, 380–382.
[12] S. Nakajima, K. I. Kawai, S. Yamada, *Phytochemistry* **1976**, *15*, 327.
[13] J. Sonnenbichler, I. Blistle, H. Peipp, O. Holdenrieder, *Biol. Chem. Hoppe-Seyler* **1989**, *370*, 1295–1303.
[14] H. Werner, *Medizinische Mikrobiologie* **1992**, de Gruyter Lehrbuch, Berlin, New York, p. 116.
[15] J. Sonnenbichler, J. Dietrich, H. Peipp, *Biol. Chem. Hoppe Seyler*, **1994**, *375*, 71–79.
[16] H. M. Rauen, *Biochemisches Taschenbuch* **1956**, Springer Verlag, Berlin, Göttingen, Heidelberg, p. 652.

[96308]