Sorbicillin Analogues and Related Dimeric Compounds from *Penicillium notatum*

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In our screening of micro-organisms for new natural products, the fungus *Penicillium notatum* delivered further members of the sorbicillin family, namely the sohirnones A [**3**, 1-(2,4-dihydroxy-5-methylphenyl)-hex-4-en-1-one], B [**4a**, 1-(2,4,5-trihydroxy-3,6-dimethylphenyl)-hexa-2,4-dien-1-one], and C [**5**, 1-(2,4,5-trihydroxy-3,6-dimethylphenyl)-hex-4-en-1-one]. A stable tautomer of oxosorbicillinol (**7**) was characterized as **6**, and the recently described 7-deacetoxyyanuthone (**8**) was re-isolated. The additionally isolated rezishanones A-D (**12**-**13c**) are the first natural Diels-Alder products of sorbicillinol (**1**) with dienophils not related with **1**. The monomers and dimers showed weak antibacterial activity, however, were inactive against fungi and algae. The structures were determined by spectroscopic methods and by comparison of the NMR data with those of the structurally related 2',3'-dihydrosorbicillin (**2**) and in the case of **4a**, by transformation into the known sorrentanone (**4b**).

Vericillium intertextum, *Penicillium* sp., *Trichoderma* sp. and some other fungi are known to produce sorbicillinol (**1**), the parent compound of more than 30 vertinoids, monomeric and dimeric C-methylated hexaketides. The dimers are forming two groups, the Diels-Alder products like bisorbicillinol (**9a**), and the Michael-type adducts. The latter are at least tricyclic due to a further intramolecular *semi*acetal formation as in bisvertinolone (**10**). At least 15 of the expected homo and hetero dimers are already known,[1] and on the basis of their origin by completely regio- and stereocontrolled reactions, the structure of further dimers is predictable.

A new isolate GWP A of the well-known source of penicillin,[2] *Penicillium notatum*, produced in our hands the monomers 2',3'-dihydrosorbicillin[3] (**2**), the new sohirnones A-C (**3**-**5**), the tautomer **6** of oxosorbicillinol (**7**),[4] 7-deacetoxyyanuthone (**8**), and a complex mixture of dimeric compounds containing the sorbicillin skeleton, i.e. the Diels-Alder dimers[5] bisorbicillinol (**9a**)[6], its dihydro derivative bisvertinoquinol (**9b**),[7] bisorbibutenolide (**11a**),[8] the new adducts rezishanone A-D (**12**-**13c**), and the Michael product bisvertinolone (**10**)[9]. The rezishanones are the first members of a new class of Diels-Alder derivatives, where sorbicillinol (**1**) reacted with alkene components not belonging to the vertinoids.

Results and Discussion

Monomers

The *Penicillium notatum* isolate GWP A was obtained from a bench top contamination and cultured in M_2^+ medium at 28 °C for 48-72 h. The ethyl acetate extract of the culture broth delivered two main fractions, by chromatography on Sephadex LH-20, of which the first yielded **8** and some known and several new sorbicillin Diels-Alder products, and the second contained the monomers **2**-**6**, and additionally 2-pyruvoylaminobenzamide.[10]

A first compound was obtained by preparative HPLC of fraction 2 as a light yellow solid which was sparingly soluble in less polar solvents like cyclohexane, $CHCl_3$ and CH_2Cl_2. A search for this compound with the molecular weight *m/z* 234 (by ESI MS) and NMR-derived substructures in AntiBase[11] led to 2',3'-dihydrosorbicillin (**2**). This phenone was, however, reported to be easily soluble in $CHCl_3$, whereas our sample was nearly insoluble in this solvent but soluble in MeOH. Reported ^{13}C

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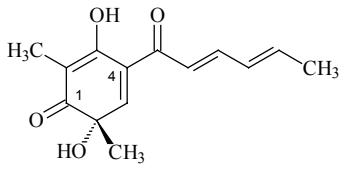

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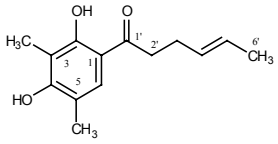

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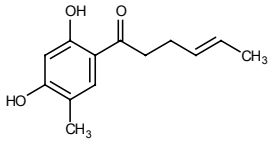

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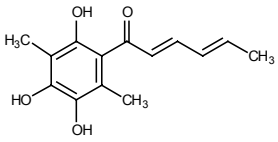
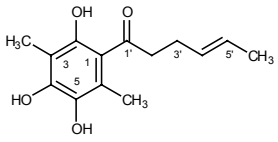


4a



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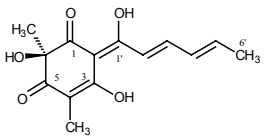
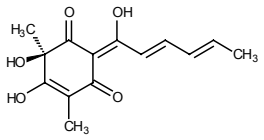
4b: quinone instead of hydroquinone



6



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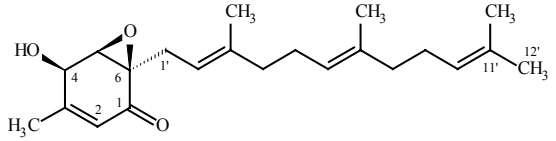

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NMR shifts ($CHCl_3$) of some of the ring atoms in **2** differed from our values in MeOH much more (C-1, $\Delta\delta$ = 7.2; C-3, $\Delta\delta$ = 10.3; C-5, $\Delta\delta$ = 10.1) than a solvent change would usually provoke. The chemical shifts of the side chain were, however, nearly the same for both samples. In spite of these differences, detailed NMR measurements of our sample confirmed an identical connectivity as in **2**. As no diastereomeric structure exists and as the *trans* configuration of the double bond was confirmed by identity with the reported NMR data of 2',3'-dihydrosorbicillin (**2**),[3] the different properties must be due to crystal effects and solvatation.

A second light yellow solid, sohirnone A (**3**), had the molecular formula $C_{13}H_{16}O_3$ (HREIMS). The ^{1}H NMR was very similar to that of 2',3'-dihydrosorbicillin (**2**), however, an additional aromatic proton signal appeared at δ 7.09 and one of the aromatic methyl groups of **2** was missing, which indicated it to be a *nor* derivative of the latter. The very small coupling between the two aromatic protons pointed to their *para* orientation. The ^{13}C NMR spectrum delivered thirteen signals as demanded by the molecular formula, and it was again similar to that of **2** except for a missing methyl signal. Sohirnone A must therefore have the structure **3**, or is an isomer where the position of the aromatic methyl and the phenolic hydroxy group (5-OH) are exchanged. The structure **3** was finally confirmed by the 2D couplings. The *trans* configuration of the double bond is shown by the same pattern of the proton signals and nearly identical ^{1}H and ^{13}C shifts for the side chain as in **2**.

Sohirnone B (**4a**) was obtained as a third yellow solid with the molecular weight 248 ($C_{14}H_{16}O_4$ by HREIMS). The ^{1}H NMR spectrum displayed four sp^2 protons between δ 7.00 - 6.20, which were assigned due to their chemical shifts, the coupling constants and the splitting pattern to a (*E,E*)-hexa-2,4-dien-1-one system. In the upfield region, the spectrum showed two aromatic methyl singlets at δ 2.19 and 2.03 and an olefinic methyl doublet at δ 1.84. Beside others, the ^{13}C spectrum depicted signals of a conjugated carbonyl group at δ 200.8. The final structure **4a** was finally derived by H,H COSY, HMQC and HMBC couplings. Due to chelation, the hydroquinone **4a** is rather stable on air and was not oxidized on silica gel. By means of cerium(IV)-ammonium nitrate, however, sohirnone B (**4a**) was easily transformed into a quinone whose NMR data confirmed the expected identity with sorrentanone (**4b**).[12]

Table 1. ^{13}C NMR data (δ) for oxosobicillinol tautomers **6** and **7**

atom	**6**[a]	**6**[b]	**7**[c,4]
1	200.4	197.4	196.3
2	104.1	102.3	104.5
3	182.8	178.8	192.2
4	99.8	96.4	106.3
5	194.1	191.9	167.4
6	80.8	79.0	75.3
1'	186.1	182.3	184.5
2'	128.7	127.9	122.5
3'	140.8	137.9	145.8
4'	132.6	131.2	131.2
5'	137.8	135.7	141.7
6'	18.8	18.3	18.9
4-Me	7.7	7.8	7.1
6-Me	31.4	30.8	30.3

[a] CD_3OD, [b] DMSO-d_6, [c] $CDCl_3$

Table 2: Comparison of the ^{13}C NMR data (δ) of bisorbibutenolide (**11a**), trichotetronine (**11b**) and of the present isomer **11a**$^{\#}$ in CD_3OD

C No.	**11a** $CDCl_3$	**11a**[1] $CDCl_3$	**11b** $CDCl_3$	**11b** CD_3OD	**11a**$^{\#}$ CD_3OD	**11a**$^{\#}$ $CDCl_3$ + CD_3OD
1	62.6	62.5	63.1	63.6	63.8	62.3
2	194.9	195.0	184.6	197.6	197.2	n.v.
3	105.5	108.4	110.5	110.1	110.6	108.6
4	42.4	42.3	45.0	43.5	43.7	42.5
5	75.0	74.9	75.2	75.9	75.9	74.6
6	208.3	208.1	213.4	210.2	210.8	209.8
7	51.3	51.3	52.5	53.0	51.8	50.6
8	43.6	43.5	47.4	44.0	44.0	42.2
9	169.8	169.8	181.0	169.8	169.1	168.1
10	117.6	117.7	126.8	119.6	119.9	117.6
11	143.9	143.9	141.4	144.0	143.2	142.6
12	130.3	130.9	132.9	131.7	132.4	130.7
13	140.9	141.0	136.8	140.9	140.1	139.7
14	18.9	19.0	18.7	19.0	18.9	18.4
15	202.7	202.6	207.5	202.3	204.0	202.6
16	127.0	126.8	129.3	128.5	129.1	127.2
17	148.0	148.0	148.4	147.9	148.0	144.3
18	131.0	130.1	131.8	132.4	131.7	130.1
19	145.5	145.6	144.8	145.2	144.8	147.0
20	19.1	19.2	19.1	19.2	19.1	18.6
21	83.2	82.5	85.1	84.5	85.1	83.1
22	176.5	175.4	194.9	178.7	190.7	n.v.
23	98.2	98.5	90.3	98.2	91.7	91.8
24	174.9	173.6	182.2	176.6	181.1	177.9
1-Me	11.0	11.0	12.9	11.4	11.5	10.5
5-Me	23.5	23.5	24.3	24.3	24.1	23.3
21-Me	23.1	23.1	24.2	23.6	24.1	22.2
23-Me	6.3	6.2	6.2	6.6	6.4	5.5

n.v. = not visible; **11a**$^{\#}$ is the isomer described here

The pale yellow sohirnone C (**5**) showed the molecular weight of 250 ($C_{14}H_{18}O_4$), which pointed to a dihydro-derivative of sohirnone B (**4a**). The ^1H NMR spectrum was indeed similar to that of **4a**, and as expected, there were signals for only two olefinic protons, which were not in conjugation with the carbonyl group. The aliphatic region delivered additional signals for two adjacent methylene groups.The ^{13}C NMR data indicated clearly that the double bond next to the carbonyl group in **4a** had been reduced to afford **5**. This compound and the corresponding quinone (2',3'-dihydrosorrentanone) have not been described previously.

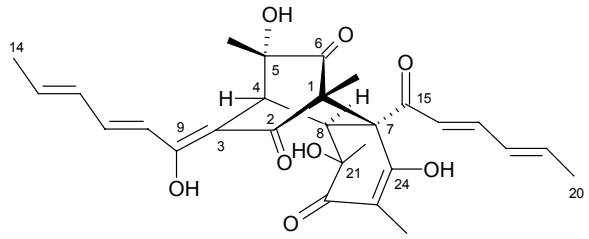
Shifts of four olefinic protons and their coupling constants in the ^1H NMR spectrum of a further yellow monomeric compound $C_{14}H_{16}O_5$, indicated two *trans* double bonds in conjugation with a carbonyl group as in **4a**. The ^{13}C NMR spectrum confirmed the conjugated ketone and delivered further four double bonds, two of which bearing oxygen, three methyl groups and one aliphatic quaternary carbon atom attached to oxygen. The search in AntiBase using the NMR and mass data led to oxosorbicillinol (**7**), however, shift differences up to Δδ 25 were observed between our ^{13}C NMR values in MeOH or DMSO and the reported data in $CHCl_3$ (Table 1).[4] As for **2**, a comparison of the shifts under identical conditions was not possible, because of the insolubility of our compound in $CHCl_3$ and the inaccessibility of an authentic sample.[13] Even repeated dissolution in MeOH and evaporation did not deliver amorphous material with a higher solubility. The 2D spectra in MeOH confirmed unequivocally the same substituent pattern as in oxosorbicillinol (**7**).[4] The strong HMBC coupling from the 6-methyl group to *both* carbonyl signals at δ 197.4 and 191.9 indicated, however, the tautomeric cyclohex-4-ene-1,3-dione **6**, where the $\Delta^{1',2}$ double bond geometry should allow a hydrogen bridge of 1'-OH with 1-CO. Polar interactions may be responsible for the stabilization of oxosorbicillinol (**7**) as this tautomer.

A further monomer with a molecular weight of 344 was obtained from the Sephadex fraction 1 as a colorless solid and identified by its IR, ^1H, ^{13}C NMR and NOESY data as the recently described 7-deacetoxyyanuthone (**8**).[14]

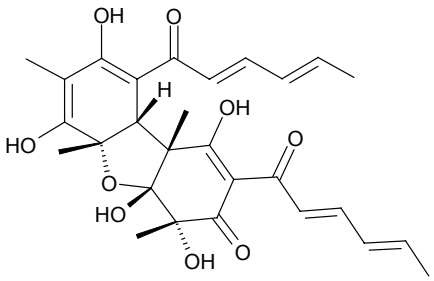
Dimers

The crude *P. notatum* extract delivered a complex mixture of higher molecular weight compounds. A search in AntiBase[11] with NMR-derived data and the formula $C_{28}H_{32}O_8$ (HRESIMS) of a light yellow solid led to bisorbibutenolide (**11a**) or the proposed diastereomeric trichotetronine[15] (**11b**), respectively. H,H COSY, HMQC and HMBC spectra of our compound resulted in the same connectivity (Figure 1) as for **11a**/**11b**, however, in contrast to these dimers, our sample was again nearly insoluble in $CHCl_3$, and deviations especially of the

^{13}C signals of C-22 – C-24 up to Δδ = 20 seemed to indicate a third isomer (Table 2).



9a
9b: 16,17-dihydro



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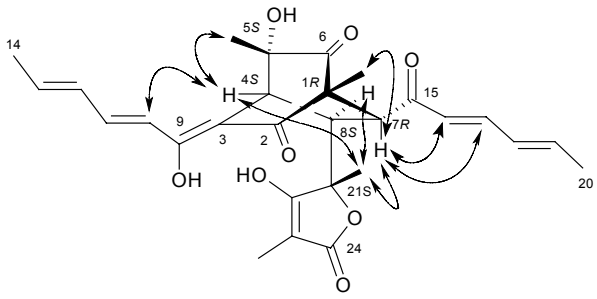
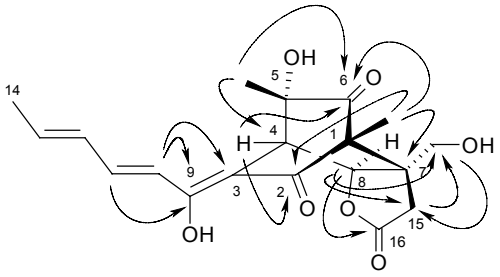
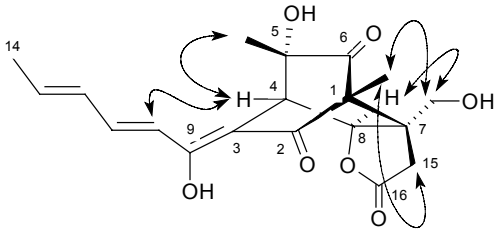


11a
11b: 21*R* instead of 21*S*

Figure 1. Structures of bisorbicillinol (**9a**), bisvertinolone (**10**), bisorbibutenolide (**11a**) and trichotetronine (**11b**). The arrows in **11** indicate the NOE couplings measured for the isomer described here.

An authentic sample of **11a** was not accessible for comparison,[16] however, when we diluted a concentrated MeOH solution of our sample with an excess of $CDCl_3$, the carbonyl signals were shifted closer to the published values.[1] As bisorbicillinol (**9a**)[6] and bisvertinoquinol (**9a**)[7] have also been isolated from our strain, the assumption of strong solvent interactions or even another prototropisomeric form at C-2,C-3,C-9,C-10 and/or C-22,C-23,C-24 are the best explanations for the different properties of our product.





12

Figure 2. Selected HMBC (→) and NOE (↔) couplings in rezishanone A (**12**)

Rezishanone A (**12**) was obtained as a colorless solid with the molecular weight of 362 Dalton ($C_{19}H_{22}O_7$ by HRESIMS). A doublet of a terminal olefinic methyl group at δ 1.88, as in bisorbibutenolide (**11a**), and signals of four *sp*^{2} methine and two aliphatic methyl groups showed that both compounds possessed a close structural relationship. The COSY correlation of the methyl doublet and cross signals of the olefinic methines indicated indeed an unsaturated side chain (C-14 – C-10), which was further extended to C-3 by HMBC couplings (see Figure 2). The assumed **1**-subunit was confirmed by HMBC and NOE couplings of 4-H and 1-Me (Figure 2), carbonyl signals at δ 208.1 and 197.4, and an enol C-9 at δ 172.4 as in bisorbibutenolide (**11a**). One of the remaining methylene groups is connected to OH, as the shift and the COSY coupling with the OH signal at δ 4.94 indicated. The second methylene group must be embedded in a ring, due to the AB splitting and the large coupling constant. The final structure **12** follows from the neighborhood of 4-H with 8-H (COSY) and from the HMBC correlations of 8-H (Figure 2).

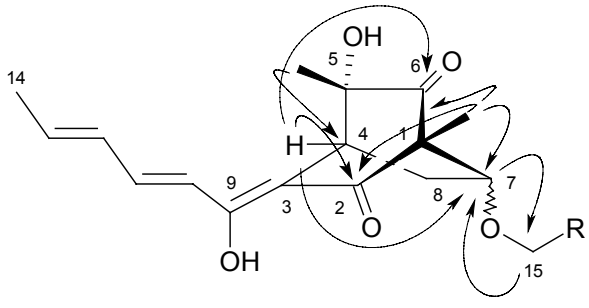
The (1*R*,4*R*,5*S*)-configuration is plausible as **12** shows a positive Cotton effect, as does **11b,** and is a **1**-derivative as well. The 7-CH_2OH showed the expected NOE coupling with 8-H; however, the ^{1}H

NMR signals of 7-CH_2OH and 4-H were overlapped, and so the crucial 4-H, 8-H NOE signal cannot be clearly assigned. The 1-methyl group also showed NOE signals with both 7-CH_2OH and 15-H_B, and the 7*R*,8*S* configuration of rezishanone A as drawn in structure **12** is based only on the assumption of an *endo* addition and could not be further confirmed.

Rezishanone B (**13a**) was obtained as a colorless solid with the molecular weight of 348 Dalton ($C_{20}H_{28}O_5$). The ^{1}H NMR spectrum was very similar to that of rezishanone A (**12**) showing their close structural relationship. It also exhibited signals of the conjugated side chain (C-10 to C14) and two methyl singlets. In **13a**, however, the C-8 methine signal of **12** was replaced by a methylene group and the methylene signals of C-15 and 7-CH_2OH were missing. A methine group bearing an oxygen function and an *n*-butyl ether residue appeared instead. Interpretation of the 2D NMR couplings (see formula) accompanied by comparison of the ^{1}H and ^{13}C NMR data with the compounds discussed above delivered structure **13a** for rezishanone B.

Rezishanone C (**13b**) and D (**13c**) showed the molecular weights of 320 ($C_{18}H_{24}O_5$) and 322 Dalton ($C_{18}H_{26}O_5$). The ^{1}H NMR spectra were in good agreement with that of **13a**, the only difference being the replacement of the *n*-butyl ether residue of **13a** by ethyl ether residues in **13b** and **13c**. Additionally, the conjugation of the side in **13c** was broken by the saturation of the C-10,11 double bond in **13a** and **13b**. The structures of rezishanone C and D were finally established by 2D NMR spectra as **13b** and **13c**. The CD spectrum of **13b** and **13c** gave the same Cotton effect as **13a**.

Figure 3. Selected HMBC couplings of rezishanones B (**13a**), C (**13b**) and D (**13c**)



13a : R = *n*-C_3H_7
13b: R = CH_3
13c: R = CH_3, $\Delta^{10,11}$ saturated

While **9a** is the result of a "normal" Diels-Alder reaction, the rezishanones must originate in Diels-Alder reactions with inverse electron demand. On the basis of density-functional calculations,[17] the observed regioselectivity of the products is correctly reproduced. While the (1*R*,4*R*,5*S*)-configuration in **13a-c** follows from the starting material **1**, the orientation of the ether substituents at C-7 could not be derived from NOE effects and remains open. The rezishanones A-D (**12**-**13c**) are the first natural Diels-Alder products of sorbicillinol (**1**) with dienophils not related with **1**. It should be mentioned, however, that none of the expected volatile precursors 4-hydroxymethyl-3*H*-furan-2-one, butylvinyl ether, and ethylvinyl ether, respectively, have been isolated as a natural product so far. While the furanone could originate from ß-hydroxyparaconic acid,[18] for the vinyl ethers an unnatural origin can also not be excluded.[19]

All vertinolides exhibit a pronounced radical-scavenging activity,[5] some of the dimers have been reported to inhibit the induction of the mitogen-induced cyclooxygenase by a polysaccharide-stimulated human monocyte cell or to inhibit the ß-1,6-glucan biosynthesis.[20] Compound **8** exhibits weak cytotoxicity against human solid tumor cells[14] and is related to oligosporon[21] and its derivatives, which show nematocidal activity. The flagranones[22] form another group of related compounds and are known to possess antibacterial and antifungal activities. The vertinolides isolated here from *P. notatum* GWP A were *semi*quantitatively tested in the agar diffusion test against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptomyces viridochromogenes* (Tü 57), *Escherichia coli*, *Candida albicans*, *Mucor miehei*, *Chlorella vulgaris*, *Chlorella sorokiniana* and *Scenedesmus subspicatus* with 20 µg of the natural products/9 mm paper disc. Dihydrosorbicillinol (**2**), the sohirnones A (**3**) and B (**4a**), oxosorbicillinol (**6**), 7-deacetoxyyanuthone (**8**), bisorbicillinol (**9a**), bisvertinoquinol (**9b**), and the rezishanones A-D (**12**-**13c**) exhibited weak activity against *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones of 12-17 mm and ca. 13 mm diameter, respectively. Fungi and algae were not inhibited by any of the isolated compounds.

Experimental Section

General Experimental Procedures. NMR spectra were measured on AMX 300 (300.135 MHz), Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers. ESIMS spectra were recorded on a Quattro Triple Quadrupole Mass Spectrometer, Finnigan TSQ 7000 with nano-ESI API ion source. EIMS spectra were recorded on a Finnigan MAT 95 spectrometer at 70 eV. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer with KBr

pellets. UV/VIS spectra were recorded on a Perkin-Elmer Lambda 15 UV/VIS spectrometer. Preparative HPLC was performed on RP18 (Eurochrom Eurospher RP 100-C18, 5 µm) using a Knauer variable wavelength monitor at 390 nm. Flash chromatography was carried out on silica gel (230-400 mesh), thin layer chromatography (TLC) was performed on Polygram SIL G/UV_{254} (Macherey-Nagel & Co, Düren, Germany). R_f values were measured with 10 % MeOH in CH_2Cl_2 when not stated otherwise. Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

M_2^+ medium. Malt extract (10 g), yeast extract (4 g) and glucose (4 g) were dissolved in 500 mL of tap water and 500 mL of artificial sea water. The medium was adjusted to pH 7.8 with 2 N NaOH and then sterilized for 33 min at 121 °C. After sterilization, an end pH 7.0 of the medium was attained.

Fermentation of *Penicillium notatum*. The strain *Penicillium notatum* was isolated from a bench top contamination and taxonomically determined by one of the authors (I. G.-W.) . The culture is kept at the Labor Grün-Wollny and in the Department of Organic and Biomolecular Chemistry, Göttingen, as strain number GWP A. The fungus was cultivated on agar plates with M_2^+ medium at 28 °C for 48-72 h where it grew with a thick greenish aerial mycelium and brown agar coloration. One hundred of 1 L-Erlenmeyer flasks each containing 250 mL of M_2^+ medium were inoculated with the well-grown agar subculture and incubated with 110 rpm at 28 °C for three days. The brownish yellow culture broth was mixed with about 1 kg diatomaceous earth and filtered through a press filter. Both phases were separately extracted with ethyl acetate. Since the extracts exhibited a similar composition by TLC, they were combined and evaporated to dryness under vacuum at 30 °C.

The crude residue (ca. 5 g) was pre-separated into two fractions on Sephadex LH-20 (4 × 120 cm, CH_2Cl_2/50 % MeOH). The first fraction delivered the dimeric rezishanones A (**12**, 12 mg), B (**13a**, 13 mg), C/D (**13b**/**13c**, 16 mg), bisorbicillinol (**9a**, 7 mg), bisvertinoquinol (**9a**), 15 mg), bisvertinolone (**10**, 81 mg) and 7-deacetoxyyanuthone (**8**, R_f = 0.73, 15 mg). Further purification of the second fraction on preparative HPLC (MeCN/20% H_2O) delivered the monomers 2',3'-dihydrosorbicillin (**2**, 12 mg), sohirnone A (**3**, 10 mg), B (**4a**, 7 mg), C (**5**, 12 mg), oxosorbicillinol (**6**, 12 mg), and 2-pyruvoylaminobenzamide (8 mg).[10]

2',3'-Dihydrosorbicillin (2). Pale yellow solid, R_f = 0.14; ^{1}H NMR (CD_3OD, 300 MHz): δ 7.56 (s, 1 H, H-6), 5.49 (m, 2 H, H-4', H-5'), 3.02 (t, J = 7.2 Hz, 2 H, H_2-2'), 2.37 (m, 2 H, H_2-3'), 2.35 (d, J = 0.8 Hz, 3 H, 5-CH_3), 2.24 (s, 3 H, 3-CH_3), 1.63 (m, 3 H, H_3-6'); ^{13}C NMR (CD_3OD, 75.5 MHz): δ 207.3 (C_q-1'), 161.4 (C_q-2), 156.6 (C_q-4), 130.0 (CH-6), 130.8 (CH-4'), 127.0 (CH-5'), 124.6 (C_q-5), 122.8 (C_q-3), 117.3 (C_q-1), 39.2 (CH_2-2'), 28.5 (CH_2-3'), 18.0 (CH_3-6'), 17.2 (5-CH_3), 10.3 (3-CH_3). Ref.:[3] ^{1}H NMR ($CDCl_3$, 80 MHz): δ 7.40 (s, 1 H, H-6), 5.7-5.4 (m, 2 H, H-4', H-5'), 2.97 (t, J = 7.5 Hz, 2 H, H_2-2'), 2.5-2.3 (m, 2 H, H_2-3'), 2.21 (s, 3 H, 5-CH_3), 2.14 (s, 3 H, 3-CH_3), 1.66 (d, 3 H, H_3-6'); ^{13}C NMR ($CDCl_3$, 20 MHz): δ 204.3 (C_q-1'), 161.5 (C_q-2), 158.6 (C_q-4), 128.8 (CH-6), 129.2 (CH-4'), 125.9 (CH-5'), 114.5 (C_q-5), 112.5 (C_q-3), 110.1 (C_q-1), 37.5 (CH_2-2'), 27.2 (CH_2-3'), 17.7 (CH_3-6'), 15.5 (5-CH_3), 7.2 (3-CH_3).

Sohirnone A (3): pale yellow solid, R_f = 0.12; UV (MeOH) λ_{max} (log ε) 265 (3.69), 327 (3.35) nm; IR (KBr) ν_{max} 3487, 2923, 1647, 1491, 1264, 1244, 1127, 1061, 961, 886, 847, 802, 718, 693, 637, 615 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz): δ 7.67 (s, 1 H, H-6), 7.09 (s, 1 H, H-3), 5.49 (m, 2 H, H-4', H-5'), 3.03 (t, J = 7.2 Hz, 2 H, H_2-2'), 2.35 (m, 2 H, H_2-3'), 2.23 (s, 3 H, 5-CH_3), 1.62 (m, 3 H, H_3-6'); ^{13}C NMR (CD_3OD, 75.5 MHz): δ 206.7 (C_q-1'), 163.0 (C_q-2), 158.4 (C_q-4), 133.0 (CH-6), 130.8 (CH-4'), 127.0 (CH-5'), 122.4 (C_q-5), 117.2 (C_q-1), 109.0 (CH-3), 39.2 (CH_2-2'), 28.5 (CH_2-3'), 18.1 (CH_3-6'), 16.0 (5-CH_3); (-)-ESIMS *m/z* 219 $[M-H]^-$; (+)-ESI HRMS *m/z* 243.0994 (calcd. for $[M+Na]^+$, $C_{13}H_{16}O_3Na$, 243.0992), 221.11741 (calcd. for $[M+H]^+$, $C_{13}H_{17}O_3$, 221.1172).

Sohirnone B (4a): yellow solid, R_f = 0.07; UV (MeOH) λ_{max} (log ε) 279 (3.93) nm; IR (KBr) ν_{max} 3283, 1643, 1609, 1558, 1290, 1243, 1051, 998, 786, 726, 640, 616 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz): δ 6.98 (dd, J = 15.6, 10.5 Hz, 1 H, H-3'), 6.34 (m, 1 H, H-4'), 6.32 (d, J = 15.6 Hz, 1 H, H-2'), 6.21 (m, 1 H, H-5'), 2.19 (s, 3 H, 3-H_3), 2.03 (s, 3 H, 6-CH_3), 1.84 (m, 3 H, H_3-6'); ^{13}C NMR (CD_3OD, 75.5 MHz): δ 200.8 (C_q-1'), 148.6 (CH-3'), 147.1 (C_q-2), 143.1 (C_q-5), 142.8 (CH-5'), 141.4 (C_q-4), 131.7 (CH-4'), 130.7 (CH-2'), 127.3 (C_q-1), 122.0 (C_q-6), 120.6 (C_q-3), 19.0 (CH_3-6'), 11.3 (6-CH_3), 10.3 (3-CH_3); (-)-ESIMS *m/z* 247 $[M-H]^-$; (+)-ESI HRMS *m/z* 271.0940 (calcd. for $[M+Na]^+$, $C_{14}H_{16}O_4Na$, 271.0941), 249.1120 (calcd. for $[M+H]^+$, $C_{14}H_{17}O_4$, 249.1121).

Sohirnone C (5): pale yellow solid, R_f = 0.08; UV (MeOH) λ_{max} (log ε) 271 (3.44), 350 (sh) nm; IR (KBr) ν_{max} 3422, 1693, 1458, 1376, 1284, 1243, 1141, 1057, 965, 771, 745, 639, 610 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz): δ 5.43 (m, 2 H, H-4', H-5'), 2.87 (t, J = 7.1 Hz, 2 H, H_2-2'), 2.52 (m, 2 H, H_2-3'), 2.19 (s, 3 H, 3-CH_3), 2.06 (s, 3 H, 6-CH_3), 1.81 (m, 3 H, H_3-6'); ^{13}C NMR (CD_3OD, 75.5 MHz): δ 210.4 (C_q-1'), 28.1 (CH_2-3'), 146.7 (C_q-2), 143.2

(C_q-5), 126.7 (CH-5'), 141.3 (C_q-4), 131.0 (CH-4'), 45.5 (CH_2-2'), 129.5 (C_q-1), 121.4 (C_q-6), 120.6 (C_q-3), 18.1 (CH_3-6'), 12.9 (6-CH_3), 10.3 (3-CH_3); (-)-ESIMS *m/z* 249 $[M-H]^-$; (+)-ESI HRMS *m/z* 273.1095 (calcd. for $[M+Na]^+$, $C_{14}H_{18}O_4Na$, 273.1097), 251.12761 (calcd. for $[M+H]^+$, $C_{14}H_{19}O_4$, 251.1278).

Oxosorbicillinol (tautomer 6): yellow solid, R_f = 0.32; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.36 (d, *J* = 15.4 Hz, 1 H, 2'-H), 7.07 (dd, *J* = 15.4, 10.8 Hz, 1 H, 3'-H), 6.24 (dd, *J* = 15.5, 10.8 Hz, 1 H, 4'-H), 6.04 (m, 1 H, 5'-H), 1.80 (d, *J* = 6.2 Hz, 3 H, 6'-H_3), 1.59 (s, 3 H, 4-CH_3), 1.24 (s, 3 H, 6-CH_3); ^{13}C NMR (DMSO-d_6, 75.5 MHz) see Table 1; (-)-ESIMS *m/z* 263 $[M-H]^-$. (+)-ESI HRMS *m/z* 287.0890 (calcd. for $[M+Na]^+$, $C_{14}H_{16}O_5Na$, 287.0890), 265.1070 (calcd. for $[M+H]^+$, $C_{14}H_{17}O_5$, 265.1070).

Oxidation of sohirnone B (4a). Sohirnone B (3.5 mg, **4a**) was dissolved in acetonitrile (0.3 mL) and a solution of cerium(IV)-ammonium nitrate (20 mg) in H_2O (0.3 mL) was added dropwise. The mixture was diluted with 10 mL of H_2O and acidified with a drop of 2N HCl. It was then extracted with ethyl acetate and purified on a silica gel (column 1 × 10 cm, 10 g) with CH_2Cl_2 to yield 1 mg of **4b**. (+)-ESIHRMS *m/z* 247.0967 (calcd. for $[M+H]^+$, $C_{14}H_{15}O_4$, 247.0965). The NMR data were identical with published values.[12]

Bisorbibutenolide (11a): yellow solid, R_f = 0.59 (CH_2Cl_2/10% MeOH); UV (MeOH) λ_{max} (log ε) 369 (3.99), 289 (4.10), 261 (4.08); CD (MeOH) $\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1} (λ/nm) +17168.8 (354), -24559.6 (315), +16935.8 (276), -18912.3 (254). IR (KBr) ν_{max} 3413, 2979, 2934, 1733, 1633, 1560, 1444, 1380, 1291, 1199, 1136, 1071, 998, 949, 870, 803, 769 cm^{-1}; ^{1}H NMR (CD_3OD, 300.2 MHz) δ 14.09 (s br, H/D exchangeable, 1 H, 9 OH), 7.29 (dd, ^3J = 14.5, 10.9 Hz, 1 H, H-11), 7.25 (dd, ^3J = 15.3, 10.5 Hz, 1 H, H-17), 6.35 (m, 4 H, H-10, 12, 18, 19), 6.23 (dq, ^3J = 15.0, 6.4 Hz, 1 H, H-13), 6.15 (d, ^3J = 15.3 Hz, 1 H, H-16), 3.43 (d, ^3J = 5.9 Hz, 1 H, H-7), 3.32 (d, ^3J = 1.2 Hz, 1 H, H-4), 3.13 (dd, ^3J = 5.9, 1.2 Hz, 1 H, H-8), 1.87 (d, ^3J = 6.4 Hz, 3 H, Me-20), 1.86 (d, ^3J = 6.4 Hz, 3 H, H-14), 1.53 (s, 3 H, 23-CH_3), 1.46 (s, 3 H, 21-CH_3), 1.19 (s, 3 H, 5-CH_3), 0.99 (s, 3 H, 1-CH_3); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 210.8 (C-6), 204.0 (C-15), 197.2 (C-2), 190.7 (C-22), 181.1 (C-24), 169.1 (C-9), 148.0 (C-17), 144.8 (C-19), 143.2 (C-11), 140.1 (C-13), 132.4 (C-12), 131.7 (C-18), 129.1 (C-16), 119.9 (C-10), 110.6 (C-3), 91.7 (C-23), 85.1 (C-21), 75.9 (C-5), 63.8 (C-1), 51.8 (C-7), 44.0 (C-8), 43.7 (C-4), 24.1 (5-Me), 24.1 (21-Me), 19.1 (C-20), 18.9 (C-14), 11.5 (1-Me), 6.4 (23-Me); (+)-ESI-MS *m/z* 541 ($[M+2Na-H]^+$, 100), 519 ($[M+Na]^+$, 30); (-)-ESI-MS *m/z* 991 ($[2M-H]^-$, 10), 495 ($[M-H]^-$, 100); (+)-ESI HRMS *m/z* 519.1989 (calcd. for $[M+Na]^+$, $C_{28}H_{32}O_8Na$, 519.1989), 497.2170 (calcd. for $[M+H]^+$, $C_{28}H_{33}O_8$, 497.2170).

Rezishanone A (12): colorless solid, R_f = 0.49 (CH_2Cl_2/10% MeOH); UV (MeOH) λ_{max} (log ε) 394 sh (3.82), 375 (4.12), 361 (4.16), 248 (3.64); CD (MeOH) $\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1} (λ/nm) +31316.5 (343), -53658.0 (307), +4225.85 (244), -11669.7 (216). IR (KBr) ν_{max} 3425, 2980, 2934, 1780, 1735, 1632, 1603, 1558, 1445, 1386, 1331, 1204, 1115, 1041, 998, 940, 908, 874, 749 cm^{-1}; ^{1}H NMR (acetone-d_6, 300.2 MHz) δ 14.32 (s br, 1 H, 9-OH), 7.32 (dd, ^3J = 15.0, 10.5 Hz, 1 H, H-11), 6.52 (d, ^3J = 15.0 Hz, 1 H, H-10), 6.40 (dd, ^3J = 14.8, 10.6 Hz, 1 H, H-12), 6.30 (dq, ^3J = 14.8, 6.4 Hz, 1 H, H-13), 5.42 (d, ^3J = 3.6 Hz, 1 H, H-8), 5.34 (s br, 1 H, 5-OH), 4.94 (s br, 1 H, CH_2OH), 3.71 (d, ^3J = 3.6 Hz, 1 H, H-4), 3.65 (m, 2 H, CH_2OH), 2.57 (d, ^2J = 19.0 Hz, 1 H, H_A-15), 2.17 (d, ^2J = 19.0 Hz, 1 H, H_B-15), 1.88 (d, ^3J = 6.4 Hz, 3 H, H-14), 1.25 (s, 3 H, 5-CH_3), 1.12 (s, 3 H, 1-CH_3); ^{13}C NMR (acetone-d_6, 75.5 MHz) δ 208.1 (C_q-6), 197.4 (C_q-2), 174.9 (C_q-16), 172.4 (C_q-9), 144.2 (CH-11), 141.1 (CH-13), 131.8 (CH-12), 119.2 (CH-10), 106.8 (C_q-3), 80.2 (CH-8), 74.0 (C_q-5), 66.4 (C_q-1), 64.9 (CH_2OH), 51.1 (C_q-7), 46.3 (CH-4), 34.7 (CH_2-15), 24.9 (5-CH_3), 18.9 (CH_3-14), 8.4 (1-CH_3); (+)-ESIMS *m/z* 747 ($[2M+Na]^+$, 100), 385 ($[M+Na]^+$, 8); (-)-ESIMS *m/z* 745 ($[2M-H]^-$, 5), 361 ($[M-H]^-$, 100); (+)-ESIHRMS *m/z* 363.1438 (calcd. for $[M+H]^+$, $C_{19}H_{23}O_7$, 363.1438).

Rezishanone B (13a): colorless solid, R_f = 0.46 (CH_2Cl_2/10% MeOH); UV (MeOH) λ_{max} (log ε) 378 sh (4.14), 359 (4.22), 247 (3.71); CD (MeOH) $\Delta\varepsilon_{max}$/dm^3 mol^{-1} cm^{-1} (λ/nm) +30281.2 (342), -54712.3 (305), +7688.0 (243), -6265.68 (218). IR (KBr) ν_{max} 3427, 2982, 2932, 1627, 1603, 1558, 1446, 1386, 1335, 1204, 1105, 1047, 998, 943, 908, 874, 750 cm^{-1}; ^{1}H NMR (C_6D_6, 300.2 MHz) δ 14.71 (s br, 1 H, 9-OH), 7.33 (dd, ^3J = 14.9, 11.0 Hz, 1 H, H-11), 5.99 (d, ^3J = 14.9 Hz, 1 H, H-10), 5.93 (ddd, ^3J = 14.9, 11.0 Hz, ^4J = 1.2 Hz, 1 H, H-12), 5.57 (dq, ^3J = 14.8, 6.9 Hz, 1 H, H-13), 3.36 (dd, ^3J = 8.4, 2.3 Hz, 1 H, H-7), 3.15 (m, 1 H, H_A-15), 3.04 (d, ^3J = 2.9 Hz, 1 H, H-4), 2.98 (m, 1 H, H_B-15), 2.91 (m, 1 H, H_A-8), 1.67 (s, 3 H, 5-CH_3), 1.61 (m, 1 H, H_B-8), 1.43 (d, ^3J = 6.4 Hz, 3 H, H_3-14), 1.25 (m, 2 H, H_2-16), 1.19 (m, 1 H, H_A-17), 1.12 (s, 3 H, 1-CH_3), 0.75 (m, 1 H, H_B-17), 0.70 (s, overlapping m, 3 H, H_3-18); ^{13}C NMR (C_6D_6, 125.7 MHz) δ 210.5 (C_q-6), 197.0 (C_q-2), 166.4 (C_q-9), 141.7 (CH-11), 138.4 (CH-13), 131.2 (CH-12), 118.6 (CH-10), 111.0 (C_q-3), 79.5 (CH-7), 74.6 (C_q-5), 69.8 (CH_2-15), 67.7 (C_q-1), 40.3 (CH-4), 32.0 (CH_2-16), 30.8 (CH_2-8), 24.1 (5-CH_3), 19.6 (CH_2-

17), 18.6 (CH_3-14), 13.9 (CH_3-18), 9.7 (1-CH_3); (+)-ESI MS *m/z* 719 ($[2M+Na]^+$, 97), 371 ($[M+Na]^+$, 100), 349 ($[M+H]^+$, 4); (-)-ESIMS *m/z* 347 ($[M-H]^-$, 100); (+)-ESIHRMS *m/z* 371.1829 (calcd. for $[M+Na]^+$, $C_{20}H_{28}O_5Na$, 371.18290), 349.2010 (calcd. for $[M+H]^+$, $C_{20}H_{29}O_5$, 349.2009).

Rezishanone C (13b): colorless solid, $R_f = 0.51$ (CH_2Cl_2/10% MeOH); UV (MeOH) λ_{max} (log ε) 342 , 318 (sh), 302 (sh); CD (MeOH); CD (MeOH) $\Delta\varepsilon_{max}/dm^3\ mol^{-1}\ cm^{-1}$ (λ/nm) +43482.9 (335), -60262.1 (294); IR (KBr) ν_{max} 3423, 2977, 2934, 1636, 1600, 1558, 1438, 1386, 1327, 1204, 1109, 1046, 998, 938, 905, 876, 746 cm^{-1}; ^{1}H NMR (C_6D_6, 300.2 MHz) δ 14.64 (s br, 1 H, 9-OH), 7.33 (dd, ^3J = 15.1, 11.3 Hz, 1 H, H-11), 5.95 (m, 2 H, H-10,12), 5.58 (dq, ^3J = 13.6, 6.8 Hz, 1 H, H-13), 3.37 (m, 1 H, H-7), 3.15 (m, 1 H, H_A-15), 3.06 (m, 1 H, H-4), 2.92 (m, 1 H, H_B-15), 2.81 (m, 1 H, H_A-8), 1.64 (s, 3 H, 1-CH_3), 1.46 (d, ^3J = 6.8 Hz, 3 H, H-14), 1.42 (m, 1 H, H_B-8), 1.01 (s, 3 H, 5-CH_3), 0.84 (t, ^3J = 7.2 Hz, 3 H, H-16); ^{13}C NMR (C_6D_6, 75.5 MHz) δ 210.5 (C_q-6), 197.0 (C_q-2), 166.4 (C_q-9), 141.7 (CH-11), 138.2 (CH-13), 131.2 (CH-12), 118.6 (CH-10), 111.0 (C_q-3), 79.3 (CH-7), 74.3 (C_q-5), 67.6 (C_q-1), 65.5 (CH_2-15), 40.2 (CH-4), 30.9 (CH_2-8), 24.1 (5-CH_3), 18.6 (CH_3-14), 15.2 (CH_3-16), 9.6 (1-CH_3); (+)-ESIMS *m/z* 343 ($[M+Na]^+$, 100); (-)-ESIMS *m/z* 319 ($[M-H]^-$, 100). (+)-ESIHRMS *m/z* 343.1516 (calcd. for $[M+Na]^+$, $C_{18}H_{24}O_5Na$, 343.1516).

Rezishanone D (13c): colorless solid, $R_f = 0.51$ (CH_2Cl_2/10% MeOH); UV (MeOH) λ_{max} (log ε) 342 (3.94), 318 sh (3.92), 302 (sh); CD (MeOH); CD (MeOH) $\Delta\varepsilon_{max}/dm^3\ mol^{-1}\ cm^{-1}$ (λ/nm) +43482.9 (335), -60262.1 (294); IR (KBr) ν_{max} 3423, 2977, 2934, 1636, 1600, 1558, 1438, 1386, 1327, 1204, 1109, 1046, 998, 938, 905, 876, 746 cm^{-1}; ^{1}H NMR (C_6D_6, 300.2 MHz) δ 14.93 (s br, 1 H, 9-OH), 5.25 (m, 2 H, H-12,13), 3.37 (m, 1 H, H-7), 3.15 (m, 1 H, H_A-15), 2.92 (m, 1 H, H_B-15), 2.81 (m, 1 H, H-4), 2.73 (m, 1 H, H_A-8), 2.15 (m, 2 H, H-11), 2.04 (m, 2 H, H-10), 1.61 (s, 3 H, 1-CH_3), 1.56 (m, 1 H, H_B-8), 1.49 (d, ^3J = 6.8 Hz, 3 H, H-14), 0.98 (s, 3 H, 5-CH_3), 0.84 (t, ^3J = 7.2 Hz, 3 H, H-16); ^{13}C NMR (C_6D_6, 75.5 MHz) δ 210.4 (C_q-6), 195.4 (C_q-2), 177.3 (C_q-9), 129.6 (CH-12), 126.5 (CH-13), 110.7 (C_q-3), 79.3 (CH-7), 74.3 (C_q-5), 67.1 (C_q-1), 65.4 (CH_2-15), 40.6 (CH-4), 32.0 (CH_2-10), 31.2 (CH_2-11), 29.4 (CH_2-8), 24.1 (5-CH_3), 17.9 (CH_3-14), 15.2 (CH_3-16), 9.5 (1-CH_3); (+)-ESI MS *m/z* 345 ($[M+Na]^+$, 100); (-)-ESIMS *m/z* 321 ($[M-H]^-$. (+)-ESIHRMS *m/z* 345.1672 (calcd. for $[M+Na]^+$, $C_{18}H_{26}O_5Na$, 345.1672).

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