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Biologically active metabolites from fungi. Part 16:☆ New preussomerins J, K and L from an endophytic fungus: structure elucidation, crystal structure analysis and determination of absolute configuration by CD calculations

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Abstract—Three known preussomerins, G (**1**), H (**2**) and I (**3**), and three new representatives, J (**4**), K (**5**) and L (**6**), were isolated from an endophytic fungus, a Mycelia sterila, from Atropa belladonna. Their absolute configuration was determined by comparison of calculated and experimental CD spectra. © 2001 Published by Elsevier Science Ltd.

1. Isolation

In connection with our ongoing search for biologically active metabolites from fungi, we investigated the constituents of an endophytic fungus, *Mycelia sterila*, isolated following surface sterilization from the root of *Atropa belladonna*. Since the fungus did not sporulate it could not be characterized taxonomically. The culture extract of the fungus was found to have antibacterial and antifungal properties. Extensive column and preparative thin layer chromatography of the ethyl ether extract afforded six pure compounds **1–6** (Scheme 1), **1** being the least polar and **6** the most polar metabolite. All spots showed a typical faint blue fluorescence on TLC plates and an identical blue-gray color of the spots on heating, indicating a common class of natural products. Compounds **1–3** are identical with the known preussomerins G–I,[2] whereas preussomerin J (**4**), preussomerin K (**5**) and preussomerin L (**6**) are new representatives of these bisspirobisnaphthalene natural products.

Preussomerins were first isolated by Gloer et al. from cultures of the coprophilous (dung-colonizing) fungus *Preussia isomera* Cain.[3,4] The six preussomerins A–F isolated by Gloer et al., showed antifungal activity toward early-successional coprophilous fungi, thus supporting the theory of interspecies competition among coprophilous fungi. Later, preussomerin D was also isolated from the endophyte *Hormonema dematioides*[5] and the new preussomerins G–I (**1–3**) from a dung-inhabiting coelomycetous fungus.[2] Singh et al. also discovered that they inhibit farnesyl-protein transferase (FPTase). FPTase inhibitors are potential agents against cancers with mutated *ras* gene such as colon or pancreatic carcinomas.[6] Very recently, the racemic preussomerins G and I[7] and several analogues[8] were prepared by chemical synthesis.

2. Structure determination

The structure elucidation is exemplified in more detail on the most polar metabolite **6**, mp 171–173°C, first isolated in pure crystalline form from methanol. The optically active compound ($[\alpha]_D^{20}=-557$) showed comparatively low solubility in nonpolar (e.g. cyclohexane) or very polar (e.g. methanol) solvents, but could easily be dissolved in dichloromethane/5% methanol. This indicated the presence of free hydroxyl groups, confirmed by a band at 3382 cm^{-1} in the IR spectrum. Another band at 1722 cm^{-1} indicated the presence of a carbonyl group. The molecular mass of $C_{20}H_{14}O_8$ (14 unsaturations) was deduced from the

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☆ For part 15 of series see Ref. 1.

Keywords: fungal metabolites; spiroketal bisnaphthalenes; preussomerins; antifungal agents.

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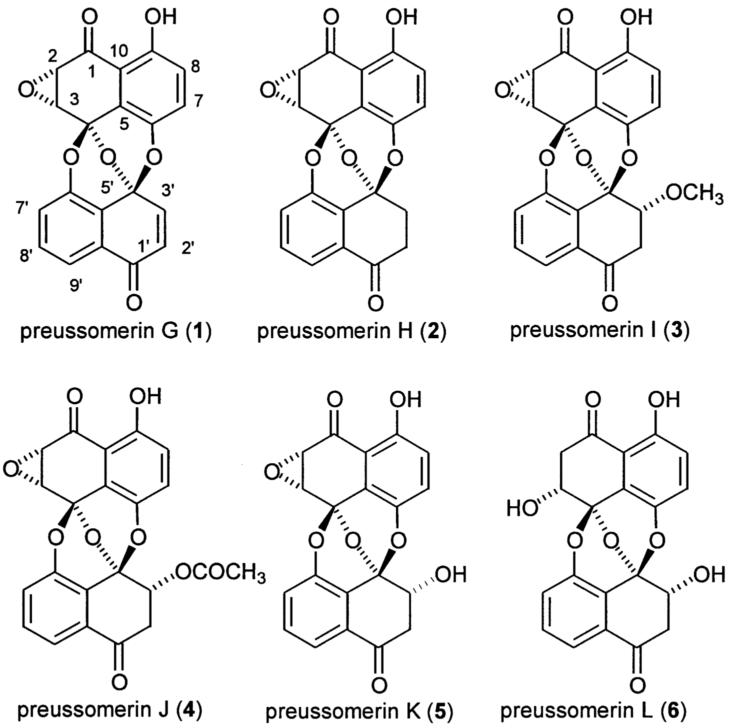


Scheme 1. Structures of preussomerins **1**–**6**.

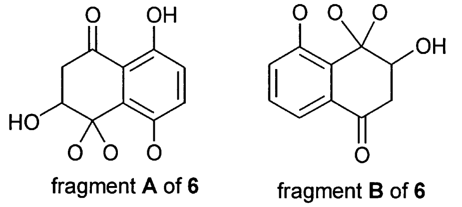
high-resolution mass spectrum (HRMS, *m/z*=382.06888) in agreement with the number of resonances for carbon and hydrogen atoms, detected in the NMR spectra (see below). The data of the ^{1}H and ^{13}C NMR spectra, together with the correlations resulting from the H,H-COSY and HMBC experiments, are listed in Table 1.

The ^{13}C NMR spectrum indicates a total number of 14 sp^{2}-hybridisized carbon atoms, three of which are directly connected to a heteroatom while two others are resonances for carbonyl groups. Very characteristic are signals at δ=95.66 and 95.71, indicating the presence of two ketal carbon atoms. Signals at δ=70.76 and 70.97 designate carbon atoms directly connected with heteroatoms and two further resonances at δ=43.21 and 43.56 are caused by aliphatic carbon atoms in the neighborhood of heteroatoms.

In addition to three singlets for hydroxy groups at δ=3.39, 3.68 and 10.16 (chelated phenolic OH), two groups of multiplets at δ=7–8 for aromatic and δ=3–5 for aliphatic protons can be detected in the ^{1}H NMR spectrum. In the aromatic region, the presence of two and three vicinal protons, respectively, can be deduced from the coupling pattern. The low-field signals at δ=4.75 are caused by two methine protons, directly bound to carbon atoms bearing oxygen atoms that couple with diastereotopic methylene protons at δ=3.62 and 2.99, respectively. Due

Table 1. ^{1}H and ^{13}C NMR data and correlations from the H,H-COSY and HMBC of preussomerin L (**6**)

C number	^{13}C	HMBC	^{1}H (*J* in Hz)	H,H COSY
1	202.24	3, 8	10.16, 10-OH, s	–
1′	195.98	9′, 3′, 2a′, 2b′	7.63, 9′-H, dd (1.0, 7.8)	8′, 7′
9	158.10	7, 8	7.48, 8′-H, t	9′, 7′
6′	152.33	7′, 8′	7.21, 7′-H, dd (1.0, 8.2)	9′, 8′
6	144.41	7, 8	7.16, 7-H, d (9.2)	8
10′ (5′)	132.22	7′, 8′, 9′	6.97, 8-H, d (9.2)	7
5′ (10′)	132.12	2a′, 2b′	4.75, 3/3′-H, 2 t	2a,2b/2a′,2b′
7	126.89	8	3.68, 3-OH, s	
7′	122.63	8′, 9′	3.59, 2a-H, dd (3.2, 18.3)	3, 2b
8′ (9′)	121.65	9′, 7′, 8′	3.49, 2a′-H, dd (3.2, 18.3)	3′, 2b′
9′ (8′)	121.55	9′	3.39, 3′-OH, s	
8	121.08	7	2.97, 2b-H, dd (2.7, 18.3)	3, 2a
5	119.55	8	2.92, 2b′-H, dd (2.7, 18.3)	3′, 2a′
10	114.15	7		
4′	95.71	3′, 2a′, 2b′		
4	95.66	3		
3 (3′)	70.97			
3′ (3)	70.76			
2 (2′)	43.56			
2′ (2)	43.21			

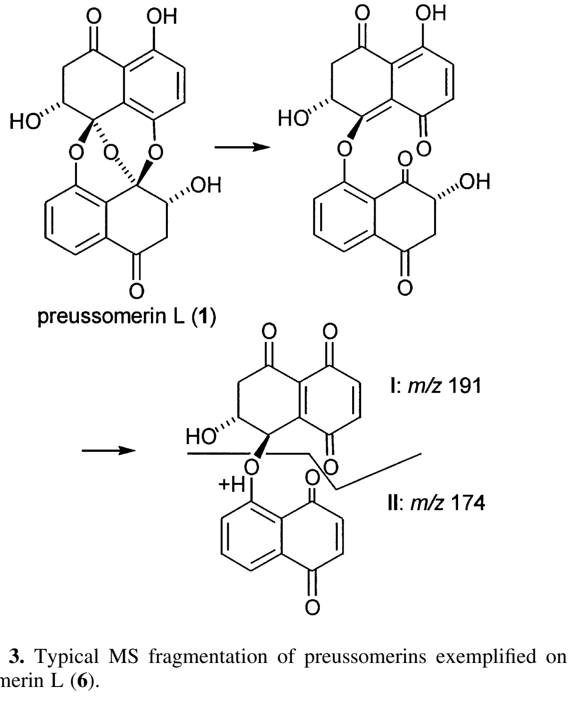


Scheme 2. Fragments **A** and **B** as deduced from the NMR spectra of **6**.

to the similarity of the top and bottom part of the molecule, many signals have nearly identical chemical shifts and represent two protons. The coupling constants of 3- and 3′-H with the neighboring methylene protons at 2- and 2′-C are J=2.7 and J=3.2 Hz, excluding 1,2-transdiaxial positions of the respective protons.

Analysis of the complete set of spin systems in combination with connectivities deduced from two-dimensional techniques (H,H-COSY, HMQC and HMBC) allowed the construction of two independent fragments **A** and **B** (Scheme 2). A combination of the fragments **A** and **B** in consideration of the total number of 14 unsaturations finally leads to structure **6** for the most polar metabolite (Scheme 1). The new preussomerin L (**6**) is the bisreduction product of a hypothetical 2,3- and 2′,3′-diepoxy preussomerin, a metabolite which has not yet been isolated from natural sources.

Interestingly, two major independent fragments can also be seen in the EI mass spectrum for all six compounds **1**–**6**, suggesting the affiliation to the same class of compounds. As proposed by Singh et al.,[2] a simplified fragmentation pattern for preussomerin L (**6**) is presented in Scheme 3. The peak of the top fragment **I** (m/z=189 or 191) indicates a hydroxylated or epoxidized fragment, respectively. In the bottom fragment **II**, peaks for both β-elimination products (m/z=174) or β-substituted fragments occur.



Scheme 3. Typical MS fragmentation of preussomerins exemplified on preussomerin L (**6**).

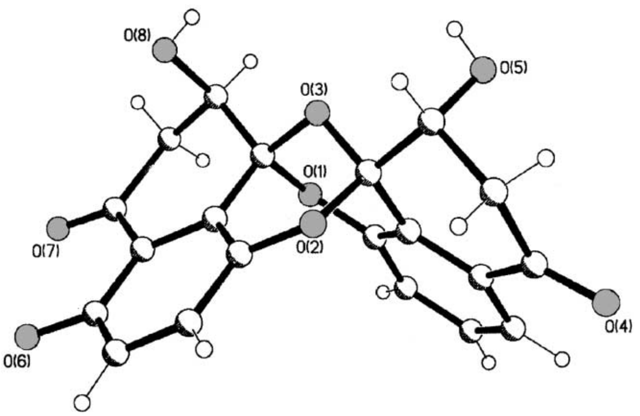


Figure 1. The molecule of preussomerin L (**6**) in the crystal.

The relative stereochemistry of preussomerin L (**6**)[9] was established by X-ray diffraction analysis of a single crystal obtained from methanol. As shown in Fig. 1, the aromatic rings are in almost perpendicular positions in the rigid but relatively strainless skeleton and quasi-boat conformations are adopted by the hydroaromatic rings in agreement with the ^{1}H NMR coupling constants.

The similarity of the spectral data of the other metabolites **1**–**5**, isolated from the endophytic fungus, confirmed membership in the preussomerin class of natural products. Structure elucidation can thus be restricted to data comparison and typical differences to those of other known preussomerins. The three unpolar compounds were identical with the preussomerins G (**1**), H (**2**) and I (**3**) (Scheme 1) as identified by Singh et al.,[2] showing desaturation or saturation at positions 2′ and 3′ or a methoxy group at C-3′. Preussomerin I (**3**), which had previously only been obtained as an oily compound, could be obtained as a crystalline material, mp 130–132°C. All spectroscopic data were in good agreement with those reported in the literature.[2]

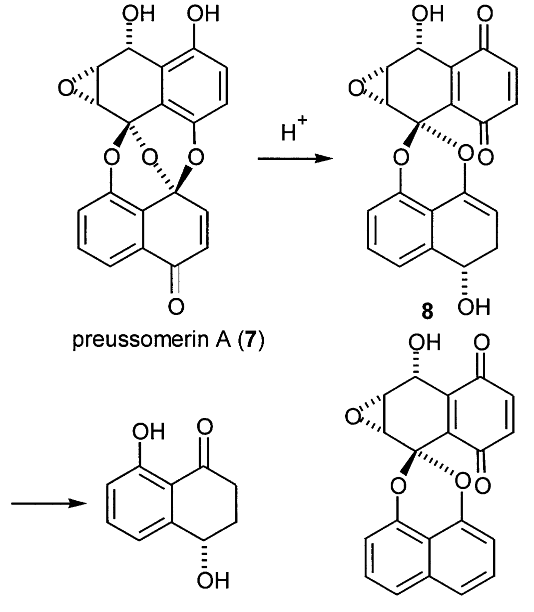
The next most polar compound, preussomerin J (**4**), was isolated in small amounts (1.3 mg). The mass spectrum and the molecular formula $C_{22}H_{14}O_9$ suggested an acetylation product of preussomerin K. This was confirmed by typical changes in the NMR spectra. Additional signals for carbon atoms appeared at δ=169.4 and 20.94 for an acetyl group. The triplet for 3′-H is typically shifted low-field from δ=4.78 for preussomerin K (**5**) to 5.93 in the acetylation product **4**. The coupling constants for the bottom aliphatic part of the molecule remain essentially unchanged ($J_{2',3'}$=2.9 Hz), indicating the same conformation for the acetylation product preussomerin J (**4**) as in **5**. It is conceivable that preussomerin J (**4**) is an artifact, formed during extraction with ethyl acetate from preussomerin K (**5**) or by Michael addition of acetic acid to preussomerin G (**1**).

The IR and UV data for the oily next polar compound, preussomerin K (**5**), are almost identical to those of preussomerin L (**6**). The molecular formula $C_{20}H_{12}O_8$, determined by HRMS, shows two hydrogen atoms fewer than the more polar metabolite **6**. In the ^{1}H NMR spectrum, two low field signals for aliphatic protons at δ=3.84 and 4.26 with a coupling constant of J=4.0 Hz can be detected. The entire pattern is typical for protons attached to an epoxide ring and is almost identical to the corresponding area in preussomerin G–I.[2] The location of the epoxide ring at C-2 and C-3 was unambiguously assigned by a long-range coupling of H-3 to the carbonyl carbon at C-1. The NMR spectra of the bottom part of **5** corresponded to that of preussomerin L (**6**) and thus structure **5** could be assigned to preussomerin K.

3. The absolute configuration

The absolute configuration of preussomerin A (**7**) was established by Weber et al.[3] by acid-catalyzed degradation (probably via intermediate (**8**) to (−)-regiolone (**9**) in combination with X-ray analysis for determination of the relative stereochemistry (Scheme 4). Interestingly, this experiment established also the stereochemical correlation to the palmarumycins as shown by the similarity of intermediate **8** to palmarumycin C_{10} (**10**).[10]

The optical rotations measured for the new compounds **4–6** and those for the preussomerins A or G are all negative. Unfortunately, the data deviate too much (preussomerin L (**1**): $[\alpha]_D^{20}=-557$; preussomerin A: $[\alpha]_D^{20}=-212$[4]; preussomerin G: $[\alpha]_D^{20}=-668$[2]) to be a solid basis for the assignment of the absolute configuration to the new preussomerins. Thus, we decided to determine their absolute configurations by comparison of calculated and experimental CD spectra. This method was previously applied successfully to elucidate the absolute configurations of several palmarumycins.[11–13] In fact, the preussomerins are ideal substrates for this kind of determination since they are conformationally very rigid molecules, fixed by their bisspiro structure. Not surprisingly, calculations using the Spartan package[14] of preussomerin L (**6**) revealed one major conformer, 18 kJ/mol higher in energy than the conformation with next lowest energy. Also, this conformation was nearly identical to that shown in Fig. 1, obtained by X-ray structure analysis. Thus, for the calculation of the CD spectrum of preussomerin L (**6**), it was sufficient to consider only the two enantiomers of this major conformation. The results of these calculations, employing the BDZDO/MCDSPD program package[15] and the comparison with the experimental CD spectrum are shown in Figs. 2 and 3.



preussomerin A (**7**)

8

(–)-regiolone (**9**)

palmarumycin C_{10} (**10**)

Scheme 4. Degradation of preussomerin A (**7**) to regiolone (**9**) and comparison with palmarumycin C_{10} (**10**).

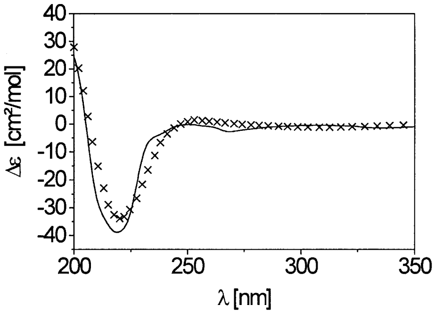


Figure 2. Experimental CD spectrum and calculated spectrum (dotted line) of structure **6** of preussomerin L.

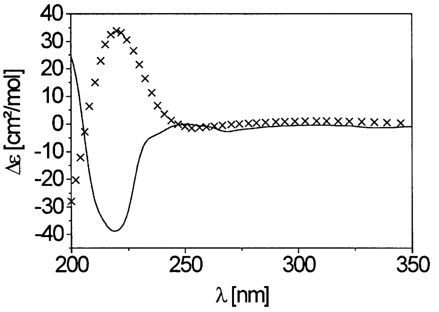


Figure 3. Experimental CD spectrum and calculated spectrum (dotted line) of structure *ent*-**6**: —, measured spectrum; ×, calculated spectra.

In Fig. 2, the experimental spectrum matches that of the calculated spectrum (dotted line) of structure **6** of preussomerin L. By contrast, the calculated spectrum for the enantiomer *ent*-**6** is almost of the opposite shape to that of the experimental spectrum. Consequently, the absolute configuration for preussomerin L (**6**) was assigned beyond any doubt to be 3*R*, 3′*R*, 4*S*, 4*S*′. The CD spectra of all of the other preussomerins G–K (**1–5**) were calculated in a similar manner. Comparison of calculated with the experimental data confirmed their absolute configuration as shown in Scheme 1 and the CD maxima are listed in Table 2.[16] The same result was obtained on the basis of the negative sign of $n\rightarrow\pi^*$ Cotton effect located at 330–334 nm, caused by the aryl ketone chromophore of these molecules using Snatzke's rule.[17]

4. Biological activity

The crude ethyl acetate extracts showed activity against the Gram-positive bacterium *Bacillus megaterium* as well as against the fungus *Microbotryum violaceum*. The activity of the purified individual preussomerins against bacterial, fungal and algal test organisms is compiled in Table 3. The activity against the fungi *Microbotryum violaceum* and *Eurotium repens* is relatively moderate as compared to those of related preussomerins A–F against coprophilous

Table 2. CD-Maxima of preussomerins G–L (**1**–**6**)

Preussomerin G (**1**)		Preussomerin H (**2**)		Preussomerin I (**3**)	
λ (nm)	$\Delta\varepsilon$ (cm^2 mol^{-1})	λ (nm)	$\Delta\varepsilon$ (cm^2 mol^{-1})	λ (nm)	$\Delta\varepsilon$ (cm^2 mol^{-1})
369	−2.75	331	−3.44	332	−5.62
318	−4.62	276	−2.87	277	−4.92
264 sh	−4.79	262	0.43	262	0.11
244	−23.38	236 sh	−7.7	238 sh	−10.22
217	−50.15	218	−37.95	218	−71.63
Preussomerin J (**4**)		Preussomerin K (**5**)		Preussomerin L (**6**)	
λ (nm)	$\Delta\varepsilon$ (cm^2 mol^{-1})	λ (nm)	$\Delta\varepsilon$ (cm^2 mol^{-1})	λ (nm)	$\Delta\varepsilon$ (cm^2 mol^{-1})
331	−3.94	331	−3.35	334	−1.37
275	−2.72	276	−3.19	269	−2.73
237 sh	−12.67	240 sh	−6.53	237 sh	−4.31
218	−54.81	218	−51.16	219	−38.82

Table 3. Biological activity of preussomerins **1**–**6** against microbial test organisms, agar diffusion assay

Compound	Bm.	Chl.	Eur.	Fu.	Mm.	Mv.
1	0.6	0	0.9	1.1	0.1	0.6–1
2	0.3–0.5	0.1–0.2	–	–	–	0.8–1.3
3	0.2	0.3	0.5–0.6	–	0.1	0.4–0.8
4	0.4–0.5	0.1	0.8	–	–	0.8–1.2
5	0.5–0.7	0.5	0.5	–	–	1.1
6	0.2–0.5	0	–	0	0.2	0.4

Concentration: 50 μl of the given 2 mg/ml solution per plate; test organisms: Bm.=*Bacillus megaterium*, Mv.=*Microbotryum violaceum*, Mm.=*Mycotypha microspora*, Eur.=*Eurotium repens*, Fu.=*Fusarium oxysporum*, Chl.=*Chlorella fusca*.

fungi as determined by Weber et al.[3] The remarkable FPTase inhibitory activity of preussomerins, notably of preussomerin G, has already been mentioned.[2]

5. Experimental

General methods and instrumentation are covered elsewhere.[18]

5.1. Cultivation and isolation

The fungus was cultivated for 70 days at ambient temperature on two different media: biomalt and malt-soya semisolid agar as described previously.[18] The combined cultures (6 l) were homogenized and extracted first with petroleum ether (0.5 l) and than four times with ethyl acetate (1 l). The biologically active metabolites were located mainly in the ethyl acetate extract (3.3 g) as shown by TLC and bioautograms. The crude extract was then suspended in a mixture of cyclohexane and dichloromethane (1:1, 30 ml), filtered, and the filtrate concentrated at reduced pressure to yield 3.0 g of soluble crude material. The residue was separated into five fractions by column chromatography on silica gel (400 g), using gradients of dichloromethane/ethyl acetate (85:15, 50:50, 0:100). The less polar fraction (2.1 g) contained mainly fatty acids and lipids. The remaining four fractions were each further purified by preparative TLC chromatography on silica gel (1 mm, Macherey and Nagel). The least polar fraction was developed twice with CH_2Cl_2/petroleum ether 2:5 to yield 13.1 mg of preussomerin G (**1**) as a yellow solid.[2] The next fraction was developed with CH_2Cl_2 to yield 5.4 mg of preussomerin I (**2**).[2] Crystallization from cyclohexane/dichloromethane gave yellow crystals, mp 130–132°C. Preparative TLC separation of the fraction next in polarity (CH_2Cl_2) gave 1.3 mg of preussomerin H (**3**).[2] The more polar fractions were separated on TLC plates and developed with increasingly polar eluent (CH_2Cl_2/2, 3, and 5% of methanol, respectively) to yield the preussomerins J (**4**) (4.0 mg), K (**5**) (8.2 mg), and L (**6**) (7.9 mg). Preussomerin L crystallized from small amounts of methanol (mp 171–173°C). All preussomerins could be detected on TLC plates by irradiation with 254 nm UV light or by spraying with a solution of 2,4-dinitrophenylhydrazine or molybdenum phosphate.

5.2. X-Ray crystallographic study of 6[19]

Crystal data: $C_{20}H_{14}O_8$, M_r=382.31, hexagonal, space group $P6_1$, a=15.274(2), c=15.074(7) Å, V=3045.5(15) Å^3, Z=6, D_c=1.251 Mg/cm^3. It was very arduous obtaining a fairly suitable crystal for data collection: Bruker AXS P4 diffractometer; Mo-K_α radiation, λ=0.71073 Å, graphite monochromator, crystal size 0.45×0.30×0.28 mm^3, T=293(2) K, ω-scan, $2.1\leq\Theta\leq25.0°$, $-16\leq h\leq16$, $-16\leq k\leq1$, $-1\leq l\leq17$, Lp correction, 3 standard reflections were recorded every 400 reflections and showed a decrease of 12%; 4523 reflections collected, 2011 independent reflections (R_{int}=0.061), μ=0.098 mm^{-1}. Structure solution by direct methods, full-matrix least-squares refinement based on 2010 F^2 values and 256 parameters, hydrogen atoms at calculated positions were refined as riding models; all but hydrogen atoms refined anisotropically. Max $(\Delta/\sigma)<0.001$, G_{oof}=1.011, $R1(I>2(\sigma)I)$=0.081, $wR2$(all data)=0.277. It was not possible to successfully refine various diffuse electron density residues which must be assigned to solvent molecules. Program used: SHELXTL NT 5.10.[20]

5.2.1. Preussomerin G (1). Yellow solid. R_f 0.62 (dichloromethane). C-10 was not correctly assigned in the ^{13}C-NMR spectrum of preussomerin G (**1**).[2] Our data, based on HMBC correlations, are given below. ^{13}C-NMR (300 MHz, $CDCl_3$): δ=52.17 (d, C-3), 53.70 (d, C-2), 89.98 (s, C-4′), 93.96 (s, C-4), 109.82 (s, C-5), 115.21 (s, C-10), 120.59 (s, C-5′), 120.70 (d, C-9′), 120.86 (d, C-7′), 121.31 (d, C-8), 126.73 (d, C-7), 130.32 (s, C-10′), 131.15 (d, C-8′), 133.57 (d, C-2′), 140.73 (d, C-3′), 143.39 (s, C-6), 148.76 (s, C-6′), 156.11 (s, C-9), 183.73 (s, C-1′), 195.45 (s, C-1).

5.2.2. Preussomerin H (2). Oil; R_f 0.5 (dichloromethane).

5.2.3. Preussomerin I (3). Mp 130–132°C (dichloromethane/cyclohexane); R_f 0.71 (dichloromethane).

5.2.4. Preussomerin J (4). Oil; $R_f=0.43$ (SiO_2, CH_2Cl_2); $[\alpha]_D^{20}=-218$ (c 0.08, CH_2Cl_2). IR: $\nu=3389$, 2919, 2852, 1734, 1641, 1263, 1098, 1031, 922, 798 cm^{-1}. UV (CH_2Cl_2): λ_{max} (log ε)=360 (2.14), 319 (2.14), 253 (2.70), 228 (2.85) nm. ^{1}H-NMR (600 MHz, $CDCl_3$): $\delta=1.99$ (s, 3H, $OCOCH_3$), 3.05 (dd, 1H, J=18.8 Hz, J=2.9 Hz, 2a′-H), 3.47 (dd, 1H, J=18.8 Hz, J=3.3 Hz, 2b′-H), 3.82 (d, 1H, J=4.0 Hz, 3-H), 4.23 (d, 1H, J=4.0, 2-H), 5.93 (t, 1H, 3′-H), 6.95 (d, 1H, J=9.1 Hz, 8-H), 7.05 (d, 1H, J=9.14 Hz, 7-H), 7.09 (dd, 1H, J=8.2 Hz, J=1.0 Hz, 7′-H), 7.44 (t, 1H, 8′-H), 7.68 (dd, 1H, J=7.9, J=1.0 Hz, 9′-H), 10.12 (s, 1H, 10-OH). ^{13}C-NMR (600 MHz, $CDCl_3$): $\delta=20.94$ (q, $OCOCH_3$), 40.26 (t, C-2′), 52.07 (d, C-3), 53.48 (d, C-2), 70.62 (d, C-3′), 92.20 (s, C-4′), 93.08 (s, C-4), 110.20 (s, C-10), 115.69 (s, C-5), 119.75 (s, C-5′), 120.74 (d, C-9′), 120.89 (d, C-7′), 121.46 (d, C-8), 121.76 (d, C-8′), 126.43 (d, C-7), 131.14 (s, C-10′), 142.49 (s, C-6), 149.66 (s, C-6′), 156.40 (s, C-9), 169.40 (s, OCO), 192.42 (s, C-1′), 195.58 (s, C-1). HRMS (EI) ($C_{22}H_{14}O_9$) calcd 422.06320; found: 422.06378 ±1.4 ppm.

5.2.5. Preussomerin K (5). $R_f=0.17$ (SiO_2, CH_2Cl_2); oil; $[\alpha]_D^{20}=-150$ (c 0.04, CH_2Cl_2). IR $\nu=3415$, 2924, 2846, 1708, 1465, 1419, 1274, 1025, 932 cm^{-1}. UV (CH_2Cl_2): λ_{max} (log ε)=363 (1.11), 314 (1.23), 250 (1.64), 260 (1.45), 230 (1.86) nm. ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta=3.03$ (dd, 1H, J=2.7 Hz, J=18.3 Hz, 2a′-H), 3.37 (dd, 1H, J=3.3 Hz, J=18.3 Hz, 2b′-H), 3.84 (d, 1H, J=4.0 Hz, 3-H), 3.53 (s, 1H, 3′-OH), 3.84 (t, 1H, 3′-H), 4.26 (d, 1H, J=4.0 Hz, 2-H), 6.94 (d, 1H, J=9.2 Hz, 8-H), 7.02 (d, 1H, J=9.2 Hz, 7-H), 7.05 (dd, 1H, J=8.1 Hz, J=1.0 Hz, 7′-H), 7.39 (t, 1H, 8′-H), 7.65 (dd, 1H, J=7.7, J=1.0 Hz, 9′-H), 10.12 (s, 1H, 1-H). ^{13}C-NMR (300 MHz, $CDCl_3$): $\delta=41.30$ (t, C-2′), 52.17 (d, C-3), 53.67 (d, C-2), 70.28 (d, C-3′), 93.58 (s, C-4), 94.38 (s, C-4′), 110.26 (s, C-10), 115.04 (s, C-5), 119.81 (s, C-5′), 121.13 (d, C-9′), 120.32 (d, C-7), 121.52 (d, C-7′), 126.46 (d, C-8), 130.85 (s, C-10′), 131.15 (d, C-8′), 142.52 (s, C-6), 149.73 (s, C-6′), 156.06 (s, C-9), 193.39 (s, C-1′), 195.64 (s, C-1). HRMS(EI) ($C_{20}H_{12}O_8$) calcd. 380.05347; found: 380.05322 ±0.8 ppm.

5.2.6. Preussomerin L (6). Mp 171–173°C; R_f 0.61 (SiO_2, CH_2Cl_2/MeOH 95:5); $[\alpha]_D^{20}=-557$ (c 0.11, CH_2Cl_2). IR (KBr) $\nu=3382$ cm^{-1}, 2925, 2851, 1722, 1649, 1434, 1044, 870. UV (CH_2Cl_2): λ_{max} (log ε)=359 nm (2.96), 315 (2.89), 258 (3.52), 226 (3.61), 194 (2.92). ^{1}H-NMR (600 MHz, $CDCl_3$): $\delta=2.92$ (dd, J=18.3 Hz, J=2.7 Hz, 2b′-H), 2.97 (dd, J=18.3 Hz, J=2.7 Hz, 2b-H), 3.39 (s, 3′-OH), 3.49 (dd, J=3.2 Hz, J=18.3 Hz, 2a′-H), 3.59 (dd, J=3.2 Hz, J=18.3 Hz, 2a-H), 3.68 (s, 1H, 3′-OH), 4.75 (dd, 2H, 3, 3′-H), 6.97 (d, 1H, J=9.2 Hz, 8-H), 7.16 (d, 1H, J=9.2 Hz, 7-H), 7.21 (dd, 1H, J=8.2 Hz, J=1 Hz, 7′-H), 7.48 (t, 1H, 8′-H), 7.63 (dd, 1H, J=7.8, J=1.0 Hz, 9′-H), 10.16 (s, 1H, 10-OH). ^{13}C-NMR (150 MHz, $CDCl_3$): $\delta=43.21$ (t, C-2/C-2′) 43.56 (t, C-2′/C-2), 70.76 (d, C-3/C-3′), 70.97 (d, C-3′/C-3), 95.66 (s, C-4), 95.71 (s, C-4′), 114.15 (s, C-10), 119.55 (s, C-5), 121.08 (d, C-8), 121.55 (d, C-9′/C-8′), 121.65 (d, C-8′/C-9′), 122.63 (d, C-7′), 126.89 (d, C-7), 132.12 (s, C-5′/C-10′), 132.22 (s, C-10′/C-5′), 144.41 (s, C-6), 152.33 (s, C-6′), 158.10 (s, C-9), 195.98 (s, C-1′), 202.24 (s, C-1). HRMS(EI) ($C_{20}H_{14}O_8$) calcd. 382.06795; found: 382.06888 ±2.4 ppm.

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References

1. Communication No. 15: Krohn, K.; Flörke, U.; Rao, M. S.; Steingröver, K.; Aust, H.-J.; Draeger, S., Schulz, B. *Nat. Prod. Lett.* 2001, submitted.
2. Singh, S. B.; Zink, D. L.; Liesch, J. M.; Ball, R. G.; Goetz, M. A.; Bolessa, E. A.; Giacobbe, R. A.; Silverman, K. C.; Bills, G. F.; Pelaez, F.; Cascales, C.; Gibbs, J. B.; Lingman, R. B. *J. Org. Chem.* **1994**, *59*, 6296–6302.
3. Weber, H. A.; Baenziger, N. C.; Gloer, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 6718–6719.
4. Weber, H. A.; Gloer, J. B. *J. Org. Chem.* **1991**, *56*, 4355–4360.
5. Polishook, J. D.; Dombrowski, A. W.; Tsou, N. N.; Salituro, G. M.; Curoto, J. E. *Mycologia* **1993**, *85*, 62–64.
6. Rodenhuis, S. *Semin. Can. Biol.* **1992**, *3*, 241.
7. Chi, S.; Heathcock, C. H. *Org. Lett.* **1999**, *1*, 3–5.
8. Ragot, J. P.; Prime, M. E.; Archiblad, S. J.; Taylor, R. J. K. *Org. Lett.* **2000**, *2*, 1613–1616.
9. Numbering of preussomerins as shown in formula **1** is in accordance with use in Ref. 4.
10. Krohn, K.; Michel, A.; Flörke, U.; Aust, H.-J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1994**, 1099–1108.
11. Bringmann, G.; Busemann, S.; Krohn, K.; Beckmann, K. *Tetrahedron* **1997**, *53*, 1655–1664.
12. Krohn, K.; Beckmann, K.; Flörke, U.; Aust, H.-J.; Draeger, S.; Schulz, B.; Busemann, S.; Bringmann, G. *Tetrahedron* **1997**, *53*, 3101–3110.
13. Krohn, K.; Beckmann, K.; Aust, H.-J.; Draeger, S.; Schulz, B.; Busemann, S.; Bringmann, G. *Liebigs Annalen/Recueil* **1997**, 2531–2534.
14. Spartan SGI Version 5.1.3; Wavefunction Inc., Irvine, USA.
15. Browning, J. W. Program package BDZDO/MCDPPD, Department of Chemistry and Biochemistry, University of Colorado, Boulder, USA. Modified by J. Fleischauer, W. Schlecker and B. Kramer, University of Aachen, Germany.
16. Plots of the experimental and calculated spectra of preussomerins G–K are available from the authors via e-mail: kk@chemie.uni-paderborn.de.
17. Snatzke, G. *Tetrahedron* **1966**, *21*, 439–448.
18. Krohn, K.; Michel, A.; Flörke, U.; Aust, H.-J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1994**, 1093–1097.
19. Data (excluding structure factors) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications no. CCDC 148953. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
20. SHELXTL NT, Ver. 5.10, Bruker Analytical X-ray Systems, Madison, Wisconsin, USA.