Online Analysis of Xestodecalactones A−C, Novel Bioactive Metabolites from the Fungus *Penicillium cf. montanense* and Their Subsequent Isolation from the Sponge *Xestospongia exigua*[#]

Ru Angelie Edrada,[†] Markus Heubes,[‡] Gernot Brauers,[†] Victor Wray,[§] Albrecht Berg,[⊥] Udo Gräfe,[⊥] Michael Wohlfarth,[‡] Jörg Mühlbacher,[‡] Karsten Schaumann,[∥] Sudarsono,[▽] Gerhard Bringmann,*[,‡] and Peter Proksch*[,†]

Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1 Geb. 26.23, D-40225 Düsseldorf, Germany, Institut für Organische Chemie, Julius-Maximilians-Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany, Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124, Braunschweig, Germany, Hans-Knöll-Institut für Naturstofforschung, Beutenbergstrasse 11, D-07745 Jena, Germany, Stiftung Alfred-Wegener-Institut für Polar und Meeresforschung, Am Handelshafen 12, D-27570, Bremerhaven, Germany, and Fakultas Biologi, Gadjah Mada University, Sekip Utara, Yogyakarta, Indonesia

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Fungal isolates of *Penicillium cf. montanense* were obtained from the marine sponge *Xestospongia exigua* collected from the Bali Sea, Indonesia. Culture filtrates of the fungi yielded three novel decalactone metabolites, xestodecalactones A, B, and C (**1**, **2a**, and **2b**), consisting of 10-membered macrolides with a fused 1,3-dihydroxybenzene ring. Online HPLC-NMR, ESI-MS/MS, and -CD spectra were acquired, and the structures of the new compounds were established and confirmed on the basis of offline NMR spectroscopic (^{1}H, ^{13}C, COSY, ROESY, ^{1}H-detected direct and long-range ^{13}C−^{1}H correlations) and mass spectrometric (EIMS) data. Quantum chemical calculations of the CD spectra proved to be difficult because of the conformational flexibility of the xestodecalactones. These compounds, of which **2a** and **2b**, due to the additional stereocenter at C-9, are diastereomeric compounds, are structurally related to a number of biologically active metabolites found in terrestrial fungal strains. Compound **2a** was found to be active against the yeast *Candida albicans*.

Marine microorganisms have evolved to biosynthesize biologically interesting and chemically diverse compounds. In the search for novel bioactive compounds more than 800 microorganisms have so far been isolated from marine sediments and organisms,[1−3] and about half of them belong to fungal genera. In the search for new pharmaceutical or agrochemical lead structures, sponge-associated fungi yielding cytotoxic metabolites have received increasing attention at a rate much faster than those of other unicellular organisms.[4,5] One group of compounds is the macrolides, which have been of much interest because of their flexible ring conformation and well-known antibiotic activities.[6] Recently, macrolides have also been reported as cytotoxic and potential antitumor agents.[7] An efficient approach for the rapid screening for novel compounds is the online analysis by hyphenated methods such as the analytical "triad" LCMS/MS−NMR−CD,[8] which often permits the elucidation of full stereostructures directly from crude extracts. This paper describes the online and offline structural analysis, isolation, and biotesting of three novel metabolites, xestodecalactones A−C (**1** and **2**) from the fungus *Penicillium cf. montanense*, which in turn was isolated from the marine sponge *Xestospongia exigua*. These new compounds are structurally related to a number of compounds isolated from terrestrial fungi. One of those, sporostatin (**3**),[9] even has the same carbon backbone. Other related compounds either contain the same substituted aromatic ring system, involving, however, not a 10- but a 12-membered macrolide system,[10−13] or are based on a similar macrocyclic ring system that, however, lacks the aromatic system.[14−16] A number of these known compounds possess pronounced toxic effects that may be related to fungal pathogenic effects in plants.[12,14]

Results and Discussion

Fungal isolates of *Penicillium cf. montanense* were isolated from the Indo-Pacific sponge *Xestospongia exigua*. The fungus was grown in liquid malt-extract medium, and the secondary metabolites were obtained from the EtOAc−MeOH total extract of the mycelia and its culture filtrate. The crude extract was subjected to an HPLC system equipped with a photodiode-array detector to qualitatively determine the major compounds. By HPLC-ESI-MS analysis (Figures 2 and 3), it was possible to assign the masses of the different monoprotonated molecular ions $[M + H]^+$ of the compounds of interest by comparison of the resulting total ion chromatogram with the corresponding UV spectra.

By this method, major metabolites with pseudomolecular masses $[M + H]^+$ *m/z* 264.9 and 281.1 were detected (Figure 2). Compounds **2a** and **2b**, both with $[M + H]^+$ peaks of *m/z* 281.1 (retention time at 17.8 and 18.12 min, respectively) showed almost identical fragmentation patterns, suggesting the presence of stereoisomers, probably diastereomers (Figure 3). Furthermore, compound **1**, with an $[M + H]^+$ peak of *m/z* 264.9 (Figure 2), displayed a fragmentation pattern similar to that of **2a** and **2b** (Figure 3), starting with the loss of one molecule of H_2O (−18 mu). The difference of the $[M + H]^+$ peaks of **1** and **2** (−16 mu) indicates the presence of an additional oxygen atom in **2a**

[#] Dedicated to Prof. Werner E. G. Müller on the occasion of his 60th birthday.

* To whom correspondence should be addressed. (P.P.) Tel: +49-211-811-4163. Fax: +49-211-811-1923. E-mail: proksch@uni-duesseldorf.de. (G.B.) Tel: +49-931-888-5323. Fax: +49-931-888-4755. E-mail: bringman@chemie.uni-wuerzburg.de.

[†] Institut für Pharmazeutische Biologie.

[‡] Institut für Organische Chemie.

[§] Gesellschaft für Biotechnologische Forschung mbH.

[⊥] Hans-Knöll-Institut für Naturstofforschung.

[∥] Stiftung Alfred-Wegener-Institut für Polar- und Meeresforschung.

[▽] Gadjah Mada University.

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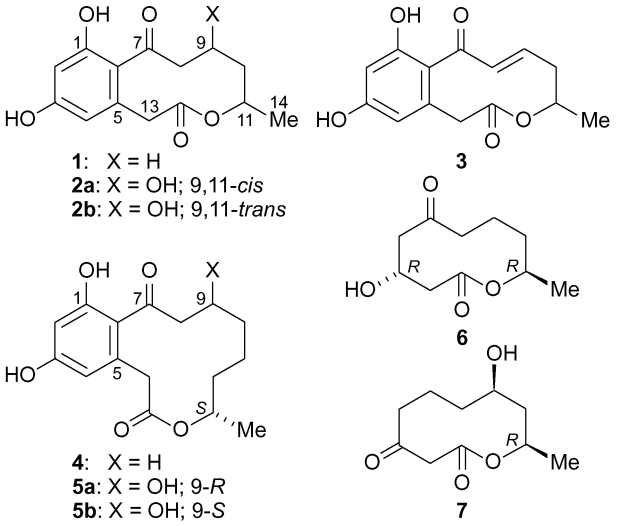

Figure 1. New xestodecalactones A−C (**1** and **2a**/**b**) from *P. cf. montanese*, sporostatin (**3**), curvularines (**4** and **5a**/**b**), diplodialide D (**6**), and decarestrictin J (**7**).

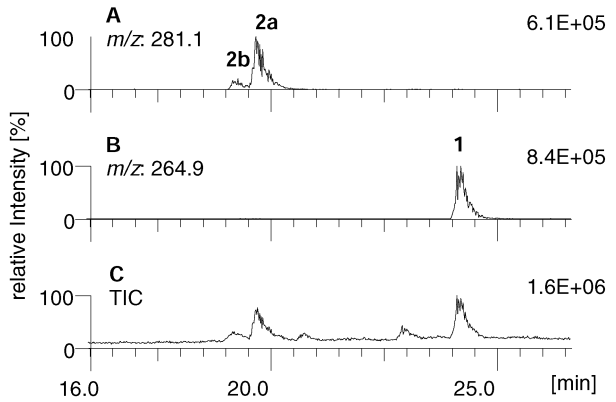

Figure 2. HPLC-MS/MS chromatogram of the crude extract: mass trace for *m/z* 281.1 (A); mass trace for *m/z* 264.9 (B); total ion chromatogram (C).

and **2b** as compared to **1**. When D_2O/MeCN was used as the eluent, compounds **2a** and **2b** gave a mass shift of +4 mu, corresponding to three exchangeable protons with $[M + D]^+$ of *m/z* 285.1, whereas compound **1** gained only 3 mu for two exchangeable protons with $[M + D]^+$ of *m/z* 267.9. Therefore compound **1** obviously lacked one hydroxy function compared to compounds **2a** and **2b**.

HPLC-NMR experiments, which were acquired using the WET sequence[17] for the solvent suppression, revealed the presence of three main metabolites in the crude fungal extract with similar retention times, as shown in Figure 2. Compounds **2** displayed two signals in the aromatic area δ 6.20, 6.32 and δ 6.26, 6.32, respectively (**2a**, Figure 5). Both gave signals at δ 4.92 and 4.91, respectively, indicating protons next to an ester group. These protons delivered couplings in WET-COSYs of **2a** and **2b** to methyl doublets at δ 1.13 (J = 6.0) and 1.10 (J = 6.2), respectively (Figure 4). The proton spectrum of **2a** provided two signals of one CH_2 group at δ 3.07 and 2.95, indicating a neighborhood to a carbonyl group. A correlation of these protons was found in the COSY to a single proton at δ 4.18, probably bound to a hydroxyl carbon. This proton showed another correlation in the COSY to a proton at δ 1.83, very close to the suppressed acetonitrile signal, which made it impossible to describe the number of protons that were represented by this signal. Furthermore the compound gave two doublets at δ 3.59 and 3.98, with an α coupling of 18.4 Hz.

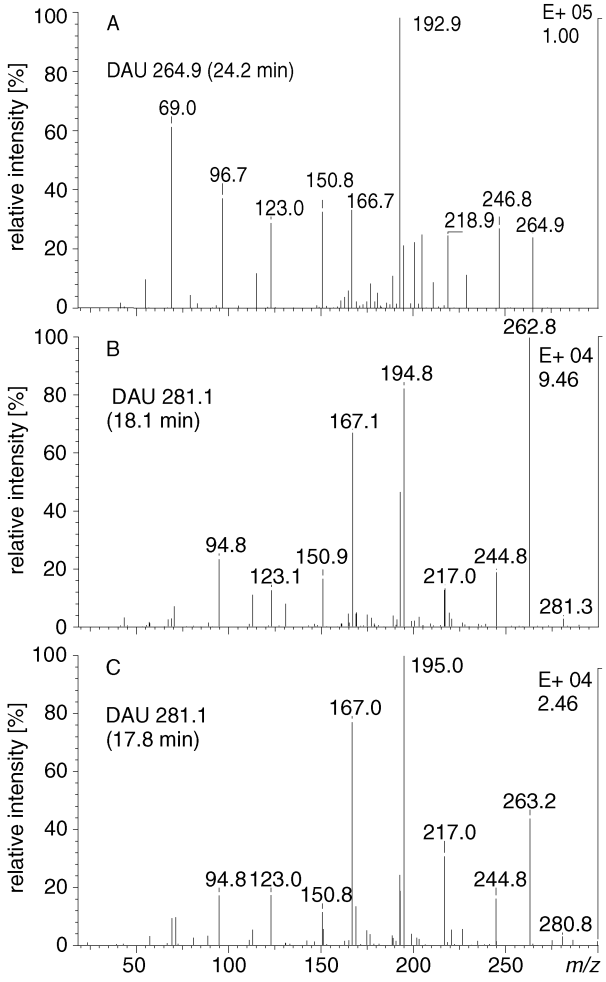

Figure 3. HPLC-MS/MS daughter-ion spectra of **1** (A), **2a** (B), and **2b** (C).

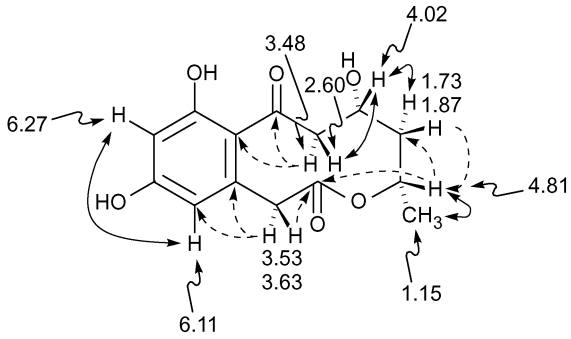

Figure 4. Selected key NMR correlations for **2a** (↔ COSY, → HMBC; − online; - - additional interaction to be seen offline). The chemical shifts are from the offline-proton spectrum in DMSO-d_6; absolute configuration arbitrary.

In the COSY they showed a correlation to the aromatic proton at δ 6.26. These protons seemed to belong to a CH_2 group next to the aromatic ring and next to an carbonyl group, probably an ester group. The splitting of the group indicated the proximity of a stereocenter.

An HPLC-WET-ROESY spectrum (Figure 6) of **2a** showed a diagnostically valuable through-space interaction of the protons at C-9 (δ 4.18) and C-11 (δ 4.91). Anticipating the results of the offline structural elucidation of the isolated xestodecalactone B (**2a**), this correlation (which was absent in **2b**) indicated a *cis* position of the protons α to the hydroxy group (9-H) relative to the methyl group (11-H) and thus gave the relative stereostructure of the xestode-

Table 1. ^{1}H NMR Data for Compounds **1** and **2a/b** (δ, multiplicity, J in Hz)

	1[a,b]				**2a**[b]				**2b**[b]		
	D_2O/MeCN	$CDCl_3$		DMSO-d_6	D_2O/MeCN	$CDCl_3$		DMSO-d_6	D_2O/MeCN		DMSO-d_6
^{1}H	δ	δ	δ	J (Hz)	δ	δ	δ	J (Hz)	δ	δ	J (Hz)
2	6.37	6.31	6.27	d, 2.1	6.32	6.28	6.27	d, 2.2	6.32	6.27	d, 2.1
4	6.28	6.20	6.10	d, 2.1	6.26	6.05	6.11	d, 2.2	6.20	6.10	d, 2.1
8α	2.63	3.62	2.60	dm[e], 15.8	3.07	3.25	3.48	bdd, 2.6,14.5	2.95	3.08	dd, 10.4,15.1
8β	2.75	4.18	2.86	dm[e], 15.8	2.95	2.97	2.60	dd, 9.5,14.4	2.63	2.81	bd, 15.1
9	1.82	1.88	1.74	bm[e]	4.18	4.17	4.02	m[d]	4.32	3.95	bt, 10.0
10α	1.22	1.50	1.74	bm[e]	1.78	1.78	1.73	ddd, 3.2,6.9, 14.6	1.91	1.65	ddd, 9.8,11.4, 14.5
10β	1.58	1.78	1.39	bm[e]	1.83	1.86	1.87	ddd, 4.0,7.3, 14.6	1.96	1.83	bd, 14.5
11	4.66	4.89	4.62	ddq, 2.1,11.0, 6.2	4.91	4.82	4.81	ddq, 4.3,6.4, 6.4	4.92	4.70	ddq, 2.5,11.4, 6.2
13α	3.37	4.18	3.82	d, 18.6	3.59	3.31	3.53	d, 17.3	3.51	3.48	d, 18.7
13β	3.20	3.62	3.55	d, 18.5	3.98	4.03	3.63	d, 17.3	3.95	3.82	d, 19.0
14	1.10	1.13	1.06	d, 6.2	1.10	1.13	1.15	d, 6.4	1.13	1.08	d, 6.2
Ar-OH			9.70	bs		9.11	~9.8[c]			9.98	s
			9.65	bs		8.85	~9.8[c]			9.87	s
9-OH							*c*			4.83	d, 2.9

[a] For **1** the sums of the coupling constants for H-8α and H-8β are 24.0 and 28.4 Hz, respectively, and the assignments of these and 10α/β are interchangeable. [b] The 2D COSY spectra of all compounds show long-range correlations between 13α and both H-2 and H-4 and less intense ones between 13β and H-4. [c] The signals at ~9.8 ppm were very broad, and the 9-OH signal was not detected. [d] The sum of the coupling constants for H-9 (dddd) is 22.6 Hz. [e] Second-order system.

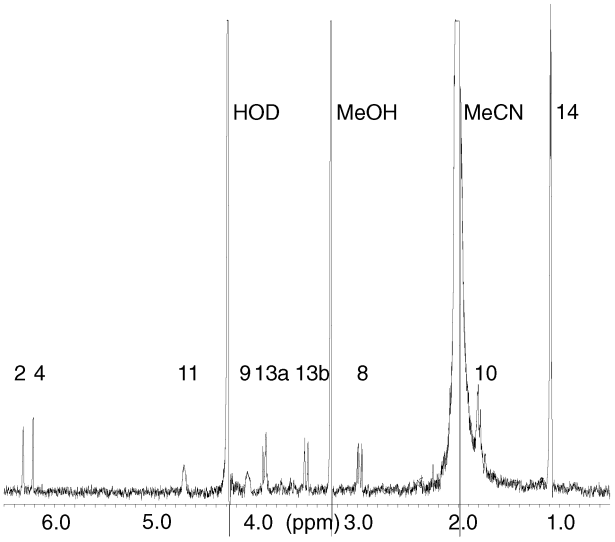

Figure 5. HPLC-NMR spectrum (using the WET solvent suppression) of **2a** in [D_2]water/acetonitrile.

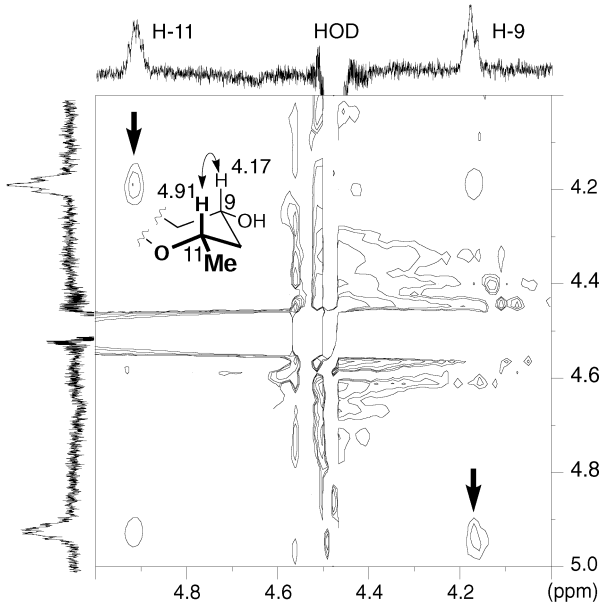

Figure 6. Online correlation of the protons H-9 and H-11 in the HPLC-WET-ROESY of **2a** directly from the extract; absolute configuration arbitrary.

calactones B (**2a**) and C (**2b**) online, directly from the extract. This result underlines the value of early online spectra.

Compound **2b**, which was much less concentrated in the extract, exhibited very similar signals in the proton spectrum.

Compound **1** showed also aromatic protons including a single proton next to a methyl group, the CH_2 group between the aromatic ring and a carbonyl group, and signals close to a carbonyl group. As shown in the HPLC-MS/MS analysis, this molecule lacks one hydroxy group compared to **2**. Therefore, the absence of the signal at δ 4.8 (see Table 1) was not surprising, and signals at δ 1.82 and at 1.22 were found instead.

HPLC-CD spectra were taken from online processing of the extract and compared to offline CD spectra from the isolated xestodecalactones (Figure 7). The compounds appeared to be stereochemically related since all displayed very similar CD spectra.

Despite these online structural hints from the analytical "triad" LCMS−NMR−CD, the isolation of these compounds was required to solve the remaining details of their structures.

Isolation of the xestodecalactones was achieved by vacuum liquid chromatography on SiO_2 gel employing gradient mixtures of dichloromethane and MeOH as the solvent system. From the nonpolar fractions the xestodecalactones A−C were obtained. All compounds were readily identified from their spectroscopic data. Their ^{1}H NMR spectra indicated that the three compounds contain the same *meta*-substituted benzene ring and differ only in the aliphatic ring system. The offline spectra showed slight differences in the chemical shifts due to the different solvent system (DMSO-d_6) compared to the online HPLC-NMR spectra ([D_2]water/acetonitrile) described above. Through-bond homonuclear (^{1}H−^{1}H COSY) and heteronuclear (long range ^{13}C−^{1}H) correlations were used to establish assignments and atom connectivities. Chemical shifts were compared with literature data for compounds containing similar structural subunits.

The isolated xestodecalactone A (**1**) was determined by HREIMS to have a molecular composition of $C_{14}H_{16}O_5$ (M^+ 264.2710). Its EI mass spectrum showed abundant ions at *m/z* 150, 166, 194, and 205. This fragmentation pattern was

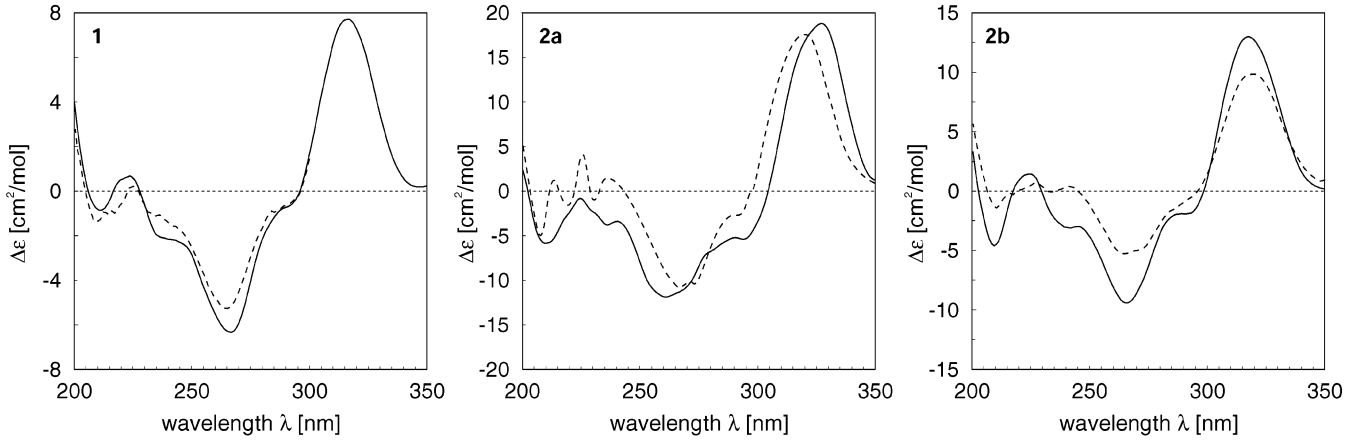

Figure 7. HPLC−CD spectra (- - -) of **1**, **2a**, and **2b** compared with the offline CD spectra (−) of the isolated compounds.

comparable to that of curvularin (**4**, see Figure 1),[13] indicating the presence of a carboxyl methyl ester (−CH_2-COO−) function and a carbonyl group adjacent to a *meta*-dihydroxybenzene ring. The new compound **1** was, however, 28 mass units smaller than **4**, which suggested the absence of two methylene groups. The ^{1}H and ^{13}C NMR spectra of **1** were also comparable to that of **4**, with a less complicated set of signals in the aliphatic region. In the ^{1}H NMR spectrum, a pair of doublets in the aromatic region with a coupling constant of 2.1 Hz hinted at the presence of two aromatic protons *meta* to each other (H-2 and H-4). The presence of two aromatic hydroxy substituents was indicated by ^{13}C resonances at δ 156.87 and 158.94. An AB pair of doublets at δ_H 3.55 and 3.82 (J = 18.5 Hz) corresponded to the methylene group (H-13α and H-13β) situated between the aryl group and the lactone carbonyl function. Its position was confirmed by a homonuclear long-range correlation with the aromatic doublet for H-4 at δ 6.10 in the COSY spectrum and by HMBC cross-peaks from H-4 to C-13 and from H-13α/β to C-12. Furthermore, the lactone carbonyl carbon showed a correlation with H-11 (Figure 4). Further correlations indicate **1** must contain the partial structure Ar−CH_2−CO−O−CH−Me as in curvularin A.[18] The ^{1}H NMR spectrum showed the presence of a methyl doublet (δ 1.06, J = 6.2 Hz) attached to a methine group (δ 4.62 ddq, J = 2.1, 11.0, and 6.2 Hz) bearing the endocyclic oxygen atom of the lactone ring. In the COSY spectrum it was also observed that H-8α and H-8β couple with those of the multiplet signals at δ 1.40−1.75 corresponding to the remaining four protons of C-9 and C-10, while the methylene group at C-10 showed correlations to the methine group at C-11, indicating the partial structure −CO−CH_2−CH_2−CH_2−CH(O−)−Me, which is similar to that found in diplodialide D (**6**).[14] By HMBC correlations, the multiplets at δ 2.60−2.90 were attributed to the C-8 methylene group, which was found to be attached to the aromatic carbonyl at δ 207.52 (C-7). The position of the carbonyl adjacent to the benzene ring was evident from the four-bond long-range "*W*" correlation of C-7 with H-2 at δ 6.27. These evidences defined the constitution of xestodecalactone A as shown in Figure 1; however, we could not obtain crystalline **1** for an investigation of the configuration at C-11 by X-ray crystallography. Therefore, we chose to use quantum mechanical CD calculations to deduce the absolute configuration (vide infra).

Xestodecalactones B (**2a**) and C (**2b**) are in agreement with a molecular composition of $C_{14}H_{16}O_6$ as determined by HREIMS (M^+ 280.0965 and M^+ 280.0927, respectively). As discussed above, the HPLC-MS/MS analysis had shown

Table 2. ^{13}C NMR Data for Compounds **1**, **2a**, and **2b** in DMSO-d_6

carbon	**1**	**2a**	**2b**
1	156.87 (s)	156.84 (s)	157.08 (s)
2	101.25 (d)	101.22 (d)	101.26 (d)
3	158.94 (s)	159.07 (s)	159.11 (s)
4	109.25 (d)	109.85 (d)	109.25 (d)
5	134.36 (s)	135.48 (s)	134.43 (s)
6	121.16 (s)	119.67 (s)	121.15 (s)
7	207.52 (s)	205.04 (s)	204.60 (s)
8	45.22 (t)	52.48 (t)	55.29 (t)
9	22.25 (t)	64.13 (d)	67.82 (d)
10	36.08 (t)	41.98 (t)	46.03 (t)
11	73.46 (d)	68.18 (d)	70.60 (d)
12	169.08 (s)	169.18 (s)	168.85 (s)
13	38.66 (t)	37.83 (t)	38.66 (t)
14	20.65 (q)	19.53 (q)	20.77 (q)

Table 3. HMBC Data for Compounds **1**, **2a**, and **2b**

proton	**1**	**2a**	**2b**
2	C-1, C-3, C-4, C-6, C-7	C-1, C-3, C-4, C-6, C-7	C-1, C-3, C-4, C-6
4	C-2, C-3, C-6, C-13	C-2, C-4, C-6, C-13	C-2, C-3, C-6, C-13
8α	C-7, C-9, C-10	C-7, C-9, C-10	C-7, C-9, C-10
8β	C-7, C-9, C-10	C-6, C-7, C-9, C-10	C-7, C-9, C-10
9	C-7, C-10, C-11		
10α/β		C-8, C-9, C-11, C-14	C-8, C-9
11	C-12	C-9, C-10, C-12	C-12
13α/β	C-4, C-5, C-6, C-12	C-4, C-5, C-6, C-12	C-4, C-5, C-6, C-12
14	C-10, C-11	C-10, C-11	C-10, C-11
9-OH			C-8, C-9, C-10

that **2a** and **2b** contain an additional hydroxyl group compared to **1**. Both compounds gave the same fragmentation pattern as **1** in their MS spectra, and their ^{1}H and ^{13}C NMR spectra were also comparable except for a more well-defined set of resonances in the aliphatic region, since the proton signals for H-8α/β and H-10 α/β were shifted to lower field. The occurrence of a ^{1}H signal at ca. 4.00 ppm for each compound indicated the presence of an oxygen-bearing methine that correlated with the signal at ca. 65 ppm in the ^{13}C NMR spectra. The COSY spectra of **2a** and **2b** indicated the presence of a partial structure CH_2−CH(OH)−CH_2−CH(O−)−Me comparable to that of decarestrictine J (**7**).[16] This partial structure and its orientation was confirmed by correlations of H-8 α/β with the carbonyl (C-7) at ca. 205 ppm and with C-6 at ca. 120 ppm, and the corresponding correlations (Table 2) described above for **1**.

Compounds **2a** and **2b** are, as found in the HPLC-NMR analyses, stereoisomers at the secondary hydroxyl function

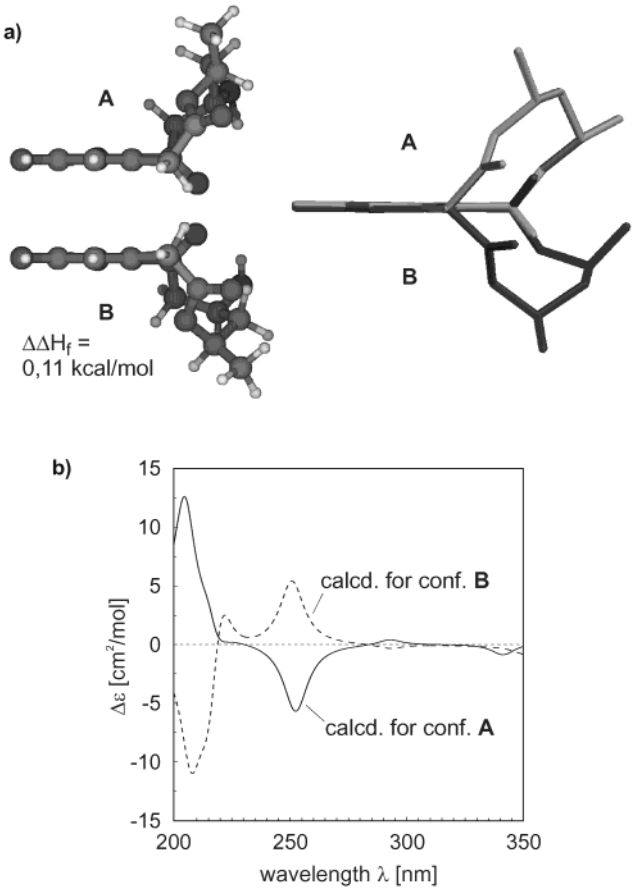

Figure 8. (a) Example of two conformers **A** and **B** of **2a** (arbitrarily for the 9*R*,11*S*-enantiomer) with mirror-image-like molecular frameworks accessible at room temperature (left) and their match with respect to the aromatic ring (right). (b) Comparison of two single CD spectra calculated as examples for the two "pseudoenantiomeric" conformers **A** (—) and **B** (- - -) with the same absolute configuration.

on C-9. The available quantities, however, were again not sufficient to establish the absolute configuration at this position. Decisive information on the absolute configurations was expected from chiroptical investigations by means of quantum chemical CD calculations, which had proved to be a particularly valuable tool in earlier studies.[19,20] Due to the flexibility of the molecular frameworks of **1** and **2**, we used an earlier established method[19] to calculate CD spectra of nonrigid molecules. For this purpose, the calculations of the CD spectra were based on molecular dynamic (MD) simulations.[19] Unfortunately, the resulting theoretical CD spectra matched only slightly with the experimental ones. To investigate the dependency of the geometry of the molecule and the CD curve, we performed a conformational analysis on **2a** (arbitrarily for the 9*R*,11*S*-enantiomer), the obviously most rigid species of the three decalactones because of the *cis*-configuration of the two substituents (9-OH, 14-CH_3) in the ring system, which can thus both adopt equatorial positions. Of the resulting minimum structures, single CD spectra were calculated. Several pairs of "pseudoenantiomeric", energetically very similar conformations were located. An example is shown in Figure 8, accessible at room temperature with mirror-image-like molecular stereoarrays. As expected from their diastereomorphous, but near-enantiomeric structural shapes and thus likewise virtually enantiomeric chromophores, the respective conformers provided pairwise opposite CD curves (Figure 8), difficult to weight according to the Boltzmann statistic, because of the *small energetic difference* between the conformers calculated. Due to these facts—the high flexibility of the molecule and the energetic similarity of conformers with enantiomeric chromophores—no unequivocal CD spectra could be calculated for all of the decalactones. Still, the calculations suggested a slight preference for an *S* configuration at C-11 for **1** and **2**, which, however, still has to be confirmed by (much more CPU-intensive) CD calculations at a higher level or, alternatively, by a stereochemically unambiguous total synthesis of the enantiopure xestodecalactones. This work is in progress.

The new xestodecalactone congeners **1** and **2a/b** are 10-membered macrolides containing a fused 1,3-dihydroxybenzene ring. As shown above, they are structurally related to sporostatin (**3**) from the fungus *Sporormiella* sp. M5032, an inhibitor of cyclic adenosine 3′,5′-monophosphate phosphodiesterase.[9] Similar compounds are the curvularins,[18,21] obtained from terrestrial strains of *Curvularia*,[10] *Penicillium* sp.,[11] *Alternaria*,[12] and *Cochliobulus*,[13] which are 12-membered macrolides (**4** and **5**) with the same fused aromatic ring system, incorporated with two additional methylene groups in the lactone ring. The new compounds **1** and **2** are also structurally related to the 10-membered lactones (**6** and **7**) devoid of the fused aromatic system first isolated from the plant-pathogenic fungus *Diplodia pinea* (diplodialides)[14] and later also from terrestrial strains of *Penicillium* sp. (decarestrictines).[16,22] Interestingly, similar compounds from the insect *Phoracanta synonyma*[15] have also been described. The 10-membered lactones isolated from the fungi of the genera *Diplodia*[14] and *Penicillium*[11,16,22] have been reported as steroid hydroxylase inhibitors and were described to exhibit antihyperlipidemic activity. Decarestrictine derivatives known as tuckolides were also isolated from the Canadian tuckahoe, *Polyporus tuberaster*, and were found to be HMGCoA reductase inhibitors.[23] Cytotoxic 10-membered lactones have been reported, which include apicularen A from the myxobacterium *Chondromyces pediculatus*[24] and humicolactone from the fungus *Gilmaniella humicola*, which inhibit proliferation of human cancer cell lines. Other bioactive 10-membered lactones are putaminoxins and pinolidoxins from the fungi *Phoma putaminum*[25] and *Ascochyta pinodes*,[26] respectively, which are of relevance to crop protection due to their selective phytotoxicity against certain weeds; mueggelone is a fish toxin isolated from the freshwater cyanobacterium *Aphanizomenon flos-aquae*;[27,28] and some examples of antibiotics include the aspinolides from *Aspergillus ochraceus*,[29] the cephalosporolides from *Cephalosporium aphidicola*,[30] and the didemnilactones from the tunicate *Didemnum moseleyi*.[31]

Compound **2a** was found to be active against the yeast *C. albicans*. Using the agar diffusion assay, it caused inhibition zones of 25, 12, and 7 mm at concentrations of 100, 50, and 20 μmol, respectively. All the isolated compounds were found to be inactive toward the bacteria *B. subtilis*, *S. aureus*, and *E. coli*.

As known for similar lactones,[32] the xestodecalactones should be formed via an acetogenic pathway, as evident from the periodically arranged oxygen atoms, which should be the remainders of a polyketide precursor.

Materials and Methods

The fungus *Penicillium cf. montanense* was isolated from fresh samples of the marine sponge *Xestospongia exigua*. Sponge samples were collected by scuba diving in the Bali Sea along the shores of Mengangan Island, Indonesia, in July 1997. Tissue samples were taken from inside the sponge body under sterile conditions and were inoculated on malt agar slants, containing malt extract (15 g/L), agar (15 g/L), and sea salt (24.4 g/L), and then incubated at 27 °C. From the growing

cultures, pure strains of *Penicillium cf. montanense* were isolated by reinoculation on malt agar plates. A voucher strain (no. HBI-3/D) is deposited at the Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven.

The taxonomic identification of the strain was effected by the standard method of Pitt,[33] i.e., growing the fungus for 7 days on three standard media (CYA, MEA, G25N) at 5, 25, and 37 °C and evaluating the colony habits and the micromorphology of the culture, hyphae, and sporulation structures. This clearly led to the anamorphic genus *Penicillium* LINK (deuteromycota, anamorphic trichocomaceae, mitosporic fungi: code group 1.A2.15). According to the monoverticillate penicilli, the strain has to be assigned to the section Aspergilloides. Entering all the data into the computer key[34] "Penname", we did not come to an unequivocal identification of the species, and the final count of "3" may only relate to the correct name. Thus, we identified our isolate HBI-3/D as *Penicillium cf. montanense*. The definite identification still has to be checked against the type culture *of P. montanense* CHRISTENSEN & BACKUS.

Prior to extraction, the cultures were grown in malt-broth medium (25 g malt extract in 1 L of seawater). After 41 days of incubation, the mycelium and culture filtrate were collected and extracted with MeOH and EtOAc. The total extract was evaporated under reduced pressure, taken to dryness (6.31 g), and chromatographed by vacuum liquid chromatography over SiO_2 gel employing CH_2Cl_2/MeOH as the solvent system in gradient ratios. The lipophilic fractions 1−3 contained fatty acids and sterols, while fractions 4−6 yielded xestodecalactone congeners **2b** (10.3 mg) and **2a** (19.2 mg) obtained at 92% CH_2Cl_2, while **1** (5.0 mg) was obtained at 90% CH_2Cl_2. Purification of the xestodecalactones was accomplished by semipreparative HPLC (Merck, Munich, Germany) on a Eurospher C-18 column using the following gradient: 0 min, 40% MeOH; 30 min, 60% MeOH; 35−40 min, 100% MeOH.

HPLC-NMR, -MS/MS, and -CD. The extract was dissolved in acetonitrile/water (2:8 v/v) and filtered through membrane filters of pore size 0.2 μm. For HPLC-MS/MS analysis, the solution was diluted to 0.1 mg mL^{-1} and 5 μL was injected. For HPLC-CD analysis, the solution was diluted to 1 mg mL^{-1} and 20 μL was injected. For HPLC-NMR experiments, a saturated solution of the crude extract was prepared in D_2O/MeCN (1:1) and 20 μL was injected.

For all hyphenated techniques, chromatographic separations were performed on a Symmetry C-1_8 column (Waters, Eschborn) (150 × 2.1 mm i.d., 5 μm for HPLC-MS/MS and 250 × 4.6 mm i.d., 5 μm for HPLC-NMR and HPLC-CD). Solvent A was 0.01% (v/v) TFA in water, and solvent B was MeCN. HPLC was programmed as follows: 0 min, 0% B; 35 min, 60% B, 40 min, 100% B. The flow rate was set to 1 mL min^{-1} for HPLC-NMR and HPLC-CD and to 0.2 mL min^{-1} for HPLC-MS/MS analysis. For HPLC-NMR, water was replaced by D_2O.

HPLC-NMR experiments were performed with a Bruker DMX 600 NMR spectrometer operating at 600.13 MHz ^1H frequency (Bruker, Rheinstetten, Germany) and controlled by the software system XWinNMR from Bruker. The outlet of the UV detector was connected to the flow-probe by a PEEK capillary via a BPSU interface (Bruker), controlling the experimental modes. The spectrometer was equipped with an inverse ^1H,^{13}C flow probe with a 3 mm (60 μL) detection cell (Bruker, Rheinstetten, Germany). The chromatographic system consisted of a Bruker LC-22 pump and a UV detector from Bischoff working at 254 nm absorption. Typically 128 scans with a sweep width of 12 kHz were accumulated. A WET solvent supression on MeCN and the residual HOD in D_2O was performed using standard Bruker pulse programs with additional carbon suppression in the proton experiments. For calibration, the residual signal of the acetonitrile was set to 2.0 ppm.

HPLC-ESI-MS/MS analyses were performed with a triple-stage quadrupole TSQ 7000 mass spectrometer equipped with an ESI interface (Finnigan MAT, Bremen, Germany), an Applied Biosystems 140b pump, and a personal DEC station 5000/33 (Digital, Unterföring, Germany), with ICIS 8.1 software (Finnigan MAT). Nitrogen served both as sheath and auxiliary gas; argon, as collision gas.

Positive ions were detected by scanning from 110 to 1000 μ with a total scan duration of 1.0 s for a single spectrum. HPLC-MS/MS experiments were performed at a collision gas pressure of 2.0 mTorr Ar (1 Torr = 133.322 Pa) and a collision energy of 0.2 s (or 1.2 s) for one experiment.

All CD spectra were measured on a J-715 CD spectrometer (JASCO, Gross-Umstadt, Germany) at room temperature in ethanol (offline) and acetonitrile/water (stop-flow) from 195 to 400 nm. Typically, four scans were accumulated. Furthermore, an LC-980-025 Ternary Gradient Unit, a PU1580 pump (JASCO), a Rheodyne 7725i injection valve, and the Borwin chromatographic software from JASCO were used for HPLC−CD coupling.

Offline ^1H NMR and ^{13}C NMR spectra (chemical shifts in ppm) were recorded in DMSO-d_6 on Bruker DMX 600 and Bruker AVANCE DPX 300 NMR spectrometers. Mass spectra (EIMS) were measured on a Finnigan MAT 8430 mass spectrometer. Optical rotations were determined on a Perkin-Elmer-241 MC polarimeter. CD spectra were recorded on a CD6 ESA Jobin-YVIN/dInstrument S.A. using MeCN as solvent. For HPLC analysis, samples were injected into an HPLC system (Gynkotek) coupled to a photodiode-array detector for an online measurement of the UV spectra. Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with Eurospher C-18. UV spectra were recorded in MeOH. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with Si gel 60 F254 (Merck, Darmstadt, Germany).

Computational Methods. Conformational Analysis. The conformational analysis of xestodecalactone C was performed on Linux *i*PII and *i*PIII workstations by means of the AM1[35] parametrization as implemented in the program package VAMP,[36] starting from preoptimized geometries generated by the Tripos[37] force field using the RandomSearch algorithm.

Molecular Dynamics (MD). The MD simulations were performed on Silicon Graphics OCTANE (R10000) workstations using the Tripos force field as implemented in the molecular modeling package Sybyl.[37] The molecules were weakly coupled to a virtual thermal bath.[38]

CD Calculations. The wave functions for the calculation of the rotational strengths for the electronic transitions from the ground state to excited states were obtained by CNDO/S-CI[39] calculations, in which the CI expansion[39] takes into account the ground state and all n and π orbitals. These calculations were carried out on Linux *i*PII and *i*PIII workstations using the BDZDO/MCDSPD[39] program package. For a better comparison of the theoretical CD spectra with the experimental ones, Gaussian band shape functions were generated over the calculated rotational strength values.

Xestodecalactone A (1): white powder residue; $[\alpha]_D$ +28.3° (*c* 0.31, MeOH); UV λ_{max} (MeOH) nm 208, 218 sh, 263, 300; CD λ_{max} (MeCN) nm 193 ($\Delta\epsilon$ +7.59), 208 ($\Delta\epsilon$ −0.74), 222 ($\Delta\epsilon$ +0.63), 238 ($\Delta\epsilon$ −2.14), 265 ($\Delta\epsilon$ −6.42), 286 ($\Delta\epsilon$ −1.05), 314 ($\Delta\epsilon$ +7.73); ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* $[M]^+$ 264 (88), $[M - H_2O]^+$ 246 (22), 217 (22), 205 (34), 194 (54), 166 (100), 150 (32), 121 (20), 97 (12), 81 (14), 69 (14), 55 (26); HREIMS *m/z* 264.2710 (calcd for $C_{14}H_{16}O_5$ 264.2738).

Xestodecalactone B (2a): white powder residue; $[\alpha]_D$ +17.3° (*c* 3.08, MeOH); UV λ_{max} (MeOH) nm 208, 218 sh, 240 sh, 288, 300; CD λ_{max} (MeCN) nm 195 ($\Delta\epsilon$ +5.27), 210 ($\Delta\epsilon$ −5.06), 224 ($\Delta\epsilon$ −0.27), 243 ($\Delta\epsilon$ −3.50), 266 ($\Delta\epsilon$ −8.67), 286 ($\Delta\epsilon$ −3.07), 318 ($\Delta\epsilon$ +9.92); ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* $[M]^+$ 280 (22), 252 (38), 195 (46), 167 (80), 150 (100), 122 (22), 69 (32), 43 (26); HREIMS *m/z* 280.0965 (calcd for $C_{14}H_{16}O_6$ 280.0947).

Xestodecalactone C (2b): white powder residue; $[\alpha]_D$ +22.5° (*c* 0.15, MeOH); UV λ_{max} (MeOH) nm 208, 218 sh, 240 sh, 288, 300; CD λ_{max} (MeCN) nm 195 ($\Delta\epsilon$ +3.56), 208 ($\Delta\epsilon$ −5.84), 223($\Delta\epsilon$ −1.11), 239 ($\Delta\epsilon$ −3.62), 257 ($\Delta\epsilon$ −11.65), 282 ($\Delta\epsilon$ −6.58), 325 ($\Delta\epsilon$ +18.93); ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* $[M]^+$ 280 (38), 262 (24), 252 (34),

236 (38), 229 (36), 195 (40), 167 (98), 150 (100), 121 (34), 69 (22), 43 (18); HREIMS *m/z* 280.0927 (calcd for $C_{14}H_{16}O_6$ 280.0947).

Bioassays. Antibacterial and antifungal activities were tested as described previously,[40] using the following microorganisms: *Bacillus subtilis* 168, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Escherichia coli* HB 101, and *Candida albicans*. The bacterial strains were from the laboratory cultures of the Institute for Hygiene, University of Würzburg, Germany.

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